20TH CONGRESS OF THE INTERNATIONAL UNION FOR PURE APPLIED BIOPHYSICS (IUPAB)

50TH ANNUAL MEETING OF THE BRAZILIAN SOCIETY FOR BIOCHEMISTRY AND MOLECULAR BIOLOGY (SBBQ)

45TH CONGRESS OF BRAZILIAN BIOPHYSICS SOCIETY (SBBF)

13TH BRAZILIAN SOCIETY ON NUCLEAR BIOSCIENCES CONGRESS

PROGRAM AND ABSTRACT BOOK

October, 2021

20th International Congress of the International Union for Pure Applied Biophysics (IUPAB)

50th Annual Meeting of the Brazilian Society for Biochemistry and Molecular Biology (SBBq)

45th Congress of Brazilian Biophysics Society (SBBf)

13th Brazilian Society on Nuclear Biosciences Congress (SBBN)

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October 4th to 8th, 2021

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Ilustração da Capa: Alexandre Takashi

Welcome Letter

On behalf of the International Union of Pure and Applied Biophysics (IUPAB), of the Brazilian biophysics and biochemistry and nuclear biosciences societies, we are honor to announce the book of abstracts of the 20th IUPAB Congress, which is occurring from 4th to 8th of October of 2021 in the virtual format.

The presence of leading scientists among the invited speakers will certainly contribute to creating a very rich scientific environment, which we hope will also allow for bringing together the best of science in biophysics and biochemistry. The most advanced platforms for virtual conferences will be used to allow a very interactive experience during the event. ExpoSBBq, which is an exhibition hall for companies and sponsors that usually is one of the greatest aspects of our past congresses, will be held during the 20th IUPAB Congress, 45th Congress of SBBf, 50th Annual Meeting of SBBq and 13th Congress of SBBN, and we are optimistic that it will likewise be a success in the virtual format.

With more than 1100 delegates, the 20th IUPAB Congress will certainly offer a broad international overview of research frontiers and recent developments in biophysics, biochemistry and radiation biology, emphasizing the importance of the transdisciplinary approach. We have organized an outstanding program with contributions in the form of keynote lectures and symposia, as well as oral and poster presentations. 14 keynote speakers, two of them Nobel prize winners, and 37 symposia will cover a wide range of interests in biophysics and biochemistry. Over 120 speakers (29 from North America, 27 from Europe, 23 from South America, 8 from Asia, Oceania and Middle East) from all continents (24 countries) in an equilibrated male:female ratio have accepted to deliver lectures. There will be two specific periods for poster presentations and we are expecting more than 500 poster presenters.

We hope you will appreciate the novelties and hard-core basic and applied science that is described in 527 abstracts presented in this book. We are certain that the congress will be an unforgettable event and we look forward to welcoming you to the virtual 20th IUPAB, 45th SBBf, 50th SBBq and 13th SBBN!

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Keynote Lectures

PL-01. - Impact of single particle electron cryo-microscopy in structural biology

Richard Henderson [1]
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In the last 8 years, single particle electron cryomicroscopy (cryoEM) has experienced rapid growth in its capability, due to improved electron microscopes, better detectors and better software, and this has revolutionised structural biology. I will describe some recent results and discuss remaining barriers to progress. CryoEM is already a very powerful method, but there are still many improvements that can be made before the approach reaches its theoretical limits. There is also a desperate need to expand access to the methodology by developing low-cost cryoEM equipment, so I will also describe some of our efforts in this direction.

Keywords: cryoEM, affordable cryoEM, remaining barriers

PL-02. - Co-temporal Force and Fluorescence Measurements Reveal a Ribosomal Gear-shift Mechanism of Translation Regulation by mRNA Secondary Structures

Carlos Bustamante [1]
[1]Department of Molecular and Cell Biology, Physics and Chemistry, University of California (Berkeley, USA)

Ribosome translocation on mRNAs is often interrupted by secondary structures that represent mechanical barriers and that play a central role in translation regulation. Here, we investigate how ribosomes couple their internal conformational changes with the activity of translocation factor EF-G to unwind mRNA secondary structures using high-resolution optical tweezers with single-molecule fluorescence capability. We find that hairpin opening occurs during EF-G catalyzed translocation and is driven by the forward rotation of the small subunit head. Moreover, we modulate the magnitude of the hairpin barrier by force and surprisingly find that ribosomes respond to strong barriers by shifting their operation to an alternative 7-fold slower kinetic pathway prior to translocation. This shift into a slow gear results from an allosteric switch in the ribosome that may allow it to exploit thermal fluctuations to overcome mechanical barriers. Finally, we observe that ribosomes occasionally open the hairpin in two successive sub- codon steps, revealing a previously unobserved translocation intermediate.

Keywords: Ribosome, Translation, Single Molecule

PL-03. - Targeting the microbiome in cancer immunotherapy

Giorgio Trinchieri [1]
[1]Laboratory of Integrative Cancer Immunology, Center for Cancer Research, National Cancer Institute, National Institutes of Health (Bethesda, MD, USA)

Commensal microorganisms colonize barrier surfaces of all multicellular organisms, including those of humans. For more than 500 million years commensal microorganisms and their hosts have coevolved and adapted to each other. As a result, the commensal microbiota affects many immune and non-immune functions of their hosts, and de facto the two together comprise one metaorganism. The commensal microbiota communicates with the host via biologically active molecules. Microbial imbalance plays a critical role in the development of multiple diseases, such as cancer, autoimmune conditions, and increased susceptibility to infection. The commensal microbiota not only may affect the development, progression, and immune evasion of cancer but it has also important effects on the response to cancer immune- and chemotherapy. In my presentation I will discuss our recent analysis of the role of the microbiome in anti-PD1 therapy in melanoma patients and the data of a fecal microbiota transfer clinical trial in anti-PD1 refractory melanoma patients that has provided proof of concept of the possibility to target the gut microbiota composition in cancer therapy.

Keywords: Microbiome, Cancer Immunotherapy, Fecal Microbiome Transfer

PL-04. Cryogenic superresolution correlative light and electron microscopy on the frontier of subcellular imaging

Tao XU [1]
[1]Institute of Biophysics, Chinese Academy of Sciences (Beijing, China)

Electron microscopy (EM) reveals cellular ultrastructure at a high definition but faces the challenges of identification of specific subcellular structures and localization of specific macromolecules, whereas fluorescence microscopy (FM) can label and localize specific molecules in the cells. Correlative light and electron microscopy (CLEM) combines the advantages of both microscopic techniques. Imaging vitreous hydrated samples at cryogenic temperatures using CLEM enables the observations of cellular components of interest and their cellular context in a near-native state. This cryo-CLEM approach is further strengthened by incorporation of superresolution fluorescence microscopy, which can precisely pinpoint the targets on electron micrographs. Cryogenic superresolution correlative light and electron microscopy (csCLEM) is an emerging and promising imaging technique that is expected to unveil its full power in ultrastructural studies. The present review describes the logic and principles behind this technique, how the method is implemented, the prospects, and the challenges.
Keywords: Cryogenic , electron microscopy, subcellular imaging

PL-05. LESSONS FROM 620 DAYS STUDYING COVID-19

Michael Levitt
Stanford University, USA

This talk will cover COVID-19 broadly in terms of the trajectory of a single outbreak, the expected burden of on multiple outbreaks, the role of interventions and how it will end.

PL-06. - Carbon dioxide redox metabolites in eustress and oxidative distress

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Life adaptation to molecular oxygen allowed evolution of complex life forms but came with a cost because oxygen is prone to one-electron transfers, producing metabolites (radicals and oxidants) that are toxic to life. In consequence, oxygen pushed an evolutionary explosion of alternative and novel metabolic networks yielding a variety of gene products, such as antioxidant enzymes that increased fitness of the organisms. Although oxygen and its metabolites imprinted the evolution of complex life forms, the cell damaging mechanisms of these metabolites received most of the attention over the years. Only recently, the participation of radicals and oxidants in both, physiological and pathological processes became widely accepted and the aged oxidative stress concept is changing to oxidative distress as opposed to the eustress concept (homeostasis). Here, I summarize investigations arguing for the influence of carbon dioxide (CO_2) redox metabolites on cells and organisms. Aerobes produce considerable amounts of this gas through respiration (humans, about 1 kg of carbon dioxide/day). The gas, in equilibrium with bicarbonate, is crucial for physiological pH control but at high level it toxic to mammals (hypercapnia) and microorganisms. Relevantly, carbon dioxide reacts with biologically ubiquitous oxygen metabolites such as peroxynitrite and hydrogen peroxide to render redox active metabolites such as the carbonate radical and peroxymonocarbonate, respectively. Several evidences indicate the participation of the carbonate radical in situations of oxidative distress (associated with nitric oxide overproduction, hypercapnia and related clinical situations). Peroxymonocarbonate attracted much less attention. Nevertheless, its formation may explain the accelerating effects of the bicarbonate buffer on the oxidation of thiol proteins, including important players in redox signaling, such as protein tyrosine phosphatase (PTP1B) and 2-Cys peroxiredoxins (Prx1 and Prx2). In times of increasing levels of atmospheric carbon dioxide, more studies are required to the understanding its impact on cellular and organisms homeostasis. **Keywords:** carbon dioxide, oxidative stress, eustress. **Supported by:** FAPESP (2013/07937-8); CNPq (300465/2009-2)

PL-07. - Calcium-driven voltage sensing and the role of charged residues in the voltage sensor domain of BK channels

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Allosteric interactions between the voltage-sensing domain (VSD), the Ca^{2+} -binding sites, and the pore domain govern the mammalian Ca^{2+} - and voltage-activated K+ (BK) channel opening. We examined the energetic interaction between Ca^{2+} binding and VSD activation by investigating the effects of internal Ca^{2+} on BK channel gating currents. Our results indicate that Ca^{2+} sensor occupancy has a strong impact on VSD activation through a coordinated interaction mechanism in which Ca^{2+} binding to a single a-subunit affects all VSDs equally. Moreover, the two distinct high-affinity Ca^{2+} -binding sites contained in the C-terminus domains, RCK1 and RCK2, contribute equally to decrease the free energy necessary to activate the VSD. We conclude that voltage-dependent gating and pore opening in BK channels is modulated to a great extent by the interaction between Ca^{2+} sensors and VSDs. On the other hand, the voltage sensing mechanism of BK channels is still unknown. Here, we demonstrate that two arginines in transmembrane segment S4 (R210, and R213) are the gating charges. The energy landscape of the gating particles is electrostatically tuned by the network of salt bridges contained in the voltage sensor domain (VSD). Molecular dynamics simulations and the hyperpolarization-activated transport of protons by the VSD mediated by the R210H mutant suggest that the electric field drops in a narrow septum whose limits are defined by the gating charges. In BK channels, unlike Kv channels, the charge movement is limited to a small displacement of the guanidinium moieties of R210 and R213, without a significant S4 movement. **Keywords:** Ca2+, voltage sensing, BK channels. **Supported by:** FONDECYT 1190203

PL-08. - The awesome power of Fluorine NMR

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^{19} F NMR is a powerful and versatile tool to study protein structure and protein–ligand interactions due to the favorable NMR characteristics of the ^{19} F atom, its small size and absence in naturally occurring biomolecules. ^{19} F atoms can be introduced readily into proteins and ligands, permitting to use them as 'beacons' to study interactions by NMR. Both, ligand and protein resonances can be exploited for this purpose. I will discuss several applications, involving ^{19} F-modified proteins and ^{19} F-containing ligands, demonstrating the awesome power of ^{19} F NMR.

Keywords: Fluorine NMR, protein–ligand interactions , protein structure

PL-09. - Next generation localization microscopy - or - how and why to ruin a perfectly good microscope

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In localization microscopy, the positions of individual nanoscale point emitters (e.g. fluorescent molecules) are determined at high precision from their point-spread functions (PSFs). This enables highly precise single/multiple-particle-tracking, as well as super-resolution microscopy, namely single molecule localization microscopy (SMLM). To obtain 3D localization, we employ PSF engineering – namely, we physically modify the standard PSF of the microscope, to encode the depth position of the emitter. In this talk I will describe how this method enables unprecedented capabilities in localization microscopy; specific applications include dense emitter fitting for super-resolution microscopy, multicolor imaging from grayscale data, volumetric multi-particle tracking/imaging, dynamic surface profiling, and high-throughput in-flow colocalization in live cells. We often combine the optical encoding method with neural nets (deep-learning) for decoding, i.e. image reconstruction; however, our use of neural nets is not limited to image processing - we use nets to design the optimal optical acquisition system in a task-specific manner.

Keywords: super-resolution microscopy, deep learning, computational imaging

PL-10. - Lipids are important: Avanti/IUPAB Award lecture
Anthony Watts [1]

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Lipid-protein interactions became a major topic of study after the now seminal description of the “fluid-mosaic model” of biomembranes by John Singer and Gareth Nicholson in 1972 [1], and it is still actively pursued – few areas have such longevity in biophysics. Initial studies [2,3] were driven by new developments in spectroscopy, in particular ESR spin-labels and wide-line (2H, 31P) NMR. Terminology such a “immobilized” and “annular” lipid were too specific, and only from an indepth description of anisotropy averaging of magnetic interactions in biomembranes [4] (eg: hyperfine splittings, quadrupolar interactions) was it realized that the time scale for any approach defined the semantics [4]. This work required synthesis of novel (at the time) spin labelled [5] and deuterated [3] phospholipids, a task taken on commercially by Walt Shaw and Avanti, to widen the use of these probes and lipids making them more readily available and bringing lipid research within the grasp of a much wider (physics, chemistry, biology) community. Reviewers were pretty tough in the beginning, and insisted on detailed functional studies to support any suggestion of specific lipid-protein interactions from biophysical approaches – much less is asked for these days. A technical challenge was the synthesis of cardiolipin [6], which we patented, and taken on by Avanti. Specific CL-interactions induce significant protein dynamics with electron transport components [7], but even today, rigid-atom crystal structures are modelled in such descriptions – membranes are dynamic encompassing multiscale motions. Early crystallographers insisted that membrane proteins should be totally free of lipids if they were to be crystallized, a totally misleading suggestion, and indeed, some lipids promote crystallization [8]. Lipids are now resolved in protein structures, often mistakenly assigned [9], but some have a major functional role, as with AR3 which we recently resolved, as the first structure of this important component in optogenetics, to 1.03Å [10]. References [1]. Singer J. & Nicholson G. (1972) Science, 175:720-731 [2]. Watts, A. (1993) In: Phospholipids Handbook (G. Cevc, ed.) 687-740, Marcel Dekker [3]. Watts, A. (1998) BBA, 1376:297-318. [4]. Watts, A (1981) Nature 294:512-513 [5]. Marsh D. & Watts A. (1982) Lipid-protein interactions. [6]. Duralski et al., (1989) Tett. Letts 30:35853588 [7]. Pinheiro et al., (1979) Biochemistry 18:5006-5013 [8]. Sternberg et al., (1983) J. St. Biol. 110:196-204 [9]. Marsh & Pali (2004) BBA 166:118-141 [10]. Juarez et al., (2021) Nature Comms 12:1-10
Keywords: Lipid-protein interactions, biomembranes, spectroscopy
Supported by: Leverhulme Trust Fellowship (to AW)

Symposia

SP-01. Drug design and delivery

SP-01.01 - Targeting Membrane Transporters for Oral Drug Delivery

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Membrane transporters play a critical role in the absorption, distribution and excretion of nutrients and drugs. Despite their importance, their structure remains poorly defined and limits the ability to effectively target transporters for drug delivery purposes. This presentation will review current applications and challenges associated with transporter-mediated drug delivery, especially as it relates to nanoparticle-mediated targeting platforms. It is well known that transporter systems can facilitate the uptake of small molecules that mimic endogenous substrates (e.g. sugars, amino acids, dipeptides, nucleoside analogs), but it has become clear recently that they can be efficient targets also for transporter substrates tethered to macromolecules or nanoparticle cargo. One such target is the intestinal bile acid transporter, which internalizes via endocytosis when presented with high-affinity substrates coupled to polymeric vesicles. An overview will be presented of this and other systems that can be exploited for macromolecular drug delivery. Additionally, functionalized nanoparticles can be utilized to modulate the gut immune system and may provide exciting new avenues for the treatment of chronic inflammatory diseases. Recent studies highlighting the potential for therapeutic treatment and intervention will be discussed.

Keywords: drug transport, membrane biology, drug delivery.
Supported by: National Institutes of Health

SP-01.02 - Polymer-Based Nanoparticles: Fabrication and Health Applications

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Nanoparticles are about one thousand times smaller than the average cell in a human body. Their small size, flexible fabrication, and high surface-area-to-volume ratio make them ideal systems for drug delivery. Nanoparticles (NPs) can be made from various materials, including lipids, metals, polysaccharides, and proteins. Biological lipid and protein-based NPs such as natural and synthetic lipids, collagen, elastin, corn zein, and soy lipid and protein-based NPs are advantageous in biodegradability, bioavailability, and relatively low cost. Many lipids and protein NPs are easy to process and can be modified to achieve desired specifications like size, morphology, and weight. Lipid and protein NPs are used in various settings and are replacing many materials that are not biocompatible and harm the environment. OBJECTIVES: An overview is given over UV and γ–irradiated diacetylene and albumin serum-based nanoparticles, their characterization structurally and functionally. MATERIALS AND METHODS: The Nps were characterized by AFM, DLS, zeta potential, TEM, gel-electrophoresis, and spectroscopy. We studied the stability of the NP at different pHs and time variation, changes in the tryptophan protein NP environment by fluorescence spectroscopy. The NPs were decorated if protein (B9) or lipids protect (DNA) and function-evaluated through its interaction with the hydrophobic drug Emodin. The binding and kinetic properties of the obtained complex were evaluated by biophysical methods and their toxicity in tumor cells. DISCUSSION AND RESULTS: According to its biophysics, the NPs are spherical nanosized vehicles. The nanoparticle is nontoxic for cancer cell lines. With Emodin, protein-NPs proved to be more active on MCF-7 cancer cell lines. Significantly, the lipid or albumin aggregates preserve the primary activity function and improved characteristics as excellent carriers of molecules. CONCLUSION: More than carrier properties, the NPs induced an immune response in macrophages which may be advantageous in vaccine and cancer therapy formulations. **Keywords:** Biophysics, Lipid /protein nanoparticles, Drug delivery. **Supported by:** CONICET, MINCyT, IAEA and UNQ

SP-01.03 - Microneedles and nanoparticles for dermal vaccination

Joke Bouwstra [1], Pieter Schipper[1], Juha Mönkäre[1], Guangsheng Du[1], Wim Jiskoot[1]
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One of the most promising strategies for the dermal vaccination are microneedles. Microneedles are microsized needles with a length less than 1000 μm. Microneedles permit pain free and minimal invasive vaccination. There are several microneedle systems under investigation, these are hollow microneedles, dissolvable microneedles, coated microneedles and microneedle pretreatment. In this presentation, we will focus on coated and hollow microneedle arrays. In case of coated microneedles, in most cases dip-coating has been used. In contrast, in our approach we use pH sensitive microneedle surfaces and coat the microneedle surface alternatingly with an anionic antigen and a cationic polymer, trimethyl chitosan, TMC. This is the so called layer by layer approach. As antigen we used diphtheria toxoid (DT). It appeared that by changing the number of coated layers, we could control the delivered dose in human skin accurately. In a subsequent vaccination study in mice, microneedle arrays coated with an increasing number of DT/TMC bilayers resulted in step-wise increasing DT-specific immune responses. Dermal immunization with microneedle arrays with a 10 times lower dose than subcutaneous immunization resulted in similar immune responses. Therefore, the layer-by-layer coating approach is highly suitable for dermal immunization. As nanoparticles can be very beneficial in increasing or shifting the immune response, nanoparticles are in principle very attractive candidates to be coated onto pH-sensitive microneedles. To select the most effective nanoparticles to redirect the immune response various nanoparticle formulations containing ovalbumin (antigen) and poly(I:C) (adjuvant) were administered intradermally using hollow microneedles. Four types of nanoparticles were compared: poly(lactic-co-glycolic acid (PLGA) nanoparticles, mesoporous silica nanoparticles (MSNs), liposomes and gelatin nanoparticles (GNPs). Release studies revealed that PLGA nanoparticles and liposomes had slower and more controlled release of OVA than the other two nanoparticle formulations. Subsequent immunization studies showed that the nanoparticles did primarily modulate IgG2a titers and improved cellular responses. These results indicated that a proper choice of nanoparticle is crucial in redirecting the immune response. PLGA, MSNs and liposomes are promising tools to be used for coating on pH sensitive microneedles. However, when studying MSNs coated microneedles, not only the type of nanoparticle, but also the interaction with the microneedles plays a role. **Keywords:** microneedles, vaccination, dermal delivery, skin, nanoparticles

SP-01.04 - Interfacial reactions in water to functionalize the surface of polymeric nanocapsules intended for drug targeting

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Biodegradable nanocarriers have been studied as a promising alternative to therapeutics contributing to expand the applications of nanotechnology. Some advantages of the nanoparticulate systems are related to the drug targeting reducing side effects and increasing therapeutic index. The presentation addresses the aspects of the synthesis of lipid-core nanocapsules, a nanocarrier useful to encapsulate poorly water-soluble drugs, which surface can be functionalized using interfacial reactions to form a chitosan-metal ion-ligand complex. Examples of physicochemical characterization of the liquid formulations and biological applications of the aqueous dispersions containing surface-functionalized lipid-core nanocapsules, including antitumor activity and atheroma inhibition, are discussed. The advantages of this new strategy to obtain surface functionalized polymeric nanocapsules are: a) easy process based on self-assembling and interfacial reactions, b) versatile surface functionalization, and c) no need of purification. This new platform was developed to obtain decorated soft nanoparticles showing the ability of the lipid-core nanocapsules as building blocks to produce functionalized multiple-wall nanocapsules having narrow size distributions with an excellent reproducibility. The results show the promising use of the formulations in nanomedicine.

Keywords: nanotechnology, nanocapsules , nanomedicine. **Supported by:** CNPq, CAPES, FAPERGS

SP-02. Protein Structure Dynamics and Functions

SP-02.01 - Structure determination of antimicrobial peptides in live bacteria

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Antimicrobial peptides (AMPs) have been extensively studied as promising alternatives to traditional antibiotics. Solid-state NMR has been used to characterise their effect on lipid bilayers, their primary target. Such studies are important to provide high-resolution details within a controlled and homogenous system but correlation with *in vivo* situations remains speculative, especially in view of the complex modulation observed with slight changes in sample conditions (pH, temperature, lipid composition or peptide concentration). Studying AMPs in live bacteria is, therefore, attractive but presents several challenges, such as sensitivity and bacterial lifetime. New strategies to study AMPs in live *E. coli* or *S. aureus* bacteria using solid-state NMR techniques will be presented. The impact of the AMP maculatin 1.1 (Mac1) on bacteria was monitored by 31P while structural details on the peptide were obtained using dynamic nuclear polarization (DNP) enhanced 13C and 15N solid-state NMR experiments. Under AMP stress, a significant change in DNA packing in *E. coli*and *S. aureus* was observed. Mac1 also modulated the lipid dynamics of the bacterial membranes. Finally, a novel strategy to perform in-cell DNP NMR experiments was established by using spin-labelled peptides; and {15N}13C REDOR measurements have been performed to measure the distance between several pairs of 13C=O and 15NH within the Mac1 amino acid sequence, which indicate that the peptide adopts a helical structure in bacteria. DNP and solid-state NMR techniques allow the structural determination of membrane-active peptides within bacteria and may lead to better understanding of their mechanism of action *in vivo.*

Keywords: membrane, solid-state NMR, antibiotics
Supported by: Australian Research Council, National Health & Medical Research Council

SP-02.02 - Time-Resolved Crystallography at X-ray Free Electron Lasers

Marius Schmidt [1]
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12 years ago, the first free electron laser for hard X-rays (XFEL), the Linac Coherent Light Source (LCLS) became available to the general user community. XFELs generate ultrashort X-ray pulses of extreme brilliance. Due to this, XFELs are exceptionally well positioned to conduct time-resolved studies on biological macromolecules. In this talk I will summarize some of our recent results on bacterial phytochromes, on the chloride ion pumping rhodopsin and on substrate diffusion in enzyme crystals.

Keywords: TR-SFX, Photoreceptors, Mix-and-Inject Serial Crystallography
Supported by: This work was supported by NFS STC 'Biology with XFELs (BioXFEL)', award number STC-1231306.

SP-02.03 - Structure, Function, and Dynamics of Voltage-Gated Sodium Channels and their Complexes with Drug

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Voltage-gated sodium channels are responsible for the conductance of sodium ions across cell membranes in neurological and cardiovascular tissues. They are essential targets for drug design, with particular relevance in epilepsy, cardiac conditions and pain diseases. Using crystallography, cryo-electron microscopy, and a range of biophysical techniques including circular dichroism spectroscopy, bioinformatics, and molecular dynamics calculations, we have examined the structure, function and dynamics of sodium channels and their complexes with a range of pharmaceutical drugs.
We have identified the binding sites and molecular interactions, and the functional effects of a wide range of both on-target and off-target drugs which bind to sodium channels. These studies provide crucial information for the development of new pharmaceuticals and for the understanding of side-effects of current drugs.
Keywords: sodium channels, structure, Drug Interactions

SP-02.04 - Structural snapshots of bacterial cell wall biosynthesis

Alexandre Martins[1], Carlos Contreras-Martel[1], Manon Janet-Maitre[1], Mayara M. Miyachiro[1], Leandro F. Estrozi[1], Daniel Maragno Trindade[2], Caíque C. Malospirito[2,3], Fernanda Rodrigues-Costa[2,3], Lionel Imbert[1], Viviana Job[1], Guy Schoehn[1], Ina Attrée[1], **Andrea Dessen** [1,2]

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The bacterial cell wall is important for survival and shape, and its biosynthetic mechanism is the target of beta-lactam antibiotics. The spread of resistant strains, however, has limited the usefulness of these drugs and calls for efforts towards studies of cell wall formation that could lead to the development of innovative treatments. The elongasome, or Rod system, is a protein complex that controls cell wall formation in rod-shaped bacteria. MreC is a membrane-associated elongasome component that co-localizes with the cytoskeletal element MreB and regulates the activity of cell wall biosynthesis enzymes. We employed electron cryo-microscopy and X-ray crystallography to determine the structure of a self-associated form of MreC from Pseudomonas aeruginosa in atomic detail. MreC monomers interact in head-to-tail fashion. Longitudinal and lateral interfaces are essential for oligomerization in vitro, and a phylogenetic analysis of proteobacterial MreC sequences indicate the prevalence of the identified interfaces. I will present results that illustrate a model where MreC's ability to alternate between self-association and interaction with the cell wall biosynthesis machinery plays a key role in the regulation of elongasome activity and sheds light on the importance of studying cell wall formation processes in light of the antibiotic resistance crisis.
Keywords: bacterial cell wall, antibiotic resistance, structural biology

SP-03. Biological Photosensors and their Applications in Optogenetics

SP-03.01 - Time-resolved detection of association/dissociation reaction and conformation changes of photosensor proteins towards applications in Optogenetics

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Photosensor proteins are important not only because of their biological functions but also because of their applications in optogenetics. To understand the molecular mechanism behind their biological functions and consequently seek possible applications to optogenetics, the dynamics of their intermolecular interaction (for example, association/dissociation reaction and conformational changes) upon photoexcitation need to be elucidated. Although it has been difficult to trace such reactions in the time domain using traditional spectroscopic techniques, the time-resolved diffusion method based on the transient grating (TG) technique has been demonstrated to possess a significant advantage in detecting such spectrally silent dynamics in a time-resoled manner. In this paper, the principle and studies on blue-light sensor proteins, phototropins (phots), is presented. The experimental method is based on the pulsed laser induced TG technique. Photoexcitation by two beams of laser light initiates a reaction, which creates spatial modulations in the refractive index. The TG method detects this refractive index change, and the temporal profile reflects reaction dynamics and the diffusion process. Reaction kinetics of dimerization, dissociation reactions, and conformational changes were measured from the signal. Phots are blue light sensor proteins found in higher plants and green algae. This protein contains the LOV domain, and the reaction has been attracting many scientists from various view points including the optogenetics. We studied the reactions in time-domain, and the reaction kinetics of dimerization, dissociation reactions, and conformational changes were determined. It is interesting to find that photochemical properties of phot from *Chlamydomonas reinhardtii* were considerably different from those of phot from *Arabidopsis* in terms of the conformational changes and their kinetics. This method can be employed to elucidate the reaction schemes and kinetics that cannot be detected by other spectroscopic methods.

Keywords: protein reaction, photosensor, diffusion

SP-03.02 - Light switchable protein engineering with photoactive yellow protein

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Photoactive yellow protein (PYP) is a 125 residue, that contains the chromophore *p*-coumaric acid linked to a Cys residue. Blue light irradiation is absorbed by the chromophore causing trans-to-cis isomerization and a series of molecular events that have been studied extensively using a variety of biophysical methods. PYP is thus a good test bed for exploring the engineering of a light switchable protein. I will describe our efforts in this area including numerous unexpected results we have obtained.

Keywords: optogenetics, light, protein engineering

SP-03.03 - Optogenetic control of biological processes: from photoreceptor engineering to their implementation in microbial, animal and plant systems

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The engineering of molecular switches using light as inducer allows the long-sought goal of remotely controlling biological systems at highest spatial and temporal resolution. The first systems, namely optogenetics 1.0, comprised the introduction and further engineering and customization of different opsins into neurons. The prompt and widespread implementation of light-regulated ion channels revolutionized experimental neurobiology within a few years, facilitating fundamental research on brain function and disease and yielding promising biomedical applications1. More recently, the development of a functionally different set of optoswitches has taken root and expanded the applicability of light as stimulus to control a plethora of cellular processes. These range from gene expression, protein stability, receptor function, subcellular localization of proteins and organelles up to the generation of biohybrid materials to manipulate extracellular environments and regulate cell viability. The non- opsin-based optogenetics or optogenetics 2.0, relies on the engineering of microbial and plant photoreceptors to transduce information in the form of photons into a molecular function, mediated e.g. by a change in protein conformation or enzymatic activity, that is in turn used to control a cellular process2. These theoretical- experimental approaches are enabling the minimally invasive study and control of biological systems at unprecedented spatio-temporal and quantitative resolution. We discuss here representative examples of the whole synthetic biology research process leading from the engineering and rewiring of the photoreceptors for the intervention of the molecular and cellular processes up to their application in vivo. We describe a wide family of tools sensitive to different wavelengths of the white light spectrum, namely UV-B, blue, green, orange, red/far-red. With hundreds of engineered photoreceptors and optoswitches being reported3, we have now entered an era in which we can combine different systems to achieve orthogonal, independent control of various cellular processes using light of different colors sequentially or simultaneously. We implement these molecular tools into microbial, yeast/fungi, mammalian cells, and in vivo in animals. We recently, we have successfully introduced optogenetic into plants, by overcoming the intrinsic experimental limitations posed by the need of plants for light to grow. We use optogenetics to precisely control metabolic and signaling networks, and introduce novel functionalities in the organisms. These synthetic biology strategies open up unforeseen perspectives in fundamental and applied research, including the biomedical and biopharmaceutical fields and crop improvement. 1Deisseroth K, Hegemann P (2017) The form and function of channelrhodopsin. Science 357: eaan5544. 2Christie JM, Zurbriggen MD (2021) Optogenetics in plants. New Phytologist 229: 3108–3115. 3Kolar K, Knobloch C, Stork H, Znidaric M, Weber W (2018). OptoBase: A web platform for molecular optogenetics. ACS Synthetic Biology 7: 1825–1828. E-mail: matias.zurbriggen@uni-duesseldorf.de, http://synthetic-biologie.hhu.de/en.html, https://www.ceplas.eu/de/forschung/principal-investigators/prof-dr-matias-zurbriggen/

Keywords: Optogenetics, Synthetic Biology, Photoreceptor engineering

SP-03.04 - An overview of the photosensitive system of the skin, a novel therapeutic target?
Leonardo Vinicius Monteiro de Assis [1]
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The skin has a system that can detect light in a fashion like the retina. Although its presence was initially reported almost 20 years ago, only in 2011 functional studies started to be reported in the literature. Initial studies suggested that opsins, a class of light sensitive proteins, were able to detect ultraviolet radiation in human skin melanocytes, leading to pigmentary responses. Opsins were also reported to participate in differentiation processes in human keratinocytes. Our group in Brazil, led by Prof Castrucci, contributed to the advancement of the field by demonstrating that murine melanocytes and melanoma cells express a functional photosensitive system that is responsive to white light and ultraviolet radiation (UVA). Interestingly, gene knockdown (siRNA) and knockout (CRISPR) strategies revealed that melanopsin (OPN4), a non-visual photopigment in the retina, participates as a UVA-sensor and regulates pigmentary and apoptosis-related processes. We also demonstrated that OPN4-dependent UVA-induced pigmentary response is dependent on cGMP pathway. Interestingly, we showed that the photosensitive melanoma cells seems to be more responsive as compared to the normal melanocytes (reviewed in de Assis et al., 2019; 2021). In addition to detecting light, we and other groups demonstrated that opsins also detect thermal energy. Such concept has been shown in sperm cells as well as in normal and malignant melanocytes. Recently, OPN4 was reported in human skin as a blue light sensor. Subsequent studies in the field suggested the role of another opsin, panopsin (OPN3), in the differentiation process of keratinocytes, the pigmentary response of human melanocytes, and hair follicle growth in response to blue light. However, the functionality of OPN3 as a light sensor has been questioned and it is still unclear (reviewed in de Assis et al., 2021). Lastly, another opsin, neuropsin (OPN5), was detected in murine skin and was shown to synchronize the molecular clock of the skin in response to UVA radiation. Although more than 20 years have passed since the discovery of the photosensitive opsin system of the skin, scientific interest has only increased in recent years, and therefore, many gaps in our knowledge still remain to be investigated. Taking the literature together, we can state that opsins are expressed in different human and murine skin cells and participate in important biological processes such as pigmentation, epidermal differentiation, and molecular clock synchronization. Within this line, the goal of this lecture will be to provide an overview of how the skin detect light and temperature via opsins, the biological processes regulated by this system, and possible manipulation for therapeutic purposes. References: de Assis LVM, Tonolli PN, Moraes MN, Baptista MS, and Castrucci AML (2021). How does the skin sense sun light? An integrative view of light sensing molecules. J Photoch Photobio C 47, 100403. de Assis LVM., Moraes MN, and Castrucci AML (2019). The molecular clock in the skin, its functionality, and how it is disrupted in cutaneous melanoma: a new pharmacological target? Cell Mol Life Sci 76, 3801-3826.
Keywords: Light detection, opsins, skin biology
Supported by: São Paulo Research Foundation (FAPESP)

SP-04 - Macromolecular Machines and Switching Devices

SP-04.01 - Molecular Mechanisms of Neuronal Exocytosis

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The central nervous system relies on electrical signals traveling along neurons at high speeds. Signals are also transmitted between two neurons, or from a neuron to a muscle fiber through synaptic junctions. Synaptic transmission relies on the release of neurotransmitter molecules into the synaptic cleft. This release in turn depends on a process called membrane fusion to ensure that the neurotransmitter molecules that are contained in synaptic vesicles are released into the synaptic cleft as quickly as possible. Membrane fusion is an important process in many areas of biology, including intracellular transport and hormone release, but it occurs much faster (< 1 millisecond) for synaptic vesicle fusion than for these other processes. Moreover, it is precisely calcium regulated. Recent structural and biophysical studies of the molecular mechanisms of neurotransmitter release will be presented.
Keywords: Exocytosis, neurotransmitter, Membrane

SP-04.02 - Honing in on motile filamentous assemblies by cryo-EM

Charles Sindelar
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Many fascinating cellular processes related to motility involve filaments (i.e., actin, microtubules, bacterial flagella) and their associated molecular motors and other cofactors. While recent developments in cryo-electron microscopy have greatly facilitated structural studies of macromolecular assemblies, filamentous structures present unique challenges. I will share several stories of how our group has developed and utilized specialized cryo-EM image-processing tools to develop a better understanding of how filament-related macromolecular machines, including the filaments themselves, work. Specific examples will include our work with kinesin and myosin molecular motors, the actin co-factor cofilin, and flagella from the spirochete bacterial phylum. A common theme in these systems is the importance of identifying and accounting for multiple types of structural heterogeneity, from single subunits to large-scale bending, flexing and twisting of filaments. Our cryo-EM approaches have provided insights into fundamental phenomena such as chemo-mechanical energy transduction by molecular motors, actin filament severing, and how bacterial flagella maintain a screw-like supercoiled shape during rotary propulsion. Given the rapidly growing capabilities for cryo-electron tomography, prospects are bright for expanding these kinds of investigations into a cellular context.
Keywords: cryo-EM, actin, microtubules, bacterial flagella

SP-04.03 - Watching bacterial sensors as they move: pliable proteins that transmit signals

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Bacteria use different protein machineries as a means to sense environmental and intracellular signals, and to respond adaptively. These sensory transduction systems include two-component systems (TCS), one-component systems (OCS), phosphotransferase systems (PTS) and extra-cytoplasmic function (ECF) sigma factors. The proteins involved act as switching devices, not only as on/off toggles, but also as modulators, able to shape different output signals according to input information. TCSs are often organized according to a minimal configuration, comprising a sensory histidine-kinase (HK) and a response regulator (RR). The HK has signal-dependent kinase activity, auto-phosphorylating a conserved His at the expense of ATP. The P~HK transfers the phosphoryl group to a conserved Asp residue on the cognate RR. The molecular bases of switching, enabling HKs and RRs to sense signals and transmit information in the form of output effects, are still a matter of intense investigation. Based on biochemical and crystallographic evidence obtained from separate HK and RR proteins, as well as from HK:RR complexes, the mechanism of on/off switching has been unveiled. A coiled-coil-driven shifting machine modifies the position of the reactive His and controls the ATP-binding domains' flexibility. We also present results that uncover molecular determinants of directionality in the phosphoryl flow. In prototypical TCSs phosphoryl-transfer generally occurs unidirectionally from the P~His to the RR's Asp. However, in phosphorelays, both P~His-to-Asp and P~Asp-to-His reactions are necessary to walk along the pathways, implying bidirectional flow in vivo. The precise configuration of the reaction center in different HK:RR complexes dictates the reversibility/irreversibility of the phosphoryl-transfer, correlated to the distance between the phosphoryl-acceptor and -donor residues. Protein malleability is key to enable signal sensing and control of output activities, be them enzymatic or protein:protein and protein:DNA association capacities. TCS switching will be compared to OCSs', showcasing the functional relevance and universality of protein dynamics features.

Keywords: Allosteric regulation, Phosphoryl-transfer, Protein structure
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SP-04.04 - Regulation of the photosynthetic AB-GAPDH via self-assembly

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Oxygenic phototrophs perform carbon fixation through the Calvin–Benson cycle. Different mechanisms adjust the concurrent metabolic reactions and light-harvesting processes to rapid environmental changes. Photosynthetic glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is a key enzyme of the cycle. In higher plants, two isoforms of GAPDH exist: the most abundant hetero-tetramer formed by A and B-subunits, and the homo-tetramer A4. Regardless of the subunit composition, the light-produced NADPH is exclusively consumed by GAPDH. For this reason, GAPDH activity is strictly regulated. Differently from the CP12-dependent regulation of A4-GAPDH, AB-isoform is autonomously regulated through the C-terminal extension (CTE) specific of B-subunit. The inactivation of AB-GAPDH occurs via oxidation of a cysteine pair located in the CTE, the substitution of NADP(H) with NAD(H) in the cofactor binding domain and therefore changes in the state of oligomerization leading to an inactive enzyme. The present study is aimed at disclosing the structural basis of the CTE-dependent regulatory mechanism. The structure of the AB-GAPDH enzyme purified from spinach and incubated in activating and inactivating conditions was studied in solution by SEC-SAXS and single particle cryo-EM analysis. The coexistence of several (A2B2)n oligomerization states (with n=2,4,5) was revealed, whose relative proportion depended on the solution conditions, showing an unexpected dynamicity. The modeling of the higher resolution cryoEM density maps of A4B4 and A8B8 oligomers showed that contacts between adjacent A2B2 tetramers are uniquely mediated by B-subunits. Moreover, the CTE of each B-subunit directly penetrates into the active site of the B-subunit of the adjacent tetramer, effectively preventing the binding of the substrate. This picture at molecular level shows how the dynamic changes in the oligomeric status of AB-GAPDH allows the modulation of the Calvin-Benson cycle in response to the fast changes of light conditions occurring in the natural environment.

Keywords: Calvin-Benson cycle GAPDH, SAXS, Cryo-EM

SP-04.05 - Functional characterization of β-lactam sensor proteins in *Staphylococcus aureus*

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Staphylococcus aureus is the main cause of intra- and extra-hospital infections and is considered a high-priority multi-resistant pathogen. The manifestation of resistance to β-lactam antibiotics in *S. aureus* is regulated by the transmembrane proteins BlaR1, MecR1 and VraS/VraT of the *bla*, *mec* and VraSRT systems. These proteins have extra-cytoplasmic domains that bind the antibiotic (BlaR1 and MecR1) or that might sense it (VraS and VraT). In this study we aim at unveiling the molecular details of the conformational change triggered by β-lactams to activate the metalloprotease domain of BlaR1 and MecR1, and elucidating the mechanism of activation of VraS by β-lactams. We combined the use of ampicillin-derived photoprobes, reporter strains, recombinant protein expression, phosphorylation assays, western blot, band-shift assays and electron microscopy. Using the *S. aureus* reporter strains we observed constitutive activation of the system for BlaR1-MN8 in contrast to inducible activation for wild type BlaR1, lower activation of the *mec* operon, and we confirmed that our ampicillin-derived photoprobes activate the VraSRT system. Incubation of recombinant VraS in *E. coli* spheroplasts with the activated photoprobes showed a band shift of VraS indicative of formation of a covalent adduct with the antibiotic, and an increase in VraS autokinase activity. Electron microscopy images of negatively stained VraS samples suggested formation of trimers or tetramers. We concluded that the mutation found in BlaR1-MN8 yields a constitutively-active metalloprotease, but with a lower activity than WT BlaR1, in agreement with the lower β-lactamase activity seen in *S. aureus* MN8 in comparison with strain NRS128. Regarding the *mec* operon, we concluded that the level of expression mediated by β-lactam-activated MecR1 is significantly lower than that mediated by BlaR1, even upon expression of MecR2. In addition, our results suggested a direct interaction of β-lactams with VraS, at a site yet to be elucidated, that upregulates autophosphorylation.

Keywords: ampicillin photoprobes, β-lactam-resistance, Staphylococcus aureus

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SP-05. Royal Society of Chemistry Chemical Biology

SP-05.01 - Probing bacterial survival strategies: inhibitors of (p)ppGpp synthesis

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Persistence is a bacterial bet hedging strategy that allows for temporary tolerance to antibiotic treatment. This phenotypic switch paves the way to the chronicity of certain infections and to the insurgence of genetic resistance. Here we present our work on targeting bacterial persisters via inhibition of the upstream of the stringent response (SR), one of the working hypothesis for their formation. The SR is triggered by the accumulation of the second messenger (p)ppGpp, promoted by a superfamily of enzymes called RSH (RelA/SpoT Homologue). e performed fragment-based virtual screening on the synthetase catalytic site of our model bifunctional protein RelSeq, selecting three main chemotypes. Thermal shift analysis on RelSeq constructs highlighted interesting affinities of some selected fragments, along with the desired selectivity over the hydrolase domain. The most promising scaffold was therefore selected for the development into a higher affinity ligand.

Keywords: Persisters, (p)ppGpp, design
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SP-05.02 - Many birds with one stone: targeting a universal signaling pathway of bacteria to improve antimicrobial therapy

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Winning the war against resistant bacteria will require a change of paradigm in antibiotic discovery. A promising new direction is the targeting of non-essential pathways required for successful infection, such as quorum-sensing, virulence and biofilm formation. Similarly important will be strategies to prevent or revert antibiotic resistance. Here we argue that the ppGpp signaling pathway should be a prime target of this effort, since its inactivation could potentially achieve all these goals simultaneously. The hyperphosphorylated guanine nucleotide ppGpp is an ancient and universal second messenger of bacteria that has pleotropic effects on the physiology of these organisms and has been implicated in the long term survival and the development of virulence and antibiotic tolerance and persistence in diverse bacteria. The cellular concentration of ppGpp is controlled by enzymes of the RSH (RelA SpoT Homology) family. Long RHS proteins are bifunctional enzymes capable of synthesizing and degrading ppGpp, whereas short RSH, also known as SAS (Small Alamone Synthetases), are single domain proteins that only synthesize ppGpp. Despite the importance of this pathway, there are remarkably few inhibitors of the RSH enzymes described in the literature. Here we will describe our efforts to develop a pathway-specific whole cell assay capable of identifying inhibitors of both the long RSH and SAS enzymes and preliminary results of the screen of two types of small molecule libraries.

Keywords: ppGpp, Rel, RSH, SAS, persistence
Supported by: FAPESP, CNPq

SP-05.03 - Chemo-Optogenetic Probes for Light-Controlled Switching of Ion Channel Activity

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Optogenetics has proven to be a transformative approach for various fields of basic research, particularly in neuroscience. It allows for a non-invasive, localized, and temporally selective optical modulation of selected cells within an animal. The use of channelrhodopsin and other opsin-based optogenetic actuators is limited, however, due in part to their low conductance, which requires that the optogenetic channel be highly overexpressed, and their algal origin, which leads to the potential for unwanted immunological responses, restricting clinical applications. We have combined automated chemical screening and scalable zebrafish behavioral assays to discover novel chemo-optogenetic compounds, including optovin and TRPswitch. These compounds bind to the vertebrate cation channel Trpa1b and convert it into a photoresponsive channel, enabling reversible and repeatable light-induced activation as well as deactivation of Trpa1b-expressing cells. Channel activation is sustained upon exposure to a short pulse of violet light illumination and is deactivated with an additional short pulse of green light. This chemo-optogenetic system exhibits high channel conductance of about 100 pS, 1000 times greater than channelrhodopsin, making it ideal for applications where high conductance or low levels of protein expression are desired. The utility of this system is demonstrated by numerous applications in the nervous system and by light-induced stopping and restarting of heartbeat *in vivo* where cardiomyocytes exogenously express Trpa1b. Therefore, Trpa1b/TRPswitch represents a novel photoswitchable step-function chemo-optogenetic system with immediate utility in biological research and potential for future clinical application.

Keywords: chemo-optogenetics, ion channels, phenotypic screening

SP-05.04 - Interaction of genetically encoded photosensitizers with scintillating nanoparticles for X-PDT

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The discovery and development of phototoxic proteins able to produce reactive oxygen species (ROS) allowed the employment of genetically encoded photosensitizers (PS) as light activated devices. Light-induced generation of ROS by chromogenic compounds has been used for single molecule inactivation and cell killing. However, for in vivo applications, optical techniques are limited by the low penetration of UV-visible light into biological tissues. To overcome this limitation, the use of X-rays has been suggested as a promising energy source for excitation of PS, due to their high penetrability in soft tissues. Because most PS have absorption coefficients that are relatively high at visible wavelength but low at X-ray frequencies, the X-ray-induced sensitizers (XS) usually comprises a traditional PS and a scintillating nanoparticle (ScNP). Therefore, understanding the physicochemical interactions and energy-transfer mechanisms between ScNP and biomolecules are of most importance to the development of X-ray activated photodynamic therapy (X-PDT). In this work, the interaction of the genetically encoded photosentizers eGFP, KillerOrange, and KillerRed proteins with LaF_3:Tb^{3+} ScNP was investigated, for the first time, in terms of physicochemical and energy-transfer properties. To do so the time-resolved and static fluorescence, TEM, DLS and radioluminescence techniques were used. The protein structure, stability and function proved to be resistant upon adverse physiological conditions (similar to the observed in cancer cells) and also upon X-ray irradiation. Energy transfer from ScNP to the three proteins was confirmed. It was also shown that the 6xHis-tag acts as a linker for protein in nanoparticles doped with Tb^{3+} , promoting the formation of stable complexes. The toxicity of these complexes upon irradiation were evaluated in *E. coli* culture. The energy transfer between the ScNP and proteins resulted in a conjugated nanocompound with a radiation-exposure-dependent toxicity that opens a new avenue on the use of genetically encoded photosensitizers for applications in X-PDT.

Keywords: genetically encoded photosensitizer, scintillating nanoparticles, X-ray irradiation
Supported by: FAPESP

SP-05.05 - Discovery of Nanomolar Myeloperoxidase Inhibitors with Anti-Arthritis Properties: A Computational, in vitro and in vivo study

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Myeloperoxidase (MPO) is an abundant enzyme in neutrophils and has an important role during inflammatory response. MPO uses hydrogen peroxide to oxidize chloride to hypochlorous acid (HOCl), a strong oxidizing agent. Pre-clinical investigations demonstrated that MPO is a key enzyme in cardiovascular and neurodegenerative diseases and in the Covid-19 severe acute respiratory syndrome (SARS), being a promising therapeutic target against this inflammatory disease. The objective this study was to discover new MPO inhibitors, that would be active in vivo, by using an inhibitor-like rule and virtual screening. Analysis of the molecular properties of know MPO inhibitors allowed the creation of an Zinc12 enriched sub database formed by 6546 compounds that after structure-based virtual screening recovers 28 computational hits. By measuring both, peroxidase and chlorinating activity of the enzyme, we found that 60% of the selected compounds were able to inhibit MPO. The IC50 of the five best inhibitors ranged from 0.3 to 16 μM and all compounds were reversible inhibitors. The inhibitors also prevented HOCl production by neutrophil-like HL-60 cells and by peripheral blood neutrophils human at the same range as known irreversible inhibitors. Four compounds were assayed in a murine model of gouty arthritis and all of them presented anti-edematous activity when administered via intraperitoneal and three of them when given orally. These results indicate that the virtual screening methods here applied recovered MPO inhibitors with a high successful rate (60%) and those that were the selected for in vivo studies presented significant anti-inflammatory properties by two different routes of administration.

Keywords: mieloperoxisase, Virtual screening, gouty arthritis. **Supported by:** CAPES

SP-06 – 24th Young Talent Award in Life Science

SP-01.01 - 3D Bioprinting Neurogenic Niches aiming the Biofabrication of In Vitro Models to Study Neurodegenerative Diseases and Treatments

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3D bioprinting technology has been a promising alternative in the tissue engineering field, due to its capacity to construct highly organized structures through the well-controlled deposition of cells and biomaterials, forming tissues with similar characteristics to those found in the in vivo model, concerning the geometry, cells density and organization, composition of biomolecules and extracellular matrix (ECM). Due to its complex architecture, the in vitro reproduction of the central nervous system (CNS) and its neurogenic niches is difficult and compromises the advances of studies involving the treatment of neural diseases. Thus, this work aimed to use the 3D bioprinting technology to fabricate neurogenic niches and obtain in vitro models to study the mechanisms involved in the development of neurodegenerative diseases. For this, the biocompatible materials metacrylated gelatin (GelMA) and fibrinogen were used as the bioink to bioprint neural stem cells (NSC) and fabricate neural tissue-like of different stiffness. Different GelMA-fibrinogen compositions were tested as their printability, mechanical, rheological and structural properties. In addition, we evaluated the effects of the biomaterials' stiffness on the NSC viability and differentiation capacity. Results showed that cells directly responded to the biomaterials' composition and stiffness, with the neuronal differentiation being favored by the softest composition, while astrocytes differentiation was preferable in the stiffest hydrogels. This work represents a preliminary study of the CNS engineering, which will greatly contribute to further studies on the development of neurodegenerative diseases treatments.

Keywords: 3D Bioprinting , Biofabrication , Neurodegenerative Diseases

SP-06.02 - Evaluation of the microRNAs in the immunopathogenesis of microcephaly caused by ZIKV

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Over the years, viral infections cause severe illness in humans. Zika Virus (ZIKV) is a flavivirus transmitted by mosquitoes that leads to notable neurological impairment, which characterizes the Congenital ZIKV Syndrome (CZS). Several regulators of biological processes are involved in CZS development, and microRNAs (miRNAs) have a fundamental role. miRNAs are important regulators once they form the RISC silencing complex and interact with complementary mRNA target sequences to further post- transcriptionally repression. The importance of miRNAs during embryonic development and antiviral immune response is unquestionable, as neuronal, and glial differentiation is finely orchestrated by miRNAs network. In this context, very little is known about the interplay between miRNAs and microcephaly caused by ZIKV. Here, we evaluated miRNAs that target neurodevelopment and antiviral immune response genes. Comparing the miRNA profile of infected primary astrocytes from SJL mice neonates, we observed that miR-295 and miR-302d were significantly upregulated by the infection. Using bioinformatics tools of prediction, we showed that ZIKV decreases Ahrr, Bcl2l11, Neurod4 and Neurod6 expression, which are miR-295 and miR-302d target genes. Conversely, the use of miR-295 and miR-302d inhibitors reverted Ahrr, Bcl2l11, Neurod4 and NeuroD6 gene expression to the same level of the non-infected group. Interestingly, this was associated with a decreased ZIKV viral load. Therefore, our data point miRNAs as potent regulators of biological processes in face of ZIKV infection, as ZIKV positively regulates miR-295 and miR-302d to consequently downregulates antiviral immune response and neurodevelopment related-genes, assisting in the establishment of microcephaly characteristic lesions. The results provided important cues not only about the pathogenesis of microcephaly caused by ZIKV but also unravels possible targets for therapeutic intervention.
Keywords: microRNAs, ZIKV, microcephaly

SP-06.03 - Vascular smooth muscle cells drive osteoblast-to- osteocyte transition via β-catenin signaling through exosome communication

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While blood vessel growth in the skeletal systems and osteogenesis are coupled, fundamental aspects of vascular function in mature osteoblast-to- osteocyte transition have not been addressed. Here we show that vascular smooth muscle cells (VSMCs), but not endothelial cells, are sufficient and necessary to drive osteoblast-to-osteocyte transition. This transition is dependent on VSMC-derived exosomes loaded with osteocyte marker transcripts encoding proteins related to osteocyte phenotype (SOST, DMP1, FGF23, miR23a), as well as Wnt/catenin signaling members. In contrast, endothelial cells-derived exosomes deliver stimuli to mature osteoblast differentiation up-reprogramming TGF gene family and osteogenic transcriptional factors Osterix and Runx2. To better identify the role of VSMCs, conditioned medium was harvested from these cells following shear stress to mimic arterial flow of blood. Curiously, unchallenged VSMCs triggered better performance, which released exosome presented smaller membrane zeta potential. Another important issue was the high level of ATP inside the exosomes, favoring mechanisms of mineralization, since ATP is a substrate for alkaline phosphatase. Osteocyte function was validated by RNAseq and activity of genes encoding proteins related to intermittent mineralization, as well as sonic hedgehog signaling reprogramming genes and significant increase of RANKL level. Our findings identify a novel and hitherto unexpected role of vascular smooth muscle cells driving osteoblast- to-osteocyte transition, with potential clinical relevance in case of bone-related systemic diseases.
Keywords: Osteogenesis, Angiogenesis, Osteocyte, Cell signalling.
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SP-06.04 - Integrated production of high-value aromatic alcohols directly from lignocellulosic biomass

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Sustainable production of fine chemicals from renewable plant biomass offers an excellent alternative to the continued use of finite geological oil reserves for fine chemistry purposes. However, in order to compete with current petrochemical refinery processes, alternative biorefinery processes must overcome significant costs and productivity barriers. The production of high-value aromatic alcohols directly from lignocellulosic biomass is an attractive alternative to add value in biorefineries worldwide. Herein, we demonstrate the biocatalytic production of the versatile chemical building block, coniferol, directly from lignocellulosic biomass. Following the biocatalytic treatment of lignocellulose to release and convert ferulic acid with feruloyl esterase (XynZ), carboxylic acid reductase (CAR) and aldo- keto reductase (AKR). This whole-cell catalytic cascade not only achieved the equivalent release of ferulic acid from lignocellulose compared to alkaline hydrolysis but also displayed efficient conversion of ferulic acid to coniferol. This system represents a consolidated biodegradation-biotransformation strategy for the production of high-value fine chemicals from waste plant biomass, offering the potential to minimize environmental waste and add value to agro-industrial residues.

Keywords: Lignocellulose, biocatalysis, Coniferol.
Supported by: FAPESP (2016/07926-4)

SP-06.05 - An N-capping asparagine-lysine-proline (NKP) motif contributes to a hybrid flexible/stable multifunctional peptide scaffold

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Structural diversity is intrinsically related to biological activities in peptide drug candidates. Therefore, numerous peptide scaffolds have been reported to date and, in some cases, correlated with a given biological activity. Here, we describe an unusual N-capping asparagine-lysine-proline (NKP) motif that confers a hybrid flexible/stable multifunctional scaffold to a computationally designed peptide (PaDBS1R7). PaDBS1R7 has a shorter α-helix segment than two other computationally designed peptides of similar sequence but with key residue substitutions. Nevertheless, although the NKP motif acts as an α-helix breaker in PaDBS1R7, the N-terminus asparagine presents exclusive N-capping effects, resulting in a highly amphipathic and stable α-helix from Pro7-Ile17. The solution nuclear magnetic resonance structures, along with temperature coefficient spectra and computational peptide mutants reinforced the role of the NKP motif for a coil/N-cap/α-helix scaffold. Biological studies revealed that all PaDBS1 peptides presented antibacterial activities, without interfering with bacterial surfaces at their minimal inhibitory concentration. However, only PaDBS1R7 displayed anticancer properties, completely eradicated Pseudomonas aeruginosa biofilms, decreased bacterial counts from 100-1,000 times in an abscess mouse model and reduced LPS-induced macrophages stress, without modulating the expression of TNF-α and IL-1β. This multifunctionality was also investigated in terms of peptide/mimetic vesicle interactions, revealing that in all conditions tested PaDBS1R7 preserved its lower α-helical content, but with higher ability to disrupt bacterial-like and cancer cells-like vesicles. Based on these results, we are confident that this study extends our understanding of an N-capping NKP motif to developing structurally hybrid peptide drug candidates with multiple biological activities.

Keywords: N-capping, peptide scaffold, multifunctionality

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SP-07. Deforming membranes

SP-07.01 - Mechanism of shaping membrane nanostructures of Endoplasmic Reticulum

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Recent advances in super-resolution microscopy revealed the previously unknown nanoscopic level of organization of endoplasmic reticulum (ER), one of the most vital intracellular organelles. Membrane nanostructures of 10-100nm intrinsic length scales, which include ER tubular matrices, ER sheet nanoholes, internal membranes of ER exit sites (ERES) and ER transport intermediates, were discovered and imaged in considerable detail, but the physical factors determining their unique geometrical features remained unknown. Here we proposed and computationally substantiated a common concept for mechanisms of all four ER nanostructures based on the membrane intrinsic curvature as a primary factor shaping the membrane and ultra-low membrane tensions as modulators of the membrane configurations. We predicted computationally the existence of a discrete series of equilibrium configurations of ER tubular matrices and recovered the one corresponding to the observations and favored by ultra-low tensions. We modeled the nanohole formation as resulting from a spontaneous collapse of elements of the ER tubular network adjacent to the ER sheet edge and calculated the nanohole dimensions. We proposed the ERES membrane to have a shape of a super-flexible membrane bead-chain, which acquires random-walk configurations unless an ultra-low tension converts it into a straight conformation of a transport intermediate. The adequacy of the proposed concept is supported by a close qualitative and quantitative similarity between the predicted and observed configurations of all four ER nanostructures.
Keywords: membrane curvature, membrane shaping, membrane elasticity

SP-07.02 - To bud or not to bud: remodeling of artificial cells

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Cell membranes exhibit a large variation in curvature. It is a common perception that curvature is caused by the activity of specific protein species. Here, we will demonstrate that it can be readily generated by various other asymmetries across the membrane, which plausibly represent a governing factor for defining shapes of membrane organelles. As a workbench for artificial cells, we employ giant vesicles (Annu. Rev. Biophys. 48:93, 2019). In this talk, we will introduce approaches employing them for the precise quantification of the membrane spontaneous curvature. Several examples for generating curvature will be considered: asymmetric distribution of ions on both sides of the membrane (Nano Lett. 18:7816, 2018), insertion/desorption of the ganglioside GM1 (PNAS 115:5756, 2018), asymmetric lipid distribution (Sci. Rep. 8:11838, 2018) and PEG adsorption (PNAS 108:4731, 2011; ACS Nano 10:463, 2016). We will also show how spontaneous curvature generation by protein adsorption at low surface density is able to modulate membrane morphology and topology to the extent of inducing vesicle fission (Nature Commun. 11:905, 2020). Finally, the process of membrane wetting by molecularly-crowded aqueous phases will be shown to induce vesicle budding and tubulation (Adv. Mater. Interfaces 4:1600451, 2016). The presented examples will demonstrate that even in the absence of proteins and active processes, the membrane is easily remodeled by simple physicochemical factors.
Keywords: curvature genaration in membranes, membrane nanotubes, spontaneous curvature
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SP-07.03 - Control of actin assembly at the cell membrane by phosphatidylinositol 4,5 bisphosphate

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Over 35 years ago, a landmark report by Lassing and Lindberg (Nature 314:472-4 (1985)) showed that phosphatidylinositol 4,5 bisphosphate (PIP2), but not other lipids nor IP3 the isolated headgroup of PIP2, was able to remove actin monomers bound to profilin and promote their assembly to actin filaments (F-actin). Since then, more than 100 different proteins, many of them actin regulators, have been shown to bind PIP2 with similar affinity and specificity. Experimentally manipulating PIP2 levels in cells shows that increasing its production leads to a large increase in cellular F-actin and decreasing PIP2 levels or sequestering it by overexpression of PIP2 scavengers leads to decreased actin assembly and detachment of the membrane from the interior cytoskeleton. How PIP2 achieves regulation of actin assembly is still not well understood but depends in part on the spatial distribution of PIP2 in either liquid disordered membrane domains or in Ca2+-mediated nanoscale clusters. The physical chemistry of PIP2 and its unique interaction with Ca2+ compared to other divalent cations is an essential element in its ability to control so many cellular functions. The structure of diverse PIP2-regulated actin binding proteins also suggests how integration of these protein functions can drive actin assembly or disassembly.

Keywords: phosphoinositide, cytoskeleton, actin
Supported by: NIH

SP-07.04 - The interaction of Dengue and Zika capsids with oligonucleotides and membranes generate liquid-liquid phase separations.

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Flaviviridae viral capsids recruit the genomic information of virus to infective viral particles the are generated from the endoplasmic reticulum (ER). The mechanism and regulation of such processes are still not known but we hypothesize that membrane physical properties and interactions with oligonucleotides should be a key player. From this perspective our objective is to understand how the interaction of Zika and Dengue capsids is, both from the Flaviviridae viral family, with biomimetic membrane systems and in the absence/presence of RNA/DNA oligonucleotides. Our results are obtained from experiments using confocal fluorescence spectral microscopy, fluorescence anisotropy and lifetime analysis and FCS of labelled proteins and DNA/RNA molecules when interacting with giant unilamellar vesicles and large liposomes. Here we show how the capsid proteins of Dengue and Zika virus not only are able to bind ER mimicking model membranes but also to dock liposomes and at the same time interact with oligonucleotides. In addition, these interactions trigger reversible liquid-liquid phase separations what could mean an important physical state of the inherent soft matter for the viral nucleation at the membrane of the ER. Dengue and Zika capsid proteins are able to undergo a liquid-liquid phase separation when interact with negatively charged membranes and/or oligonucleotides. This finding may be a key step not only the first recruitment of the viral genomic information but also for the correct in-cell localization of the RNA

Keywords: Dengue Zika capsids, protein membrane interaction, protein oligonucleotide interaction
Supported by: Secyt-UNC, FONCYT-Argentina

SP-07.05 - The SARS-CoV-2 nucleocapsid protein N-terminal domain phase separation is triggered by the serine-rich region and modulated by TRS binding

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The SARS-CoV-2 nucleocapsid protein (N) is a multifunctional promiscuous nucleic acid-binding protein, which plays a major role in nucleocapsid assembly and discontinuous RNA transcription, facilitating the template switch of transcriptional regulatory sequences (TRSs). We investigated the ability of the N protein N-terminal domain (N-NTD), either with or without the C-terminal serine-rich (SR) region, to undergo liquid-liquid phase separation (LLPS). Recombinant N-NTD and N-NTD-SR from SARS-CoV-2 were obtained by expression in E. coli. Images were acquired by DIC microscopy. N-NTD-SR but not N-NTD formed spherical, micron-szied droplets upon nucleic acid binding, suggesting that the SR-rich region is necessary for nucleic acid-driven LLPS under macromolecular crowding. In the presence of a long, non-specific RNA ligand, the amount of liquid droplets increased progressively with protein concentration. TRS duplex triggered significant N-NTD-SR LLPS at equimolar concentration; however, at DNA excess, the condensates dissolved. In contrast, in the presence of single-stranded TRSs (ssTRS(+), ssTRS(-)), N-NTD-SR condensates were smaller and more homogenous in size. dsTRS was demixed together with N-NTD-SR as evidenced by DAPI staining. Addition of 10% 1,6-hexanediol decreased the number of droplets by about 30%, while 300 mM NaCl completely disassembled N-NTD-SR condensates, suggesting that electrostatic contacts play a major role in LLPS. Interestingly, acidic pH (5.5) led to more numerous, larger droplets with circular morphology, suggesting that lower pH induces LLPS. To investigate the role of sequence specificity, we followed condesation by a non-specific (NS) DNA sequence. Similar to dsTRS, dsNS induced N-NTD-SR LLPS at 1:1 stoichimetry, and DNA excess dissolved the droplets. However, condensates were not spherical and wetted the coverslip surface. In addition, ssNSs were not capable of inducing LLPS at the concentrations tested, highlighting binding specificity. These results provide a mechanism by which SARS-Cov-2 N regulates viral transcription and replication.

Keywords: SARS-CoV-2, Nucleocapsid, Phase Separation

SP-08 - Systems biology and biomarkers for human disorders

SP-08.01 - Systems Biology of Mammalian and Human Sleep/Wake Cycles ~Phosphorylation Hypothesis of Sleep~

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The detailed molecular and cellular mechanisms underlying NREM sleep (slow-wave sleep) and REM sleep (paradoxical sleep) in mammals are still elusive. To address these challenges, we first constructed a mathematical model, Averaged Neuron Model (AN Model), which recapitulates the electrophysiological characteristics of the slow-wave sleep. Comprehensive bifurcation analysis predicted that a Ca^{2+} -dependent hyperpolarization pathway may play a role in slow-wave sleep. To experimentally validate this prediction, we generate and analyze 26 KO mice, and found that impaired Ca^{2+} -dependent K^+ channels (*Kcnn2* and *Kcnn3*), voltage-gated Ca^{2+} channels (Cacna1g and Cacna1h), or Ca^{2+} /calmodulin-dependent kinases (*Camk2a* and *Camk2b*) decrease sleep duration, while impaired plasma membrane Ca^{2+} ATPase (*Atp2b3*) increases sleep duration. Genetical (Nr3a) and pharmacological intervention (PCP, MK-801 for *Nr1/Nr2b*) and whole-brain imaging validated that impaired NMDA receptors reduce sleep duration and directly increase the excitability of cells. Based on these results, we propose phoshporylation hypothesis of sleep that phosphorylation-dependent regulation of Ca^{2+} -dependent hyperpolarization pathway underlies the regulation of sleep duration in mammals. We also recently developed a simplified mathematical model, Simplified Averaged Neuron Model (SAN Model), which uncover the important role of K^+ leak channels in NREM sleep. In this talk, I will also describe how we identify essential genes (*Chrm1* and *Chrm3*) in REM sleep regulation, as well as present how we enable accurate and comprehensive measurement of human sleep in society. References: 1. Tatsuki et al. Neuron, 90(1) : 70–85 (2016). 2. Sunagawa et al, Cell Reports, 14(3):662-77 (2016). 3. Susaki et al. Cell, 157(3): 726–39, (2014). 4. Tainaka et al. Cell, 159(6):911-24(2014). 5. Susaki et al. Nature Protocols, 10(11):1709-27(2015). 6. Susaki and Ueda. Cell Chemical Biology, 23(1):137-57 (2016). 7. Tainaka et al. Ann. Rev. of Cell and Devel. Biol. 32: 713-741 (2016). 8. Ode et al. Mol. Cell, 65, 176–190 (2017). 9. Tatsuki et al, Neurosci. Res. 118, 48-55 (2017). 10.Ode et al, Curr. Opin. Neurobiol. 44, 212-221 (2017). 11. Susaki et al, NPJ. Syst. Biol. Appl. 3, 15 (2017). 12. Shinohara et al, Mol. Cell 67, 783-798 (2017). 13. Ukai et al, Nat. Protoc. 12, 2513-2530 (2017).14. Shi and Ueda.BioEssays 40, 1700105 (2018). 15. Yoshida et al, PNAS 115, E9459-E9468 (2018). 16. Niwa et al, Cell report, 24, 2231-2247. e7 (2018)
Keywords: Sleep, phosphorylation, Calcium

SP-08.02 - The effects of COVID-19 in the human brain

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One increasingly documented tendency of COVID-19 patients is to exhibit neuropsychiatric and neurological symptoms. Here we found that anxiety and cognitive impairment are manifested by 28-56% of COVID-19 convalescent individuals with mild respiratory symptoms and are associated with altered cerebral cortical thickness. Using an independent cohort, we found histopathological signs of brain damage in 25% of individuals who died of COVID-19. All of the affected brain tissue studied exhibited foci of SARS-CoV-2 infection and replication, particularly astrocytes. We also found that neural stem cell-derived human astrocytes in vitro are susceptible to SARS-CoV-2 infection through a mechanism that involves spike-NRP1 interaction. SARS-CoV-2-infected astrocytes manifested changes in energy metabolism and in key proteins and metabolites used to fuel neurons, as well as in the biogenesis of neurotransmitters. Moreover, infection elicits a secretory phenotype that reduces neuronal viability. Our data support the model in which SARS-CoV-2 reaches the brain, infects astrocytes and consequently leads to neuronal death or dysfunction. These deregulated processes are also likely to contribute to the structural and functional alterations seen in the brains of COVID-19 patients.

Keywords: COVID-19, SARS-CoV-2, Proteomics
Supported by: Fapesp, CAPES, CNPq

SP-08.03 - Development and utilization of a highly specific and sensitive multiplex serological COVID-19 assay

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The COVID-19 pandemic poses an immense need for accurate, sensitive and high-throughput clinical tests, and serological assays are needed for both overarching epidemiological studies and evaluating vaccines. Here, we present the development and validation of highly specific and sensitive high-throughput multiplex bead-based serological assay. More than 100 representations of SARS-CoV-2 proteins were included for initial evaluation, including antigens produced in bacterial and mammalian hosts as well as synthetic peptides. The five best-performing antigens, three representing the spike glycoprotein and two representing the nucleocapsid protein, were further evaluated for detection of IgG antibodies in samples from 331 COVID-19 patients and convalescents, and in 2090 negative controls sampled before 2020. Three antigens were finally selected, represented by a soluble trimeric form and the S1-domain of the spike glycoprotein as well as by the C-terminal domain of the nucleocapsid. The sensitivity for these three antigens individually was found to be 99.7%, 99.1% and 99.7%, and the specificity was found to be 98.1%, 98.7% and 95.7%. The best assay performance was although achieved when utilizing two antigens in combination, enabling a sensitivity of up to 99.7% combined with a specificity of 100%. Requiring any two of the three antigens resulted in a sensitivity of 99.7% and a specificity of 99.4%. These observations demonstrate that a serological test based on a combination of several SARS-CoV-2 antigens enables a highly specific and sensitive multiplex serological COVID-19 assay.

Keywords: COVID-19, serology, proteomics

SP-08.04. - Urease of Helicobacter pylori: role in neuroinflammation

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Alzheimer's disease (AD) is considered the most common neurodegenerative disorder in people above 60 years old, a tauopathy characterized by dementia and memory loss. AD's characteristic histopathological alterations are the presence of plaques consisting of deposits of beta-amyloid peptide, and neurofibrillary tangles, formed by the deposition of hyperphosphorylated Tau protein. Tau's function is associated with microtubules in the neuronal axons, and it is regulated by phosphorylation/dephosphorylation mechanisms. *Helicobacter pylori* is a gram-negative pathogen responsible for chronic gastritis, peptic ulcer, and gastric cancer, infecting ca. 60% of the world's population. These bacteria produce large amounts of urease (HPU), an important virulence factor. Our group has shown that HPU displays ureolysis-independent pro-inflammatory properties eliciting cytokines production, platelet and neutrophil activation, promoting tissue damage. It has been reported that a filtrate of *H. pylori* induces hyperphosphorylation of Tau protein in different sites. It was suggested that exotoxins produced by the bacteria could break the blood-brain barrier (BBB) and directly induce tau's phosphorylation. This work aimed to investigate possible alterations promoted by HPU in neuroinflammation and tau phosphorylation. 30 days old Wistar male rats received i.p. 5 µg purified HPU daily for 7 days. After euthanasia, their brains were stored at -80 ºC for further analysis. In the control group, sterile saline solution was given. Western blotting assays were performed using anti-totalTau, anti-pTauThr205 and anti-pTauSer199 antibodies. The release of IL-1β and TNFα by HPU-activated BV-2 murine microglial cells was evaluated by ELISA. HPU given i.p. to young rats increased the phosphorylation of tau on the Thr205 and Ser199 sites as compared to controls. HPU also induced the production of pro-inflammatory cytokines by microglial cells. Our findings reassure previous data suggesting an association between infection by H. pylori and tauopathies such as AD, mediated by the bacterial urease.

Keywords: Helicobacter pylori, Urease, Alzheimer's Disease. **Supported by:** Fapergs, CNPq, CAPES

SP-08.05 - Invasive behaviour of breast cancer cells as a response to hypoxic signalling via extracellular vesicles

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Metastasis, the most frequent cause of mortality in breast cancer, is a multifactorial process in which tumour cells detach from the primary site, go through an epithelial-mesenchymal transition, and invade the adjacent tissue. Metastasis can be triggered by oxidative stress as a direct result of the increased tumour mass. Also, this low-oxygen environment signals for an increased communication between tumour and stromal cells, which is performed majorly via extracellular vesicles. In this work, we aimed to identify the cellular and biochemical alterations in triple-negative breast cancer by hypoxic small extracellular vesicles (SEV) at an invasive setting. For SEV isolation, we have used the differential ultracentrifugation method, followed by characterization via transmission electron microscopy, nanoparticle tracking analysis, protein quantification, western blotting, and large-scale proteome analysis. We have used in vitro and in silico approaches, such as invasion and morphology assays, label-free proteomics, flow cytometry and western blotting to investigate cellular responses. Our SEV samples are enriched with proteins ALIX, CD63 and flotillin-1 but lack Cytochrome C, which confirms its origin and absence of contamination with other organelles. Our initial results show an increase in the invasive behaviour of breast cancer cells treated with hypoxic SEV, plated on both matrigel and gelatine coating. Cell morphology is altered to a mesenchymal phenotype, losing circularity 24 h after SEV treatment, with differentiated expression of integrins. We were able to identify an increase in ECM-degrading enzymes (MMP-2 and MMP-9) in cell lysates under SEV treatment. Overall, our results indicate an important role of hypoxia in triggering metastasis, by facilitating epithelial-mesenchymal transition, ECM degradation and intracellular pathways leading to invasion. **Keywords:** extracellular vesicles, invasion, hypoxia
Supported by: FAPESP

SP-09. PABMB Symposium: Metabolism and Bioenergetics

SP-09.01 - Mitochondrial fusion proteins and their role in metabolic disorders.

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Mitochondrial fusion and fission are key processes that regulate mitochondrial morphology. Mitochondrial fusion is catalyzed by MFN1, MFN2 (Mitofusins) and OPA1 proteins in human cells. However, some of these proteins show a complex biology. In this connection, OPA1 is a key protein involved in cristae formation. MFN2 protein not only regulates mitochondrial morphology but it also controls the morphology and function of the endoplasmic reticulum, and also the mitochondrial import of phosphatidylserine. Expression of MFN2 is exquisitely regulated in tissues. Thus, it is induced in skeletal muscle in response to chronic exercise and after exposure to cold. In contrast, MFN2 is repressed in different tissues of mice fed a high fat diet or during aging. MFN2 is repressed in muscle from type 2 diabetic patients, and in liver biopsies from NASH subjects. In turn, changes in MFN2 expression have a marked impact on mitochondrial metabolism. The use of MFN2 mutant mice has revealed a wealth of information on the metabolic role of this protein in mouse tissues. Some of the mechanisms of MFN2 function will be also discussed.

Keywords: mitochondrial homeostasis, type 2 diabetes, NASH

SP-09.02 - A role for mitofusins in oocyte development: impact on fertility and offspring viability

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The mitochondrion is inextricably linked to the oocyte health, corroborating the key role of this organelle in energy fueling, metabolite supplying, calcium buffering, and regulation of apoptosis. Although oocytes contain the largest mitochondrial content across mammalian cells, characteristics such as a highly-fragmented network and poorly-developed cristae suggest mitochondria are quiescent in oocytes. Mitochondrial architecture and, in turn, function, are under the control of opposing processes of fusion and fission, determining mitochondrial dynamics. Mitofusins 1 (MFN1) and 2 (MFN2) are key factors regulating outer mitochondrial membrane fusion, with their expression levels and functions being regulated in a tissue-specific manner. MFN2 also locates at the endoplasmic reticulum (ER) membrane, where from it interacts with mitofusins in mitochondria to promote mitochondria-ER juxtaposition. The aim of this study was to investigate the role of mitofusins in oocytes as well as their impact on fertility and offspring viability. Towards that, we used a mouse model with targeted deletion of *Mfn1* and/or *Mfn2* in oocytes. In wild-type oocytes, *Mfn1* expression is 5-fold higher than that of *Mfn2*, being *Mfn1* essential to female fertility; *Mfn1* deficiency leads to arrested folliculogenesis and failed ovulation, phenotypes secondary to impaired PI3K-AKT signaling and disrupted intercellular communication. Although *Mfn2* deficiency has little impact on oocyte development and fertility, *Mfn2*-null oocytes show a profound transcriptomic change besides evidence of mitochondrial and ER dysfunction. In addition, mice born to females with *Mfn2*-deficient oocytes present glucose intolerance, decreased insulinemia and defective insulin signaling. Interestingly, the double loss of *Mfn1* and *Mfn2* alleviates the impact on oogenesis, partially rescuing mitochondrial function, PI3K-AKT signaling, and intercellular communication as compared to the single loss of *Mfn1*. These findings suggest that MFN1 and MFN2 have distinct, non-redundant roles, in oocytes, with MFN1 acting downstream of MFN2 to counter its activity.

Keywords: mitofusin, mitochondria, oocyte
Supported by: FAPESP, CNPq and CAPES

SP-09.03 - Systems Biology Approach of the Down Syndrome Critical Region 1 gene, RCAN1: implications in mitochondrial biology, cellular proliferation, and differentiation

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Down Syndrome (DS) is the product of an extra copy of chromosome 21 and is related to different neuronal and cardiac pathologies. DS patients present increased oxidative stress and, therefore, increased DNA damage; in addition to altered cell differentiation that would lead to failures in organogenesis. In humans, RCAN1, located in the critical DS region of chromosome 21, is responsible of the enlarged and over functional mitochondria observed in DS iPSC, however, the relation between these alterations and the dysfunctional cardiac organogenesis observed in DS patients is still unknown. To analyze *in silico* and *in vitro* the effect of RCAN1 on mitochondrial dynamics, proliferation, and DNA damage of DS iPSCs; and to evaluate the role of this protein in the differentiation process of iPSC-derived cardiomyocytes (iPSC-CM). Using a system biology approach, we constructed a transcriptional regulatory network for RCAN1 that shows the over-representation of processes related with organelle dynamics, cellular proliferation, and organ differentiation, all of them connected by genes related with the response to DNA damage. Moreover, *in vitro* microscopy and Western blot analysis showed that DS iPSCs present lower rates of mitochondrial fission, as well as decreased levels of PINK1. RCAN1 overexpression in DS iPSC induced an enhanced proliferation and cumulative DNA damage observed by immunofluorescence and qRT-PCR, which were dependent on the expression levels of RCAN1. Finally, DS iPSC-CM also expressed RCAN1-dependant lower levels of cardiac differentiation markers than control cells after 15 days of culture. RCAN1 overexpression regulates the increased mitochondrial fusion, proliferation and DNA damage observed in 3S iPSC; together with a decrease in the 3S iPSC differentiation ability towards a cardiomyocyte lineage. **Keywords:** Down syndrome, RCAN1, iPSC. **Funding:** This project was funded by FONDECYT 1190743 and FONDAP 15130011. U-Redes G_2018-35 and CRP-ICGEB CHL18-04.

SP-09.04. - Mechanism of rotenone inhibition of respiratory complex I

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Respiratory complex I in the inner mitochondrial membrane plays an essential role in cell metabolism. It catalyses oxidation of NADH in concert to reduction of ubiquinone (Q). This electron transfer process is coupled with translocation of protons across the membrane, creating an electrochemical gradient for ATP synthesis. Rotenone is a natural compound that strongly inhibits complex I activity and cryo-EM structures indicate it binds in three different sites inside complex I. Two of them are located in the 30 Å long Q-chamber: the first binding site (BS) is near the chamber exit and the second is the Q reactive site (RS), near the iron-sulfur cluster N2. A distant third site (PS) was found in the membrane domain. Evaluate the relative binding affinity of each site and the role of ligand internal conformation (either in straight or in bent geometry) for binding of rotenone and three derivatives with variable conformational restrictions. We applied molecular dynamics simulations and the free energy methods umbrella sampling, metadynamics and linear integration energy. We find that rotenone has similar affinities to either RS and BS sites. All derivatives have low affinity, between +4 and -3 kJ/mol, to the third PS. This result indicates that the PS may be an experimental artifact due to the high rotenone concentrations used in the cryoEM preparation. Two conformationally restricted derivatives show low affinity to the RS, suggesting that the bent rotenone conformation is stabilized in the RS, favoring complex I inhibition. Considering these, rotenone probably inhibits complex I by binding in the two sites (RS and BS) located in the Q-chamber and RS binding requires an internal flexibility to a bent geometry. We are now analysing how this internal flexibility affects rotenone transit inside the chamber. **Keywords:** Molecular dynamics, Electron transport chain, Free energy methods. **Supported by:** FAPESP

SP-10. Biophotonics

SP-10.01 - Light-based non-thermal therapy: from basis to clinical applications

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Light-based non-thermal therapies are evolving as promising non-invasive and cost-effective medical technologies. These therapeutic platforms mainly encompass photobiomodulation (PBM) and photodynamic therapy (PDT), which use visible or near infrared (NIR) light to induce biological responses without any significant heating effects. For PBM, it is most commonly used red or NIR light to optimize light penetration into biological tissues. The photon absorption by natural chromophores at these spectral regions cause photophysical and photochemical reactions inside cells that trigger several biological effects such as to accelerate wound healing, reduce inflammation and relief pain, depending on light parameters and target tissue. On the other hand, PDT makes use of photoactivated drugs, also called as photosensitizers, which absorb light to induce chemical reactions that kill microbial or cancer cells by oxidative stress. Our group have been investigating the mechanisms and several applications of PBM and antimicrobial PDT (APDT) for almost 20 years. In this lecture I will share our experience in the area to discuss how PBM and APDT could be used to revolutionize health care in the photonics era. An integrated perspective from the basic mechanisms, preclinical and clinical trials for both therapies will be presented, including PBM on cancer management and APDT against drug-resistant pathogens. The lecture will also highlight future perspectives. **Keywords:** antimicrobial photodynamic therapy, photobiomodulation therapy, preclinical and clinical assays. **Supported by:** FAPESP, CNEN, CNPq

SP-10.02 - The water-isotopologue deuterium oxide (D2O; 'heavy' water): From biophysical properties to experimental cancer therapeutic

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Since its initial discovery as a natural heavy isotope variant of dihydrogen oxide (^1H_2O), extensive research has focused on the biophysical, biochemical, and pharmacological effects of deuterated water [^{2} H_2O (D_2O, also referred to as 'heavy water')]. Here, we provide a D_2O-centered perspective on biophysical properties and potential therapeutic use targeting cancer cells. Due to its unique physicochemical properties, D_2O has become a valuable biochemical probe examining various physiological parameters using MRI and isotope ratio-mass spectrometry. Biological effects of D_2O are generally attributed to altered isotopic and solvent properties, associated with an increased strength of deuterium-based hydrogen bonds. Indeed, deuteron- (versus proton-) dependent biological impact is largely attributed to alteration of biophysical properties including (i) conformational stability of proteins, (ii) fidelity of nucleic acid base pairing, and (iii) other proton-sensitive effectors including mitochondrial energy metabolism and solute channels (e.g. aquaporin and calcium). Importantly, shortly after its initial discovery by Urey in 1932, cancer-directed effects of D_2O (administered systemically) have been examined in vivo, and inhibitory effects on murine tumor growth were described as early as 1938, documenting growth inhibition of implanted carcinomas using D_2O drinking water supplementation. Cumulative evidence now confirms tumor-directed activity of D_2O supplementation in murine cancer models including pancreatic, colorectal, squamous cell carcinoma, and malignant melanoma. Using a panel of cultured melanoma and pancreatic ductal adenocarcinoma cells we have recently profiled apoptogenicity, stress response gene array expression (redox-, metabolism, and proteotoxicity-related), and phosphoprotein-signaling substantiating the chemotherapeutic efficacy of systemic D_2O administration targeting human malignancy in relevant murine models. **Keywords:** deuterium oxide, water-isotopologue, cancer therapeutic. **Supported by:** NIH (National Cancer Institute)

SP-10.03 - Wavelength, dose skin type and skin model related radical formation in skin

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The exposure to sun radiation is indispensable to our health, however, a long term and high exposure could lead to cell damage, erythema, premature skin aging and promotion of skin tumors. An underlying pathomechanism is the formation of free radicals which may induce oxidative stress at elevated concentrations. Different skin models, such as porcine-, murine-, human- ex vivo skin, reconstructed human skin (RHS) and human skin in vivo, were investigated during and after irradiation using X- and L-band EPR spectroscopy within different spectral regions (UVC to NIR) [1,2]. The amount of radical formation was quantified with the spin probe PCA and the radical types were measured ex vivo with the spin trap DMPO. The radiation dose influences the types of radicals formed in the skin. While reactive oxygen species (ROS) are always pronounced at low doses, there is an increase in lipid oxygen species (LOS) at high doses. Furthermore, the radical types arise independent from the irradiation wavelength, whereas the general amount of radical formation differs with the irradiation wavelength. Heat pre-stressed porcine skin already starts with higher LOS values. Thus, the radical type ratio might be an indicator of stress and the reversal of ROS/LOS constitutes the point where positive stress turns into negative stress [3]. Compared to light skin types, darker types produce less radicals in the ultraviolet, similar amounts in the visible and higher ones in the infrared spectral region, rendering skin type-specific sun protection a necessity [4]. References [1] Albrecht, S., Meinke M. C. et al. (2019). "Quantification and characterization of radical production in human, animal and 3D skin models during sun irradiation measured by EPR spectroscopy." Free Radic Biol Med 131: 299-308. [2] Zwicker, P., Meinke M. C. et al. (2021). "Application of 233 nm far-UVC LEDs for eradication of MRSA and MSSA and risk assessment on skin models." Scientific reports submitted. [3] Lohan, S. B., Meinke M. C. et al. (2021). "Switching from healthy to unhealthy oxidative stress - does the radical type can be used as an indicator?" Free Radic Biol Med 162: 401-411. [4] Albrecht S, Meinke M. C. et al. (2019) Skin type differences in solar simulated radiation-induced oxidative stress. Br J Dermatol. 180(3):597-603.

Keywords: Electron paramagnetic resonance (EPR), spectroscopy, reactive oxygen species, lipid oxygen species

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SP-10.04 - Low power light triggers opposite effects on stem cells: influence of the wavelength and culture conditions

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Photobiomodulation (PBM) has been gaining importance in a wide range of medical fields in the past few years, particularly in stem cell-based regenerative medicine. Improving in vitro cell proliferation, differentiation and viability are ways where PBM could play a pivotal role optimizing biotechnological and bioengineering applications. Here we investigated whether different wavelengths (blue, green and red) would promote distinct outcomes in human adipose-derived stem cells (hADSCs) cultured in regular and supplemented media for tenocyte differentiation. MATERIALS AND METHODS. Freshly isolated hADSCs were cultured in a specific stem cell medium (MSCGM, Lonza), DMEM or a tenogenic medium (TEN-M: DMEM supplemented with growth factors and ascorbic acid). Cells were irradiated every 48 h (23.28 mW/cm ^{2}, 17 min 10 s delivering 24 J/cm ^{2} per session) using a LED irradiator (LEDbox, BioLambda). MTT and crystal violet assays were used to evaluate cell metabolic activity and proliferation. Red wavelength (660 nm) significantly increased metabolic activity after five irradiations, but only for cells cultured in TEN-M. Oppositely, blue (450 nm) and green (520 nm) light decreased both cell proliferation and metabolic rate, with more pronounced effects for blue light in TEN-M. Considering these findings, we examined whether irradiating only the media would generate toxic compounds that could impair cell viability. We therefore assessed reactive oxygen species (ROS) production by p-nitrosodimethylaniline/histidine assay while irradiating the three different media under the same conditions as mentioned above. Immediately after blue and green light exposure, an increment in ROS production was observed for DMEM and TEN-M, that continuously increased until reaching between 4.5 and 7.1 μM one-hour after irradiation – with higher values for TEN-M exposed to blue light. Since no significant ROS formation was observed following red light exposure, we concluded that medium composition was responsible for the different effects on metabolic activity and proliferation observed after irradiation with different wavelengths.

Keywords: oxidative stress, culture media, photosensitivity. **Supported by:** CNPq

SP-10.05 - Breast tissue diagnosis using artificial intelligence applied to FTIR spectroscopy images

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The estimative of new breast cancer cases was of 2.1 million of new breast cancer cases in 2018, hence being the most incident type of cancer in women. The improvement of its diagnosis has been the aim of many researchers, including vibrational spectroscopy teams. With the advancement of the artificial intelligence, a field of computer science to enhance intelligence into computer systems, specially of the deep learning, big data acquired from spectroscopy image has entered a new era. Therefore, the proposal of this work was to diagnose breast tissue samples as malignant (cancer) or benign (adenosis) using deep learning techniques. Micro-FTIR spectroscopy images were acquired from BR804b human breast tissue microarray (Biomax, USA), resulting in more than 100 thousand spectra for each group. A k-means approach was established to separate spectra into three clusters: tissue, paraffin and slide. A preprocessing step was applied by the following pipeline: outlier removal; biofingerprint truncation; Savitzky–Golay filter to smooth and to obtain the second derivative; extended multiplicative signal correction to correct spectra and remove the paraffin contribution. The deep learning algorithm was built using two-layers of one-dimensional convolutional neural network (CNN) connected to a two-layers (100 and 50 neurons) feedforward network (FFN). Both networks used dropout layers of 50% and rectified linear unit activations. CNN kernel size was set to 5. The output neuron used a sigmoid activation. Adam optimizer was applied to train the networks, using a binary cross-entropy loss to improve the weights. A 4-fold cross-validation of 20 epochs and batch size of 250 was performed. The networks exhibited an accuracy of (97.8 ± 0.4)% during the training stage, and (96.9 ± 0.8)% during the testing stage, demonstrating a generalized classification. Accuracies of almost 100% indicates this approach as a potential technique for the breast diagnosis.

Keywords: FTIR images, artificial intelligence, breast cancer.
Supported by: FAPESP, CNPq and CAPES

SP-11. Microbiomes: human and enviromental

SP-11.01 - Studies of the human microbiome in health and disease

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The Centre for Translational Microbiome Research (CTMR) started in 2016 as a collaboration between Karolinska Institutet, Science for Life Laboratory and Ferring Pharmaceuticals. Since then, a broad technical, biological, clinical and epidemiological platform for studying complex microbiological communities in well-defined human materials has been established. CTMR aims to better understand the contribution of the human microbiome to physiology and pathophysiology with the goal to open opportunities for development of novel therapies in the area of cancer, gastroenterology and reproductive health. The talk will present details on CTMR´s efforts to define what is healthy in the human gut and vaginal microbiome based on samples obtained in hospital and population-based studies. Furthermore, approaches to develop therapies or lifestyle interventions to change a dysbiotic profile back to normal again will be presented. **Keywords:** Human microbiome, Dysbiosis, Intervention

SP-11.02 - Metagenome-assembled genomes and their contribution to microbiome studies

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Metagenome-assembled genomes (MAGs) are microbial genomes reconstructed from metagenome data. In the last few years many thousands of MAGs have been reported in the literature, for a variety of environments and host-associated microbiota, including humans. These MAGs have helped us better understand microbial populations and their interactions with the environment where they live; moreover most MAGs belong to novel species, therefore helping decrease the so-called microbial dark matter. However, not much effort has been invested in the quality of these reconstructions, which means that many of the reported MAGs may be artefacts. This talk will be a MAG survey, in which some key issues and specific examples will be presented. **Keywords:** Metagenome-assembled genomes, microbial genomes, microbiome

SP-11.03 - Microbiome studies of the built environment: from commensals, to cancer & COVID-19

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The cost reduction recently seen for large-scale sequencing allowed the implementation of new, ambitious projects that have provided information that are impacting our lives will allow a better understanding of the life in the planet. One of these projects started in 2015 with the creation of the MetaSub consortium (www.metasub.org), which aimed to provide the first detailed map of the microorganisms that inhabit the built environment around the globe. The recent publication of the first article from this consortium - based on about 5,000 samples collected over a three-year period across 60 cities in 32 countries and six continents – allowed a detailed map of the distribution of microorganisms, including hundreds of new bacteria and viruses, as well as the mapping of antimicrobial resistance genes and microorganisms relevant for human health. This includes microorganisms of interest, such as Helicobacter pylori, a carcinogen type-1 according to the World Health Organization, related to gastric cancer. The metadata collected in these cities, including temperature, humidity, surface type and others may be used to better design public transportation systems and hospitals, helping to control the survival and spread of contagious agents. The protocols validated in the project have been used during the current COVID-19 pandemics, revealing the distribution of SARS-CoV-2 in different cities and providing the basis for a global genomic surveillance.

Keywords: microbiota, sars-cov-2, Helicobacter pylori
Supported by: FAPESP (2021/05316-2), Fundação Araucária (17.921.164-5), Metasub consortium

SP-11.04 - Microbial Potlatch: The advantage of leakage of essential metabolites and resultant symbiosis of diverse species

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How can diverse species or strains coexist in microbial communities? Besides the fittest strain under isolation conditions, a variety of strains or species coexist even when limited by a single resource. It has been argued that metabolite secretion creates new niches and facilitates such diversity. Nonetheless, it is still a controversial topic why cells secrete even essential metabolites so often; in fact, even under isolation conditions, microbial cells secrete various metabolites, including those essential for their growth. To reveal a possible origin of metabolite secretion and microbial symbiosis, we analytically and numerically investigated mathematical models that incorporate multilevel dynamics at the intercellular (population) and intracellular (metabolic) levels. First, we demonstrate that leaking essential metabolites can be advantageous. If the intracellular chemical reactions include multibody reactions like catalytic reactions, this advantageous leakage of essential metabolites is possible and indeed typical for most metabolic networks via 'flux control' and 'growth-dilution' mechanisms; the later is a result of the balance between synthesis and growth-induced dilution with autocatalytic reactions. Counterintuitively, the mechanisms can work even when the supplied resource is scarce. Next, when such cells are crowded, the presence of another cell species, which consumes the leaked chemicals is beneficial for both cell species, so that their coexistence enhances the growth of both. The latter part of the paper is devoted to the analysis of such an unusual form of symbiosis: 'consumer' cell species benefit from the uptake of metabolites secreted by 'leaker' cell species, and such consumption reduces the concentration of metabolites accumulated in the environment; this environmental change enables further secretion from the leaker cell species. This situation leads to resilient coexistence of diverse cell species, as supported by extensive simulations. A new look at the diversity in a microbial ecosystem is thus presented.
Keywords: ecology, metabolite, secretion

SP-11.05 - **Molecular mechanisms underlying the role of the centriolar CEP164-TTBK2 complex in human ciliopathies**

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Cilia formation is essential for human life. One of the earliest events in the ciliogenesis program is the recruitment of tau-tubulin kinase 2 (TTBK2) by the centriole distal appendage component CEP164. Due to the lack of high-resolution structural information on this complex, it is unclear how it is affected in human ciliopathies such as nephronophthisis. Furthermore, it is poorly understood if binding to CEP164 influences TTBK2 activities. OBJECTIVES In this study, we aimed at investigating the structure and function of the human CEP164-TTBK2 complex in health and disease. MATERIALS AND METHODS: We present a detailed structural analysis by X-ray crystallography and NMR of the CEP164-TTBK2 complex. We further dissect the importance of individual residues in the TTBK2 binding domain for CEP164 by mutating individual residues. DISCUSSION AND RESULTS: We show that the CEP164 N-terminal region (amino acid residues 1-104) contains a canonical WW-domain inserted into an α-helical bundle. The N-terminal region of CEP164 preceding the WW-domain is partly unstructured and highly flexible. We demonstrate that the CEP164 WW-domain binds to the TTBK2 C-terminal proline-rich region (amino acid residues 1074-1085) and that this interaction is significantly reduced for the CEP164 Q11P ciliopathic mutant. We further demonstrate that CEP164 R93W ciliopathic mutation located at the α-helical bundle destabilizes the CEP164 N-terminal domain negatively affecting its interaction with TTBK2. We also show that both CEP164 Q11P and R93W mutants fail to rescue ciliogenesis in RPE-1 cells. Moreover, we provide novel insights into how binding to CEP164 is coordinated with TTBK2 activities. We demonstrate that CEP164 binding inhibits EB1 engagement by TTBK2 but does not stimulate TTBK2 autophosphorylation. CONCLUSION: Together, our data deepen our understanding of a crucial step in cilia formation and will inform future studies aimed at restoring CEP164 functionality in a debilitating human ciliopathy.

Keywords: Centriole, Cilia, Ciliopathy

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SP-12 - Molecular and Cell Imaging

SP-12.01 - Far-field fluorescence nanoscopy with sub-10 nm resolution

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Far-field fluorescence nanoscopy is a family of methods that has revolutionized biological imaging by providing sub-diffraction spatial resolution while keeping the low invasiveness of visible light interrogation. Making use of on-off switching of molecular emission, these methods break any fundamental limitation to the achievable spatial resolution. In practice, however, the resolution is limited by the total number of excitation-emission or on-off cycles that a molecule can perform or withstand. Under biological conditions, the lateral resolution is typically limited to about 20 – 50 nm. Axial resolution is typically worse, in the range of 60 – 120 nm. Resolving supramolecular protein structures, as well as the spatial organization of protein-protein interactions requires another push to the resolution to get into sub-10 nm regime, which is the typical size of structural proteins and complexes. Here, three recent methodological advances from our lab will be presented that enable biological imaging with sub-10 nm resolution. First, a new and simpler implementation of MINFLUX1 will be described which provides sub-10 nm lateral resolution and gives access to fluorescence excited state lifetime. Second, a successful combination of STED-FRET will be shown, which is able to super-resolve biomolecular direct interactions. Finally, a TIRF nanoscopy method will be presented which can be implemented on any wide-field single-molecule fluorescence microscope and is able to deliver sub-10 nm axial resolution (2). (1) Balzarotti, F. et al. Science 355 (2017) 606–612. (2) Szalai, A. M. bioRxiv 693994 (2019). doi:10.1101/693994
Keywords: Super-resolution, MINFLUX, FRET

SP-12.02 - Single cell physiological characterization in living tissue. Determination of cell fate

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Optical super-resolution has been around for more than 10 years. Yet, superresolution is mainly applied to produce stunning images at the 10-20 nanometer scale of the interior of cell. This kind of superresolution imaging had limited applications to reveal the dynamics, motion and interactions of molecules at the nanoscale, which is at the basis of life. In This talk we show work done in our lab to filling this gap by developing enabling technologies that will open the potential of superresolution imaging to dynamic at the microsecond-millisecond- temporal scale.
Keywords: Single cell , tissue, Optical super-resolution

SP-12.03 - Alpha-catenin forms a cooperative and asymmetric catch bond with F-actin to regulate cell junction fluidity

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Cell adhesions dynamically tune their mechanical properties during tissue development and homeostasis. Fluid connections required for cell mobility can switch to solid links to maintain the mechanical rigidity of epithelial layers. Changes in the composition and clustering of adhesion molecules have been proposed to modulate cell junction fluidity, but the underlying mechanisms are unclear. α-catenin has been shown to play a fundamental role in different adhesion sites. At adherens cell-cell junctions (AJ), α-catenin localizes in cadherin-catenin complexes, where it provides a mechanical link between α-catenin and the actin cytoskeleton. However, its function is controversial owing to the low affinity between actin and the α-β-catenin heterodimer. Outside AJ, α-catenin binds itself to form homodimers that connect the cell membrane to the actin cytoskeleton to promote adhesion and migration, but its mechanosensitive properties are inherently unknown. Here, using ultra-fast laser tweezers (Capitanio et al., Nature Methods, 2012) we show that a single mammalian α-catenin molecule displays very different force-bearing properties depending on whether it is associated to α-catenin or not. We found that a single α-β-catenin heterodimer slips along an actin filament in the direction of force, while a single α-catenin homodimer forms a strong asymmetric catch-bond with actin, in which the bond lifetime increases, and the protein unfolds with force directed toward the F-actin pointed end. Importantly, assemblies of multiple α-β-catenin heterodimers show asymmetric force-bearing and unfolding properties similar to the α-catenin homodimer. Our results indicate that, outside AJ, single α-catenin homodimers act as a mechanical link with the actin cytoskeleton that resists force efficiently. Nonetheless, inside AJ, α-catenin's capability to hold cell-cell connections under physiological loads critically depends on the recruitment of multiple (5-10) complexes. Our data support a molecular model in which α-catenin clustering and intercellular tension engage a fluid-to-solid phase transition at the membrane-cytoskeleton interface.

Keywords: optical tweezers, alpha-catenin, adherens junctions, single molecule biophysics
Supported by: MIUR, Horizon 2020, University of Florence, Ente Cassa di Risparmio di Firenze

SP-12-04 - Advanced fluorescence microscopy techniques to study the interaction of amphiphilic peptides with model membranes

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The interest on the detailed analysis on peptide-membrane interaction is multifold in applied sciences as it underlies both functional and pathogenic phenomena. Peculiar properties of membranes and peptide structural details, together with environmental conditions, may select different events at the membrane interface, which drive the fate of the peptide-membrane system. Due to the complexity of these highly dynamic and spatially heterogeneous processes, a mechanistic description of these phenomena is still far from being achieved. Here we use an experimental approach based on the combination of spectroscopy and fluorescence microscopy methods to characterize the interactions of the multifunctional amphiphilic peptide Transportan 10 (TP10) with model membranes. Our approach, based on the use of suitable fluorescence reporters, exploits the advantages of phasor plot analysis of Fluorescence Lifetime Imaging (FLIM) measurements to highlight the molecular details of membrane modifications in terms of rigidity and hydration simultaneously to the ability to distinguish whether the peptide is adsorbed or inserted in the membrane with high spatial resolution. Our results show that while TP10 does not interacts with the POPC:POPG membranes enriched with cholesterol, it interacts with cholesterol-free ones and in a concentration dependent way. TP10 is absorbed or inserted in the membrane inducing pores formation but not however affecting the membrane morphology at the micronscale. By means of the use of Laurdan and di-4-ANEPPDHQ, fluorescent dyes, that sense physico-chemical aspects of the membranes at different length scales, we analyze what happens at molecular scale at different depth of phospholipid bilayers. Results indicate how the complementary use of multiple molecular reporters and FLIM analysis, by means of phasor approach, highlight diverging aspects of such complex phenomenon as peptide-membrane interaction allowing the possibility of following dynamic events in real time without sample manipulation. **Keywords:** Phasor approach, membrane hydration, antimicrobial peptides

SP-12-05 - Study of SARS-CoV-2 morphogenesis and interaction with the cell by transmission and high resolution scanning electron microscopy

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SARS-CoV-2 is a single strand RNA virus, belonging to the betacoronavirus genus, within the Coronaviridae family. This pathogen, transmitted mainly through droplets and aerosol, is responsible for the COVID-19. As occur to many of the positive sensed RNA viruses, its cellular cycle involves a robust membranar rearrangement in the cytosol of the infected cells. This structure delimits and protects the locus of replication and morphogenesis of SARS-CoV-2. In the present study, we approached the main steps of SARS-CoV-2 morphogenesis and interaction with the cell by transmission and high resolution scanning electron microscopy (HR-SEM). The sites of viral replication, and assembly were documented in Vero cells at 24, 48 and 72 hours post-infection by using both electron microscopy modes. In addition, the interactions of this virus with the cell surface, as well as the viral factory and the details of its main components, were also investigated with the aim of HR-SEM, after the removal of infected cells plasma membrane. This allowed the visualization of unprecedented features of the interactions between this virus and the cell, such as the so-called “virus surfing”, which enables a relatively safe cell-to-cell viral propagation in the tissue. On the other hand, the electron-tomography of these samples showed, for the first time, the presence of SARS-CoV-2 particles in the space between the inner and the outer nuclear envelope. The data obtained in this work contribute to the knowledge of the route of SARS-CoV-2 within the infected cell and the cell biology of their interactions. **Keywords:** SARS-CoV-2, Electron Microscopy, Morphogenesis **Supported by:** FAPERJ and FINEP

SP-13. Ionic channels and membrane transporters

SP-13.01 - Sensing voltage and opening of ion channels

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The nerve impulse (action potential) generation depends on voltage-dependent sodium channels that must open before voltage-dependent potassium channels. We will review structure-function relation of the voltage sensors that give voltage dependence of the ion channels. The voltage sensors have intrinsic charges in the channel protein which move in the cell membrane electric field and generate gating currents. Experiments with voltage clamp and site-directed fluorescence describe molecular details of the voltage sensor operation indicating the paths followed by the charged arginine residues within the protein core. A detailed study of the residues in the core show that the nature of the side chains determine that Na channels are faster than K channels. The canonical coupling of the voltage sensor to the conduction pore is via the linker between transmembrane segments S3 and S4. We will describe that the proximity of the S4 segment of the voltage sensor and the S5 segment of the pore region makes another (noncanonical) coupling pathway. The molecular basis of this pathway will be described.
Keywords: Nerve impulse, voltage-dependent channels, gating currents
Supported by: NIH R01GM030376

SP-13.02 - Structural mechanism of heat-induced opening of a temperature-sensitive TRP channel

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Numerous physiological functions rely on distinguishing temperature by temperature-sensitive transient receptor potential channels (thermo-TRPs). While thermo-TRP function has been studied extensively, structural determination of their heat- and cold-activated states has remained a challenge. Here, we present cryo-EM structures of the nanodisc-reconstituted wild-type mouse TRPV3 in three distinct conformations: closed, heat-activated sensitized and open states. The heat-induced transformations of TRPV3 are accompanied by changes in the secondary structure of the S2-S3 linker, N- and C-termini and represent a conformational wave that links these parts of the protein to a lipid occupying the vanilloid binding site. State-dependent differences in the behavior of bound lipids suggest their active role in thermo-TRP temperature-dependent gating. Our structural data supported by physiological recordings and molecular dynamics simulations provide an insight for understanding the molecular mechanism of temperature sensing.
Keywords: TRP channels, cryo-EM, temperature sensitivity

SP-13.03 - Glutamate transporters contain a conserved chloride channel with two hydrophobic gates

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Glutamate is the most abundant excitatory neurotransmitter in the central nervous system, therefore its precise control is vital for maintaining normal brain function and preventing excitotoxicity. Removal of extracellular glutamate is achieved by plasma membrane-bound transporters, which couple glutamate transport to sodium, potassium and pH gradients using an elevator mechanism. Glutamate transporters, known as Excitatory Amino Acid Transporters (EAATs), also conduct chloride ions via a channel-like process that is thermodynamically uncoupled from transport. However, the molecular mechanisms that allow these dual-function transporters to carry out two seemingly contradictory roles are unknown. I will describe the cryo-electron microscopy structure of a glutamate transporter homologue in an open-channel state, revealing an aqueous cavity that is formed during the transport cycle. Using functional studies and molecular dynamics simulations, we show that this cavity is an aqueous-accessible chloride permeation pathway gated by two hydrophobic regions and is conserved across mammalian and archaeal glutamate transporters. Our findings provide insight into the mechanism by which glutamate transporters support their dual functions and add a crucial piece of information to aid mapping of the complete transport cycle shared by the SLC1A transporter family. Furthermore, this work assists in understanding the functional roles the chloride channel plays, notably, in maintaining cell excitability and osmotic balance and provides a framework for the rational development of therapeutics that can differentially modulate substrate transport or channel properties for the treatment of neurological disorders caused by EAAT dysfunction such as Episodic Ataxia. **Keywords:** Glutamate transporter, cryo-EM, channels

SP-13.04 - Conformational transitions and ligand-binding to a lipid-sensitive muscle-type acetylcholine receptor

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Fast synaptic communication requires receptors that respond to the presence of neurotransmitter by opening an ion channel across the post-synaptic membrane. The muscle-type nicotinic acetylcholine receptor from the electric fish, Torpedo, is the prototypic ligand-gated ion channel, yet the structural changes underlying channel activation remain undefined. Here we have used cryo-EM to solve apo and agonist-bound structures of the Torpedo nicotinic receptor embedded in a lipid nanodisc. Using both a direct biochemical assay to define the conformational landscape and molecular dynamics simulations to assay flux through the pore, we correlate structures with functional states and for the first time elucidate the motions that lead to pore activation of a heteromeric nicotinic receptor. We highlight an underappreciated role for the complementary subunit in channel gating, establish the structural basis for the differential agonist affinities of α/δ versus α/γ sites and explain why nicotine is less potent at muscle nicotinic receptors. We also identify numerous lipid binding sites at the periphery of the nAChR that could underlie the exquisite sensitivity of this pentameric ligand-gated ion channel to lipids.

Keywords: nicotinic acetylcholine receptor, ligand-induced conformational transition, cryo-electron microscopy

SP-14. Biomolecular association and dynamics

SP-14.01 - Time-resolved cryo-EM visualizes the structural dynamics of translation

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Accurate protein synthesis (translation) relies on translation factors that rectify ribosome fluctuations into a unidirectional process. Understanding this process requires structural characterization of the ribosome and translation-factor dynamics. Recent developments in single-particle cryo-EM enable near-atomic resolution of numerous structures sampled in heterogeneous complexes (ensembles). Ensemble and time-resolved cryo-EM have now revealed ribosome transitions during mRNA decoding and translocation. This presentation focuses on how elongation factors EF-Tu and EF-G help achieve high accuracy and efficiency of translation.
Keywords: structural dynamics, translation, ribosome

SP-14.02 - Theory of Protein Phase Separation in Biomolecular Condensates

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Compartmentalization at the cellular and sub-cellular levels is essential for biological functions. Organelles bound by lipid membrane, e.g., mitochondria and nuclei, serve this purpose. Compartments can also be non-membrane-bound. These include stress granules, germ granules, nucleoli, and many others. These bodies possess physical properties similar to those of mesoscopic liquid droplets. Referred to collectively as “biomolecular condensates”, their formation is underpinned largely by liquid-liquid phase separation (LLPS) of intrinsically disordered proteins (IDPs), intrinsically disordered regions (IDRs) of proteins, and nucleic acids. Behaviors of biomolecular condensates are fundamentally governed by the information encoded in the sequences of proteins and nucleic acids involved. We aim to gain basic physical understanding of this fascinating phenomenon. Accordingly, we developed analytical theories---including Flory-Huggins formulations, random phase approximation, Kuhn-length renormalization, and field theory simulation---as well as coarse-grained explicit-chain simulation models for sequence-specific LLPS of IDPs/IDRs. Our theoretical predictions rationalize experimental data, including those of an IDR of the DEAD-box helicase Ddx4, and elucidate the effects of net charge, sequence charge pattern, π-related aromatic interactions, pH, salt, and osmolyte on biomolecular LLPS. Our results point further to a “fuzzy” mode of molecular recognition by charge pattern matching, which should afford physical insights into how different IDP species may be miscible or demix upon LLPS to achieve biologically functional compartmentalization and sub-compartmentalization. We have also taken a first step toward rationalizing the temperature and pressure dependence of LLPS by considering empirical and atomic models of solvent-mediated hydrophobic interactions. Biological and biomedical ramifications of our findings are discussed, including how the experimentally measured pressure sensitivity of an *in vitro* model of postsynaptic densities might provide novel insights into the biophysical basis of pressure-related neurological disorders in terrestrial vertebrates.
Keywords: biomolecular condensates, membrane-less organelles, phase separation
Supported by: Canadian Institutes of Health Research; Natural Sciences and Engineering Research Council of Canada

SP-14.03 - 40 Years Learning from the Sequence-Dependent Mechanical Properties of B-DNA

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DNA is a flexible and structurally polymorphic polymer whose overall equilibrium geometry strongly depends on its sequence, the solvent environment, and the presence of ligands. Conformational changes in DNA are mediated by a complex choreography of backbone and base rearrangements. Such static and dynamic structural heterogeneities lead to local and global changes in the helix geometry impacting the ability of the DNA to recognize ligands, and consequently on its functionality. The study of the sequence-dependent mechanical properties of DNA started 40 years ago, after the first X-ray structure of a B-DNA crystal was determined. Since then, several works focused on learning about DNA flexibility by analyzing experimental structures deposited in public databases. In 2001, research groups of theoreticians and some experimentalists from all over the world decided to join efforts creating the Ascona B-DNA Consortium, with the goal of systematically describe the structural and dynamical properties of B-DNA under physiological conditions using atomistic Molecular Dynamics simulations. During the last 20 years, we characterized the sequence-dependent choreography of backbone and base movements modulating the non-Gaussian or anharmonic effects manifested in the higher moments of the dynamics of the duplex when sampling the equilibrium distribution. Contrary to prior assumptions, such anharmonic deformations are not rare in DNA and can play a significant role in determining DNA conformation within complexes. Polymorphisms in helical geometries are particularly prevalent for certain tetranucleotide sequence contexts and are always coupled to a complex network of coordinated changes in the backbone. The analysis of our simulations, which contain instances of all tetranucleotide sequences, allowed us to extend Calladine–Dickerson rules used for decades to interpret the average geometry of DNA, leading to a set of rules with quantitative predictive power that encompass nonlocal sequence-dependence and anharmonic fluctuations.

Keywords: DNA structure, Flexibility, Conformational space

Supported by: ABC, ANII, CSIC, PEDECIBA and UDELAR.

SP-14.04 - Diffusion of proteins along biopolymers: from biophysics to function

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Proteins, which are at the heart of many biological processes, are involved in a variety of self-assembly processes that are controlled by various chemical and physical interactions. Quantifying the driving forces that govern these processes and particularly the trade-offs between them is essential to obtaining a more complete understanding of protein dynamics and function. In my lecture, I will discuss the molecular determinants that govern linear diffusion of proteins along DNA or along microtubules. These and other cellular processes, such as protein folding, are subject to conflicting forces some of which are regulated by post-translational modifications. Understanding the trade-offs between the stability, affinity and mobility is not only essential to decipher transport processes in the cell but also for formulating concepts for their engineering. I will discuss the power of computational models in formulating fundamental biomolecular concepts and in predicting novel principles of cellular function or for its optimization.

Keywords: Diffusion coefficient, Intrinsically disordered proteins, Coarse-grained models, electrostatics

SP-16. Protein Folding Misfolding and Unfolding

SP-16.01 - Protein conformational dynamics and phenotypic switching

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Intrinsically disordered proteins (IDPs) are proteins that lack rigid 3D structure. Instead, IDPs exist as conformational ensembles that are highly malleable, facilitating their interactions with multiple partners. These interactions are “wired” to form scale-free protein interaction networks (PINs) that represent the main conduit of information flow in the cell. Because IDPs are extremely malleable, they typically occupy hub positions in cellular PINs. Furthermore, their conformational dynamics and propensity for post-translational modifications, contributes to ‘conformational’ noise which is distinct from the well-recognized transcriptional noise. Therefore, upregulation of IDPs in response to a specific input such as stress, contributes to increased noise and hence, an increase in stochastic, ‘promiscuous’ interactions. These interactions lead to activation of latent pathways or can induce ‘rewiring’ of the PIN to yield an optimal output underscoring the critical role of IDPs in regulating information flow. We have used PAGE4, a highly intrinsically disordered stress-response protein as a paradigm. Employing a variety of biochemical, biophysical, and computational techniques as well as mathematical modeling, we have elucidated the role of PAGE4 in phenotypic switching of prostate cancer cells at a systems level. These cumulative studies over the past decade, provide a conceptual framework to better understand how IDP conformational dynamics and conformational noise might facilitate cellular decision making.
Keywords: Protein conformational dynamics, Intrinsically disordered proteins, phenotypic switching

SP-16.02 - Liquid-liquid phase separation and assembly of viral factories: molten globule does the trick

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Dynamic spatiotemporal distribution of biomolecules that share a biochemical path within cells takes place through liquid-liquid phase separation (LLPS) driven biomolecular condensates. “Viral factories” are liquid-like structures within the cytosol of infected cells and sites for transcription and replication. The respiratory syncytial virus (RSV) is a member of the mononegavirales order which include several serious pathogens. The replication complexes of these viruses consist of an RNA polymerase, L, the nucleoprotein N, which wraps the viral genome, and a phosphoprotein, P. The RSV P tetramer has N-terminal disordered and C-terminal molten globule-like (MG) domains, and we hypothesize is the driver of LLPS in viral factories. Indeed, purified P undergoes homotypic LLPS with a thermal transition superimposable with the folding of the MG domain, suggesting that stable MG structure is required for LLPS. Moreover, solvent stabilization of the α-helical content within the MG domain potentiates demixing. Heterotypic LLPS is triggered when P and N are mixed at much lower concentrations, consistent with the biology. Co-transfection of P and N yields liquid granules as judged by FRAP experiments, where the C-term MG domain is absolutely required. Live fluorescence microscopy show minimum granules acting as condensation nuclei that gradually coalesce to yield large granules within the cell. Finally, time course of infection experiments show small granular nuclei which grow in size to render large viral factory granules observed for RSV and other mononegavirales. The N-P proteins are the minimal components for LLPS granules, modeling the assembly of viral factories, which we can recapitulate in the tube from the pure components. Weak MG-like structure must be present for the LLPS to take place in vitro and in cell, providing physicochemical grounds for phase separation behind viral a replication factory.

Keywords: virus replication, phase separation, molten globule

SP-16.03 - In Vivo Effects in Alzheimer's and Parkinson's Diseases: A Computational Biophysicist's Perspective

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Intrinsically disordered proteins amyloid-β and α-synuclein are at the center of Alzheimer's and Parkinson's diseases. The main challenge in biophysics and biochemistry is the understanding of the fundamental principles governing intrinsically disordered protein misfolding and aggregation, which represent complex conditions and sensitive processes and these pro-cesses operate at various length and time-scales. Amyloid-β and α-synuclein misfolding and aggregation processes produce products ranging from dimers to fibrils. Aggregations of amyloid-β and/or α-synuclein have been studied mostly in the test tube where the conditions were far from physiological. Therefore, there is an urgent need to extend these studies to in vivo conditions where the formation of amyloid-β and α-synuclein is affected by numerous biochemical reactions. Such interac-tions need to be understood in detail to develop therapeutics because millions of people worldwide suffer from neurodegen-erative diseases. Here, we describe recent advances in research on amyloid-β and α-synuclein formation from a physio-chemical perspective, focusing on the physiological factors that influence amyloid-β and α-synuclein aggregation processes in Alzheimer's and Parkinson's diseases, respectively. A detailed emphasis is provided for computational biophysics studies that help us to understand the in vivo effects on amyloid-β and α-synuclein.

Keywords: In vivo effects, Alzheimer's, Parkinson's

SP-16.04 - The new view of PML-bodies formation

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It is accepted the formation of one of the best-studied membrane-less organelles, PML-bodies is caused by oxidative dimerization of PML isoforms due to the formation of disulfide bonds between monomers of this protein initiates the formation of an insoluble “aggregate” to which, due to SUMO/SIM interactions, client proteins are recruited. Such views formed a certain "canon" due to the exceptional biological significance of PML-bodies for many cellular processes in health and disease, the study of these compartments began long before the emergence of fundamentally new ideas about the role of weak intermolecular nonspecific interactions and phase transitions of the liquid-liquid type in the formation of membrane-less organelles. However, oxidative dimerization of PML requires a high concentration of PML molecules and enzymes that catalyze the formation of disulfide bonds “in the right place at the right time”. Accordingly, for the de novo formation of PML bodies, PML pre-condensation is required. In our opinion, this can occur as a result of liquid-liquid phase separation of PML isoforms, which appears due to multiple weak nonspecific interactions of intrinsically disordered regions of PML isoforms. We found a population of “small” PML bodies of spherical topology with high exchange dynamics of PML isoforms with nucleoplasm and a low proportion of immobilized proteins, which suggests their liquid state unrelated to the multivalent SUM/SIM interactions. Such structures can act as “seeds” or “embryos” of functionally active PML bodies, providing the necessary concentration of PML isoforms to attract client proteins and, in particular, enzymes that provide SUMOylation of PML molecules, as well as, possibly, the formation of intermolecular disulfide bonds between PML monomers. FRAP analysis of larger bodies with toroidal topology showed the existence of the insoluble scaffold in the structure of such organelles. Taken together, our data create the prerequisites for revising the currently accepted model of PML body biogenesis, according to which the formation of PML bodies is initiated by the oligomerization of PML isoforms that form an insoluble scaffold, to which, due to polyvalent, primarily SUMO/SIM interactions, client proteins are attracted, thereby forming a dynamic layer that exchanges its content with the environment. *Author to whom correspondence should be addressed alexfonin@incras.ru; Tel.: +7 812 2971957; Fax: +7 812 2970341. **Keywords:** membrane, PML-bodies, organelles
Supported by: Russian Science Foundation RSCF 19-15-00107

SP-17 - EBSA Symposium on "Translational Biophysics"

SP-17.01 - Cholesterol-dependent Oligomerization and Endocytosis of GPCRs: Novel Insights in Therapeutics

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G protein-coupled receptors (GPCRs) are cellular nanomachines that allow the transfer of information from the cellular exterior to inside the cell. The biomedical relevance of GPCRs stems from the fact that GPCRs represent ~40% of current drug targets across all clinical areas. The focus of our work is to understand the role of membrane cholesterol in GPCR, oligomerization and endocytosis with implications in health and disease. The GPCR of choice is the serotonin-1A receptor, an important neurotransmitter receptor implicated in the generation and modulation of cognitive, behavioral and developmental functions, and an important drug target. We previously demonstrated cholesterol-dependent oligomerization of the serotonin1A receptor utilizing photobleaching image correlation spectroscopy (pbICS). I will discuss how the difference in dimer forming propensity with membrane cholesterol, which is developmentally regulated, has potential implications in drug development. We recently showed that upon chronic cholesterol depletion by statin, the endocytic route and intracellular trafficking of the serotonin-1A receptor exhibits a switch, from clathrin- to caveolin-mediated endocytosis. In addition, while the receptor is recycled back to the plasma membrane in normal condition, it gets degraded in the lysosome in statin treated condition. To the best of our knowledge, our results constitute one of the first reports on the role of membrane cholesterol in GPCR endocytosis and trafficking. From a translational angle, our results could be useful in developing novel therapeutic interventions that could tap into the modulatory role of membrane cholesterol in GPCR endocytosis. For example, our results could provide novel insight on the underlying mechanistic basis of recently reported improved antidepressant activity of antidepressant drugs in combination with statins. Taken together, insights from our results could be useful in developing novel therapeutic interventions that could tap into the modulatory role of membrane cholesterol in GPCR oligomerization and endocytosis.

Keywords: Cholesterol, GPCR endocytosis, GPCR oligomerization

SP-17.02 - Drug discovery in parasitic and viral diseases using protein lipidation as a target

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Resistance continues to undermine the efficacy of front-line drugs used in the treatment of malaria and neglected tropical diseases. The need for new therapies is being approached with cell based and targeted inhibitor screens, with enzymes of post- translational modification systems presenting appealing targets. Here, collaborative studies underpinning the investigation of N-myristoyltransferases (NMTs) will be described. NMT catalyses the co-translational transfer of a C14 fatty acid from myristoyl-CoA onto the N-terminal glycine residue of a significant subset of proteins in eukaryotic cells. This covalent modification influences the interactions of the substrate proteins with lipids and partner proteins. Structure-guided development of new lead compounds emerging from high throughput screening campaigns targeting Plasmodium and kinetoplastid NMTs has led to the discovery of potent inhibitors which have been used (i) to gain insights into the role of protein myristoylation in these parasites, and (ii) to validate NMT as a drug target [1,2]. As part of these studies, compounds were tested against human NMT leading to their repurposing as inhibitors of capsid assembly in picornaviruses, a process that relies on myristoylation of the viral polyprotein by the host cell [3]. These inhibitors block the replication of the multiple strains of the common cold virus protecting cells from virus-induced killing. References: [1] Wright, M. H. et al. Validation of N-myristoyltransferase as an antimalarial drug target using an integrated chemical biology approach. Nat. Chem. 6, 112-121 (2014). [2] Brannigan, J. A. et al. Diverse modes of binding in structures of Leishmania major N- myristoyltransferase with selective inhibitors. IUCrJ 1, 250-260 (2014). [3] Mousnier, A., et al., Fragment-derived inhibitors of human N-myristoyltransferase block virus capsid assembly and replication of the common cold virus. Nat. Chem. 10, 599-606 (2018). **Keywords:** N-myristoyltransferase, NTD, inhibitor discovery.**Supported by:** This work was funded by MRC and The Wellcome Trust

SP-17.03 - Water transport through membrane channels

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Introduction and objectives: Water scarcity affects the majority of the global population. Significantly improved membranes for water desalinization and purification are required to soften its impact. In the ideal case, these membranes contain selective water channels and reject all other solutes and solvents. Plasma membrane channels may reveal the design principles for synthetic water channels. While size exclusion and the lack of surrogates for the waters of ion hydration are important for water selectivity, water confinement may reduce the transport rate. Since the macroscopic laws of hydrodynamics do not apply [1], we were looking for the major determinants of water transport through pores so narrow that the water molecules cannot overtake each other. Materials and methods: Using scanning electrochemical microscopy, light scattering, fluorescence correlation spectroscopy, and microaspiration of giant vesicles [2], we observed rate differences that are several orders of magnitude in size for water transport through various narrow pores. Results and conclusion: The unitary water permeability, pf of water channel proteins (aquaporins, AQPs), potassium channels (KcsA), and antibiotics (gramicidin-A derivatives) increases exponentially with a decreasing number, NH, of hydrogen bond donating or accepting residues in the channel wall [3]. The Gibbs activation energy for water transport commonly agrees well with the variance in NH and pf [4] - contrasting examples from recently reported synthetic channels notwithstanding. [1] Horner and Pohl, Faraday Discuss. 2018, 209, 9-33. [2] Boytsov et al., Biotechnology Journal 2020, 15, 1900450. [3] Horner et al., Science Advances 2015, 1, e1400083. [4] Horner and Pohl, Science 2018, 359. **Keywords:** single-file transport, aquaporins, lipid bilayers. **Supported by:** Austrian Science Fund (FWF, grant number TAI181)

SP-17.04 - Interfacial Biophysics to Restore the Respiratory Surface under Breathing Mechanics

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Decades of research have revealed the crucial role played by pulmonary surfactant, a lipid-protein complex synthetized and secreted by the respiratory epithelium of the mammalian lung, to stabilize the large surface exposed to gas exchange and thus minimizing the work of breathing. Surfactant forms multilayered lipid-based interfacial films at the air-liquid interface of alveoli, reducing surface tension, particularly at the end of expiration, to very low values in a mechanically stable manner. Lack or alteration of these surfactant films is associated with severe respiratory pathologies, many of them still unresolved. The talk will review biophysical setups designed to mimic interfacial breathing mechanics under physiologically meaningful conditions. These models have been used to design clinical surfactant preparations that are now being used to replace natural surfactant in preterm babies born before their lungs have matured. Other models have been designed to challenge lung surfactant preparations in similar ways to how surfactant is inactivated as a consequence of lung injury. Surfactant impairment as a consequence of lung injury associated to inflammation and acute respiratory distress (ARDS), including that associated to COVID-19, is a major pathogenic factor. Surfactant replacement is starting to be applied in these patients once new therapeutic materials with enhanced resistance to inactivation are being developed using these biophysical models that mimic the demanding conditions associated with lung injury. Finally, other recent biophysical models have allowed revealing the intrinsic ability of pulmonary surfactant to act as a drug delivery vehicle, which uses the air-liquid interface to promote rapid and efficient diffusion of associated molecules and assemblies through the airways. Extensive research using these interfacial breathing-like setups has fuelled the generation of our current molecular and biophysical models on the crucial role played by pulmonary surfactant-associated proteins in forming and sustaining the efficient surface active alveolar films.
Keywords: Air-liquid interface, lipid-protein interactions, surface tension

SP-18. Autophagy: mechanisms and applications

SP-18.01 - Identification of E3 ligase functions in recognizing damaged lysosomes cleared by autophagy

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Lysophagy is one type of selective autophagy which engulfs damaged lysosomes by autophagosomes. Lysosomes can be damaged by many factors, such as cholesterol, fatty acids, detergents, pathogens and so on. Lysosomes hold digestive enzymes and leaving them in a damaged state is harmful to cells and affects cell growth. Autophagy plays an important role to sequester them. How cells recognize the damage is still unknown. Here, I will introduce the recent finding on the involvement of newly identified E3 in recognition step of lysophagy.

Keywords: Autophagy, lysosomes, selective autophagy

SP-18.02 - Targeting autophagy in skeletal muscle diseases

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Increased proteolytic activity has been widely associated with skeletal muscle atrophy. However, elevated proteolysis is also critical for the maintenance of cellular homeostasis by disposing of cytotoxic proteins and non-functioning organelles. We recently demonstrated that exercise activates autophagy and re-establishes proteostasis in cardiac diseases. Here, we will describe the impact of exercise on skeletal muscle autophagy and proteostasis during skeletal muscle disuse.

Keywords: autophagy, muscle, proteostasis

SP-18.03 - Location, location, location: Autophagy proteins interact with organelles to modulate lifespan.

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The last decade of research solidified the importance of the process of autophagy in health, human diseases and aging. Here, using the nematode C. elegans, we have identified key regulators of the transcriptional regulation of autophagy and lysosomal genes. In long-lived animals, we found that enhanced autophagy is accompanied by differential nucleo-cytoplasmic of proteins. Mapping out subcellular protein enrichment revealed that certain autophagy proteins have unique roles in organelle dynamics, which is key to maintain proteostasis and extend lifespan. Overall, we have found novel and conserved functions for autophagy proteins that have impact on aging. Our studies provide new points of entry to target autophagy and improve proteostasis in order to alleviate diseases of aging.

Keywords: Aging, autophagy, proteostasis, lifespan, longevity

SP-18.04 - Autophagic pathways in neuronal physiology and pathology during ageing

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Numerous gene mutations and treatments have been shown to extend the lifespan of diverse organisms ranging from the unicellular Saccharomyces cerevisiae to primates. It is becoming increasingly apparent that most such interventions ultimately interface with cellular stress response mechanisms, suggesting that longevity is intimately related to the ability of the organism to effectively cope with both intrinsic and extrinsic stress. Key determinants of this capacity are the molecular mechanisms that link ageing to main stress response pathways. How each pathway contributes to modulate the ageing process is not fully elucidated. Mitochondrial impairment is a major hallmark of several age-related neurodegenerative pathologies, including Alzheimer's disease. Accumulation of damaged mitochondria has been observed in post-mortem brain of Alzheimer's disease patients. Although disease-associated tau and amyloid β are known to deregulate mitochondrial function, it remains elusive whether they also directly influence the efficiency of mitophagy. Mitophagy is a selective type of autophagy mediating elimination of damaged mitochondria, and the major degradation pathway, by which cells regulate mitochondrial number in response to their metabolic state. However, little is known about the role of mitophagy in the pathogenesis of Alzheimer's disease. To address this question, we developed an in vivo imaging system to monitor mitophagy in neurons. We demonstrated that neuronal mitophagy is impaired in C. elegans models of Alzheimer's disease. Urolithin A- and nicotinamide mononucleotide-induced mitophagy ameliorates several pathological features of Alzheimer's disease, including cognitive defects. Mitophagy stimulation restores memory impairment through PINK-1-, PDR-1 or DCT-1-dependent pathways. A better understanding of the dynamics and reciprocal interplay between stress responses and ageing is critical for the development of novel therapeutic strategies that exploit endogenous stress combat pathways against age-associated pathologies. Our findings suggest that impaired removal of damaged mitochondria is a pivotal event in Alzheimer's disease pathogenesis highlighting mitophagy as a potential therapeutic intervention.

Keywords: Ageing, Metabolism, Neurodegeneration

SP-19. Membrane Simulation

SP-19.01 - Insights in lipid-protein interactions from computer simulations

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Lipid-protein interactions play an important direct role in the function of many membrane proteins. We argue they are key players in membrane structure, modulate membrane proteins in more subtle ways than direct binding, and are important for understanding the mechanism of classes of hydrophobic drugs. In a direct comparison of a panel of membrane proteins from different families in the same complex lipid mixture we found a unique lipid environment for every protein [1]. Extending this work, we found both differences and similarities in the environment of GPCRs, dependent on which family they came from and in some cases their conformation [2], with particular emphasis on the distribution of cholesterol. More recently, we have been studying the effect of protein conformation on local membrane properties using the ABC transporter P-gp as a model system. In more applied approaches, we determined how ceramides modulate the hERG1 potassium channel [3] and how poly-unsaturated fatty acids may modulate the properties of other potassium channels [4]. A new more sophisticated coarse grained forcefield (Martini 3) [5] and improved interactive visual exploration methods should enable further interesting applications [6]. [1] Corradi et al. 2018. ACS Central Science 4, 709–717 [2] B.I. Sejdiu, D.P. Tieleman. 2020. Biophysical Journal 118, 1887-1900 [3] W.E. Miranda et al. 2021. Nature Comm. 12, 1-10 [4] S. Yazi et al. 2021. Journal of General Physiology1 53, e202012850 [5] P.C.T. Souza et al. 2021. Nature Methods 18, 382-388 [6] B.I. Sejdiu, D.P. Tieleman. 2021. Nucleic Acids Research 49, W544–W550
Keywords: lipid-protein interactions, molecular dynamics, membrane proteins

SP-19.02 - Nanocellulose-membrane contacts, insights from Molecular Dynamics simulations

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Cellulose is a versatile and abundant biopolymer. Due to being biocompatible and nontoxic it has found its way to various applications in tissue engineering, bone wound dressing, to mention some. One the practical aspects in such application is that all of them involve contact between tissues and the cellulose-based material. Thus, controlling the strength of the contact is of utmost importance. We have used molecular dynamics (MD) simulations to study membrane-nanocellulose interfaces, and the mechanisms that control the binding strength [1-3]. This involves both substitution (acetylation) and different membranes (model stratum corneum and phospholipids). The balance between hydrogen bonding of different groups was found to have a major effect and in the case of stratum corneum, electrostatics and the level of fatty acid protonation and ceramides turned out to be critical [3]. [1] Gurtovenko, A. A.; Mukhamadiarov, E. I.; Kostritskii, A. Y.; Karttunen, M. Phospholipid-Cellulose Interactions: Insight from Atomistic Computer Simulations for Understanding the Impact of Cellulose-Based Materials on Plasma Membranes. J. Phys. Chem. B 2018, 122, 9973–9981 [2] Gurtovenko, A. A.; Karttunen, M. Controlled On-Off Switching of Tight-Binding Hydrogen Bonds between Model Cell Membranes and Acetylated Cellulose Surfaces. Langmuir 2019, 35, 13753–13760 [3] Gurtovenko, A. A.; Karttunen, M. How to Control Interactions of Cellulose-Based Biomaterials with Skin: The Role of Acidity in the Contact Area. Soft Matter 2021, 17, 6507–6518
Keywords: Nanocellulose-membrane, Molecular Dynamics simulations, biopolymer

SP-19.03 - Computational assays of bacterial cell envelopes: doing microbiology with computers
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Gram-negative bacteria are protected by a complex, tripartite cell envelope. This consists of two membranes separated by the aqueous periplasmic space. All three regions are crowded with proteins and the periplasm also contains a range of small molecules known collectively as osmolytes. The details of molecular interactions within each region, but also across all three regions that lead to the correct functioning of the cell envelope as a whole are largely still elusive. We are using atomistic level and more coarse-grained models and molecular dynamics simulations to model the molecular interactions within the two membrane and the periplasmic space of E. coli. Our results show that the crowded environments give rise to many expected but also unexpected behaviors, some of which may have mechanistic impact on e.g. movement of antibiotics through the periplasm. I will discuss the models, results and also some challenges we face in system preparation and analysis as the complexity of the simulated systems increases.
Keywords: bacterial, cell envelope, simulation

SP-19.04- SuAVE (Surface Assessment via Grid Evaluation) for Every Surface Curvature and Every Cavity Shape
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Curvature is an intrinsic feature of biological membranes underlying vital cellular processes such as endocytosis, membrane fusion–fission, trafficking, and remodeling. The continuous expansion of the spatiotemporal scales accessible to computational simulations nowadays makes possible quasi-atomistic molecular dynamics simulations of these processes. In despite of that, computation of the shapes and curvatures associated with the dynamics of biological membranes remains challenging. For this reason, the effect of curvature is often neglected in the analysis of quantities essential for the accurate description of membrane properties (e.g., area and volume per lipid, density profiles, membrane thickness). We have previously proposed an algorithm for surface assessment via grid evaluation (SuAVE)1 that relies on the application of a radial base function to interpolate points scattered across an interface of any shape and able to analyze geometrical and physical properties of surfaces taking into account its structural morphology. The SuAVE program can efficiently calculate the area and volume per molecule composing an interface, membrane thickness, surface topology maps, density profiles, curvature order parameters, Gaussian and Mean curvatures and even accessible volume in porous materials. We have now implemented new functionalities in SuAVE that makes possible calculations of thermodynamic variables and the evaluation of the energy underlying the curvature forming process in surfaces. These functionalities are demonstrated through applications of SuAVE to lipid with different degrees of curvature (membranes, vesicles, micelles). Furthermore, the new functionalities of SuAVE can also be used to quantify volume-dependent properties for closed interfaces, which we showcase for porous materials with complex inner cavity shapes. Th SuAVE software is an open source code which can be download from https://www.biomatsite.net/software.
Keywords: Software Development, Gaussian and Mean curvatures, Soft Matter and Porous Materials
Supported by: FACEPE, CAPES, CNPq

SP-20. Systems Biologics: At the interfaces of engineered proteins, their cell surface receptors and cellular molecular networks.

SP-20.01 - Systems Biologics: Large-Scale Engineering of Modulators of Protein Networks

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Over the past two decades, genomics technologies have revolutionized basic research and are also having a significant impact on understanding, predicting and diagnosing disease. Over the same period, the biologics revolution, lead by therapeutic antibodies, has greatly expanded our ability to target proteins that drive cancer and other diseases. To date, however, the academic genomics revolution and the industrial biologics revolutions have not been combined, so that the vast amounts of data generated by genomics technology have not been effectively translated to drug development, which remains a slow, case-by-case process. We established the Toronto Recombinant Antibody Centre (TRAC) to combine large-scale systems biology approaches with the discovery and development of new antibody drugs. The efficient pipeline of (1) basic research, connected to (2) translational science, and (3) commercialization, constitutes a new model for research and drug development, which we have termed “Systems Biologics”. Through this model, cutting-edge systems biology basic research can be seamlessly translated into systems biologics: novel, multi-functional drugs and diagnostics that take advantage of the complexities of human biology revealed by genomics data.

Keywords: Systems Biologics, genomics technologies, antibody drugs

SP-20.02 - Variation in GPCR signaling: Implications for drug discovery

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G protein-coupled receptors (GPCRs) participate in diverse physiological processes, ranging from sensory responses such as vision, taste and smell to those regulating behavior, the immune and the cardiac system among others. The ~800 human GPCRs sense diverse signaling molecules such as hormones and neurotransmitters to allosterically activate the associated G proteins, which in turn regulate diverse intracellular signaling pathways. In this manner, GPCRs regulate virtually every aspect of human physiology. Not surprisingly, GPCRs are the targets of over one-third of all prescribed human drugs. In this presentation, I will first discuss how one could leverage data from diverse species to infer selectivity determinants of GPCR-G protein binding, which is critical to elicit the right intracellular response. I will then discuss how one could utilize data on completely sequenced genomes of over 60,000 individuals from the human population to gain insights into natural receptor variation, which can result in variable drug response. Finally, I will present our recent work wherein by studying transcriptome data from over 30 different tissues in humans, one could begin to understand how alternative splicing creates diversity in GPCR signaling components, which may contribute to tissue-specific differences in receptor signaling. Such variations not only present challenges but also opportunities for drug development. I will conclude by discussing how understanding variation at these different dimensions, i.e., across different species, among different individuals of a species, and between tissues of a species, can provide a rich source of new hypotheses with implications for personalized medicine, drug development and understanding basic receptor biology.

Keywords: Drug Discovery, GPCR signaling, Data Science, Genetic Variation

SP-20.03 – Biophysics of peptiplexes based on cell penetrating peptides

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Cell-penetrating peptides (CPPs) are promising candidates for intracellular delivery of bioactive molecules, with strong impact in future development of nanotherapeutics. When complexed with DNA, these species form the so-called “peptiplexes”, non-covalent assemblies able to promote intracellular delivery of nucleic acids. Despite their great potential in nanotherapeutics, detailed information on spatial organization of peptiplexes and structure-activity relationships are still lacking in literature. Herein, we present results from recent publications from our group approaching the structure of peptiplexes and their delivery capabilities [Soft Matter (2016) 12:9158-9169, J. Phys. Chem. B (2019) 123:8861-8871, J. Mat. Chem. B (2020) DOI: 10.1039/C9TB02219H]. We aimed to provide information on the nanoscale structure of DNA/CPPs peptiplexes. Archetypical CPPs including Penetratin, TAT-HIV and SIV40 nuclear localization sequences have been investigated. Correlations with delivery capabilities have been determined through in vitro cell assays, and our results unveil a close relationship between spatial organization and DNA delivery. The mesoscopic structure has been unveiled through a range of biophysical techniques including small-angle scattering, X-ray diffraction, electron microscopy and infrared nanospectroscopy assays. Cytotoxicity and delivery capacity have been probed through MTT, flow cytometry and fluorescence microscopy assays. Our findings demonstrate strong capacity of CPPs to condense DNA strands into highly compacted assemblies exhibiting a rich polymorphism at the nanoscale. Importantly, organization into β-sheet intermediates upon complexation is regularly found in peptiplexes and seems to be an important step to translocate cell membranes. The spatial distribution of the DNA load across the assemblies plays a paramount role for delivery efficiency. The nanoscopic structure of non-covalent assemblies based on CPPs presents strong dependence on both amino acid sequence and load characteristics. A close relationship between spatial organization and DNA intracell delivery is found. Physicochemical parameters such as amphiphilicity, charge ratio and sequence pattern are key steps for optimizing complexes intended for gene therapy. **Keywords:** Cell penetrating peptides, DNA, peptiplexes

SP-20.04 – Changes of Cell Biochemical Network States Revealed in Protein Homomeric Complex Dynamics

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The interplay of environment and genome on the traits of an organism are reflected in how variations in either act on the biochemical networks that underlie all cellular processes. Current evidence suggests that predicting how environmental or genome variation affect specific cellular processes is most accurately determined by their effects on biochemical networks of the cell. It is impossible to measure, let alone predict, how entire molecular networks function, but we can choose useful surrogates of the network to act as reporters, such as protein interaction networks (PINs). We have developed general strategies to measure spatiotemporal dynamics of PINs in living cells, using Protein-fragment Complementation Assays (PCA) (Tarassov, et al. Science, 2008) to measure dynamics of PINs at whole proteome scales in response to environmental perturbations and to map novel biochemical pathways and predict genes associated with human diseases (MacDonald, et al., Nat. Chem. Biol., 2006; Messier et al. Cell, 2013; Tchenda, et al., Nat. Meth., 2014; Stynem, et al. Cell, 2018). I will present a simple and global strategy to map out gene functions and target pathways of drugs, toxins, or other small molecules based on ‘‘homomer dynamics’’ protein-fragment complementation assays (hdPCA). hdPCA measures changes in self- association (homomerization) of over 3,500 yeast proteins in yeast grown under different conditions. hdPCA complements genetic interaction measure-ments while eliminating the confounding effects of gene ablation. We demonstrate that hdPCA accurately predicts the effects of two longevity and health span-affecting drugs, the immunosuppressant rapamycin and the type 2 diabetes drug metformin, on cellular pathways. We also discovered an unsuspected global cellular response to metformin that resembles iron deficiency and includes a change in protein-bound iron levels. This discovery opens a new avenue to investigate molecular mechanisms for the prevention or treatment of diabetes, cancers, and other chronic diseases of aging. **Keywords:** Protein Interaction Networks, Network Propagation, predicting drug mechanisms

SP-21. IUBMB Symposium: Science Education

SP-21.01 - Course-based undergraduate research experiences: what if the treatment is a CURE?

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Introduction: Calls to improve undergraduate education of the next generation of scientists have emphasized the importance of undergraduate research experiences. Historically, undergraduates have engaged in research through internships that are mentored by faculty. This is problematic because the number of internships is limited. Furthermore, the ways students access internships often exclude those from backgrounds that remain underrepresented in the sciences, including students of color and students who are first in their families to go to university. Course-based Undergraduate Research Experiences, or CUREs, involve groups of students in addressing research problems or questions in the context of a class. These integrated research and learning experiences have been proposed as more scalable, equitable, and inclusive ways of involving undergraduates in research. **Objectives:** The objectives of this work were to: - Define the features of CUREs that make them distinctive as learning experiences - Test the effects of CUREs on students' likelihood of completing an undergraduate degree and majoring in science - Connect the features of CUREs with student outcomes **Materials and Methods:** Qualitative methods were used to formulate hypotheses regarding the features of CUREs that make them distinctive. Regression models with propensity score matched samples were used to assess the effects of CURE participation on students' likelihood of graduating and completing a science major. Structural models were used to connect features of CUREs with student outcomes. **Results and Discussion:** The results include a definition of CUREs, a description of what makes them distinctive from other learning experiences, and student outcomes from the Freshman Research Initiative at the University of Texas at Austin as a unique and highly impactful CURE model. **Conclusions:** A growing body of evidence is showing that CUREs are more equitable and inclusive than internships as a starting point for integrating undergraduates into the scientific community.

Keywords: undergraduate research, equity, inclusion
Supported by: US National Science Foundation

SP-21.02 - Reflecting and evidencing transferable skills

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A longstanding challenge for educators in Higher Education is the need to prepare students for their career journey after graduation. While theoretical foundations are needed, students should be able to apply knowledge in new contexts and be able to demonstrate and evidence life- and employability-skills valuable to employers. Many degrees provide students with the opportunity to develop transferable skills, for instance through giving presentations, working in teams in labs and field courses, and applying numeracy skills to analyse biology data. Nevertheless, students are not always able to reflect on their skills development, and on the connection between theory, practice and their learning. Authentic assessments can create links between theory and practice preparing students for the workplace. However, it is common to see the product of a particular activity being assessed, and not the process through which the product was produced. This may encourage students to value the end product over skills development, and therefore not appreciate how their University experiences prepare them for the workplace. Science students can struggle with self-reflection, and therefore may find it difficult to articulate and evidence skills during job applications. We need to find new ways of assessing students to help them develop their ability to self-reflect.

Keywords: transferable skills, reflection, employability

SP-21.03 - Evidence-based post-pandemic biochemistry and molecular biology education: redesigning courses to enhance the student and teacher experiences

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Teachers and students in biochemistry and molecular biology courses have lived through several months of remote or "socially-distanced" COVID-19 pandemic education. Mitigating the undesirable pandemic impacts on student learning, success or well-being has proved to be immensely challenging. There are concerns that students are lagging on crucial outcomes such as the development of experimental and research-related skills. No wonder, there is a generalized expectation that teaching and learning after the pandemic - post-pandemic teaching - "returns" to the "pre-pandemic" routines. Unquestionably, the pandemic added new difficulties to course design but it also exposed important problems of traditional teaching for student engagement and success. This talk will argue that, as we reconsider how we will approach our courses in the near future, we must redesign both the face-to-face and the digital post-pandemic biochemistry and molecular biology teaching experiences. Simply infusing additional digital teaching without intentionally redesigning student experiences will likely be insufficient. Exploring the research that demonstrates the importance of active learning in class to enhance student success and equity, the talk will critically consider what each of us can do, no matter what our context and role, to engage students in fulfilling biochemistry and molecular biology learning experiences.

Keywords: education, post-pandemic, teaching

SP-21.04 - Biokimi App: Interactive Study of Hepatic Glycolysis and Gluconeogenesis Regulations

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Biokimi is an App in android system to aid in Biochemistry learning. It focuses on the regulation of hepatic glycolysis and gluconeogenesis processes that correspond to opposite and non-identical pathways. They are formed by reversible reactions that act on the two processes, but in reverse directions according to different physiological situations, and by irreversible reactions that act in steps considered to be regulatory, as they have very negative free energy variations. This App shows these steps using images, associated texts and cognitive challenges. The scientific content was developed by the Creation Group of Educational Objects in Biochemistry (GCOEB-UFRGS). The programming and graphic art were carried out by the team of the Pedagogical Support Core for Distance Education (NAPEAD-UFRGS) using the Unity and Adobe Illustrator softwares. The App consists of many screens, divided into 10 modules (M), subdivided into topics. The modules are arranged in a lateral numeric summary. Navigation is performed by scrolling via touch and by appropriate buttons. Textual content screens often have images or animations to aid the understanding. Some texts have numeric buttons inserting the sentences. As the user reads the sentences, he can click on the buttons to make image actualization and to follow the text content. Topics that have challenging question screens are indicated by an identifier button on the side. Each screen presents a question and several answer alternatives, where only one is correct. Version 1.3 of this educational APP is free of charge, available on the Play Store. In the period from 01/14/2019 to 08/14/2019 it had more than 100 downloads. The evaluations of scientific-pedagogical contents, navigation characteristics, design, and interactivity of Biokimi App were considered excellent by undergraduate students from Biochemistry Department and graduate students from Biochemistry-PPG at UFRGS. **Keywords:** educational app, regulation of glycolysis, control of gluconeogenesis
Supported by: Capes

SP-22. Scissioning membranes

SP-22.01 - Intrinsically disordered proteins organize and shape cellular membranes

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Membrane curvature is required for many cellular processes, from assembly of highly curved trafficking vesicles to extension of needle-like filopodia. Consequently, defects in membrane curvature play a role in most human diseases, including altered recycling of receptors in cancer and diabetes, targeting of filopodia by pathogens, and hijacking of vesicle traffic during virus replication. Therefore, understanding the basic molecular mechanisms that drive membrane remodeling is essential to our knowledge of cellular physiology and human disease. Research on membrane curvature has primarily focused on individual protein domains with specialized structures, such as crescent-shaped scaffolds and wedge-like amphipathic insertions. While this work has provided invaluable insights, it overlooks two essential elements. First, most membrane remodeling proteins contain large intrinsically disordered domains in addition to structured domains. And second these disordered domains drive assembly of large, multi-valent protein networks. Recent work in our group supports the hypothesis that disordered protein networks are essential drivers of membrane remodeling in the cell. Specifically, using clathrin-mediated endocytosis as a model pathway, we have shown that intrinsically disordered domains generate steric pressure at membrane surfaces. This pressure provides a surprisingly potent driving force for membrane bending, especially when coupled synergistically to the contributions of structured domains. Additionally, we have recently found that disordered domains within endocytic proteins drive assembly of liquid-like protein networks which efficiently initiate endocytosis. Importantly, this liquid-like behavior has the potential to resolve a long-standing paradox by explaining how curved membrane

Keywords: membrane bending, endocytosis, intrinsically disordered proteins

SP-22.02 - ESCRT-III complexes assembling on membranes

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The multi-proteins ESCRT-III complexes are involved in membrane scission in many different cellular processes. In contrast to dynamin polymers that assemble outside budding vesicle/tubule necks, ESCRT-III assemble inside the bud necks. The organization of the proteins of this complex and even more the mechanism of membrane scission remain highly debated. By combining membrane nanotube pulling experiments, confocal microscopy, CryoEM and high-speed AFM on a minimal set of human ESCRT proteins, we have obtained unexpected results regarding their assembly and affinity for curved membranes. We show that CHMP4B filaments preferentially bind to flat membranes or to tubes with positive mean curvature. Although CHP2A are CHP2B are considered as homologues, CHMP2A requires CHMP3 for membrane binding and they induce different mechanical effects to membranes. Nevertheless, both CHMP2B and CHMP2A/CHMP3 assemble on positively curved membrane tubes, but not inside them. However, combinations of CHMP4B/CHMP2B and CHMP4B/CHMP2A/CHMP3 are recruited inside the neck of membrane tubes pulled from GUVs. In addition, they reshape vesicles into helical “corkscrew-like” membrane tubes when incubated together. Sub- tomogram averaging reveals that the ESCRT-III filaments assemble parallel and locally perpendicular to the tube axis, highlighting the mechanical stresses imposed by ESCRT-III. Our results underline the versatile membrane remodeling activity of ESCRT-III that may be a general feature required for cellular membrane remodeling. References: N. de Franceschi et al., J. Cell Sci. 132 jcs217968 (2019); A. Bertin et al., Nat. Commun. 11, 2663 (2020); M. Alqabandi et al., BMC Biol. 19, 66 (2021). **Keywords:** ESCRT complexes, membrane fission, membrane shaping

SP-22.03 - The role of scaffold reshaping and disassembly in dynamin driven membrane fission

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The large GTPase dynamin catalyzes membrane fission in eukaryotic cells, but despite three decades of experimental work, competing and partially conflicting models persist regarding some of its most basic actions. In this talk I will investigate the mechanical and functional consequences of dynamin scaffold shape changes and disassembly with the help of a geometrically and elastically realistic simulation model of helical dynamin-membrane complexes. Beyond changes of radius and pitch, I will emphasize the crucial role of a third functional motion: an effective rotation of the filament around its longitudinal axis, which reflects alternate tilting of dynamin's PH binding domains and creates a membrane torque. I will also show that helix elongation impedes fission, hemifission is reached via a small transient pore, and coat disassembly assists fission. These results have several testable structural consequences and help to reconcile mutual conflicting aspects between the two main present models of dynamin fission—the two-stage and the constrictase model.
Keywords: membrane fission, dynamin, simulation
Supported by: NSF (USA)

SP-22.04 - Lipid bilayer membrane as a possible target for inhibition of the SARS-CoV-2 Spike-mediated membrane fusion process

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Enveloped viruses infect cells through the fusion of the viral and the target cell membranes. This process is initiated by the interaction of the functionally relevant fusion peptide (FP) domain from the surface-attached Spike glycoprotein with the host lipid bilayer. Upon binding to the host membrane, the FP establishes a "bridge" that connects the viral and cell membranes, triggering the refolding of the fusion protein. The mechanism of action of viral FPs in membranes includes ordering of the lipid bilayers, induction of negative curvature, reduction of the membrane fluidity, and removal of water molecules from the membrane surface. Since these parameters can be regulated by different membranotropic drugs, the lipid bilayer has the potential to be exploited as a universal target for a wide range of viruses comprising a lipid envelope protecting their genetic material, including not only the coronaviruses but also viruses such as influenza, HIV, Zika, Dengue, Hepatitis, Ebola, among others. Therefore, we examined whether membranotropic drugs that cause effects on membranes that oppose those promoted by viral FPs could putatively inhibit the FP-induced membrane fusion process. As a proof of principle, different antimalarial drugs displaying membrane properties were selected and tested as antivirals. Using a series of biophysical studies such as fluorescence-based lipid mixing assays, electron spin resonance, and differential scanning calorimetry, we showed that mefloquine inhibits the membrane fusion process induced by the SARS-CoV-2 fusion peptide by promoting lipid disordering, membrane fluidity, and cholesterol segregation. This result suggests phospholipid membranes as putative sites for mefloquine antiviral action and the lipid bilayer as a potential new target for membrane fusion inhibition. The understanding of the physicochemical parameters affecting the membrane fusion process can help in the rational design of broad-spectrum inhibitors capable of blocking enveloped viruses' infection and may be successful in controlling future outbreaks. **Keywords:** antiviral, fusion peptide, SARS-CoV-2

SP-23. Redox Biology

SP-23.01 - Mitochondrial formation, catabolism and toxicity of peroxynitrite

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Mitochondria are sources and targets of peroxynitrite ($ONOO^-$), an oxidant and nucleophile generated from the diffusion-controlled reaction of superoxide radical ($O_2^{\bullet-}$) and nitric oxide ($^\bullet NO$). Peroxynitrite is a pathogenic mediator in inflammation and degenerative diseases and it contributes to the aging process. The mitochondrial effects of peroxynitrite are largely due to initial oxidation and nitration events on key target biomolecules that, in turn, promote downstream events. In the presentation I will briefly comment on the following aspects of mitochondrial peroxynitrite: 1) mechanisms of generation, 2) methods of detection, 3) key intramitochondrial targets, 4) catabolic pathways and 5) redox-based therapeutics. The talk will provide evidence at the *in vitro* and *in vivo* levels to underscore the role that peroxynitrite has in mitochondrial dysfunction and the related opportunities for mitochondrial-targeted therapeutics.
Keywords: free radicals, mitochondria, peroxynitrite
Supported by: UDELAR

SP-23.02 - Redox control of mitochondria biogenesis as a cellular stress response mechanism

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Cellular H2O2 homeostasis and signalling in S. cerevisiae are mediated by a specific H2O2-inducible transcriptional response engaging the YAP1 transcription factor. Activation of YAP1 in the cytosol depends on the thiol peroxidase Gpx3/Orp1. We have found that, upon H2O2 stress, Gpx3 activates another, YAP1-independent and compartment-specific defence pathway in mitochondria. This is initiated by a stress-dependent mitochondrial targeting of Gpx3 guided by an N-terminal targeting peptide that is appended to the protein by alternative translation in the cytosol. This extended form of Gpx3 follows a novel import pathway that targets the protein to the mitochondrial intermembrane space (IMS) independently of the known pathways for this sub-compartment. This novel pathway is independent of ATP hydrolysis or the inner membrane potential and can still operate in dysfunctional mitochondria with a damaged inner membrane. Trapping of Gpx3 in the intermembrane space (IMS) bypasses the main Mia40 pathway that recognises cysteine-rich proteins of the IMS but requires the Tim9-10 IMS chaperone complex. Gpx3 in the IMS engages in both protein-protein and protein-lipid interactions facilitating optimal operation of the oxidative folding machinery and protecting the inner mitochondrial membrane from oxidative damage. The IMS form of Gpx3 provides a back-up mechanism under oxidative stress to the operation of the oxidative folding machinery by functional complementation of Erv1, which is the critical oxidase in the MIA pathway under non-stress conditions. Additionally, the cytosolic reductive machinery consisting of thioredoxin and thioredoxin reductase are also dually localised to the IMS following unconventional import pathways.
Our results reveal for the first time the presence of a complete redox machinery in the mitochondrial IMS. Novel import pathways operate to ensure import and function of this machinery, providing a critical, mitochondria-specific stress defence mechanism to safeguard mitochondrial fitness under stress.
Keywords: mitochondria biogenesis, redox control, stress response
Supported by: UKRI-BBSRC, Royal Society, EU COST, SFC-SULSA

SP-23.03 - Mechanisms of peroxiredoxins targeting to mitochondrial subcompartments
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Mitochondria are mostly known as the powerhouses of the eukaryotic cells, playing roles in processes such as aging, cancer and neurodegenerative diseases. These organelles are also the major source or Reactive Oxygen Species (ROS), among them H2O2, which is also well known to mediate signaling pathways. Therefore, the comprehension of the mechanisms that control H2O2 levels generated into the distinct mitochondrial subcompartments under physiological and pathological conditions is highly relevant. Peroxiredoxins (Prxs) are by far the most relevant enzymatic systems responsible for H2O2 decomposition because they are abundant and highly reactive towards hydroperoxides. Surprisingly, the knowledge on the localization of Prx in the four mitochondrial subcompartments (outer membrane, intermembrane space (IMS), inner membrane and matrix) is scarce. 1) Identify the molecular mechanisms that control the targeting of Prxs from the cytosol to the mitochondrial subcompartments in yeast and mammalian cells. 2) Gain insights on the physiological roles of Prxs in the IMS and matrix. Purification and subfractionation of yeast and mammalian mitochondria, followed by western blot analysis, employing appropriate standards for the distinct submitochondrial compartments. We already constructed yeast strains that specifically express mitochondrial Prx from Saccharomyces cerevisiae (ScPrx1) in the IMS or in the matrix. Previously, we showed that mitochondrial Prx from Saccharomyces cerevisiae (ScPrx1) displays double localization: IMS and matrix. We also identified the proteins involved in the transport of ScPrx1 to these two mitochondrial subcompartments. Furthermore, we showed that the import of human Prdx3 from cytosol to the mitochondrial matrix is dependent on MPP and Oct1/MIP proteases, through heterologous expression in yeast cells. Preliminary results employing mammalian cells indicated that Prdx3 and Prdx5 are both located in matrix, while Prdx3 is also present in the IMS. As the four mitochondrial subcompartments house distinct physiological processes, the knowledge gained in this study may improve our understanding on fundamental aspects of redox and cell biology.
Keywords: peroxiredoxin, mitochondria, protein target
Supported by: FAPESP

SP-23.04- Experimental Studies and Computational Modeling on Cytochrome C Reduction by Quercetin: the role of oxidability and binding affinity

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Quercetin is a potent reducing agent of cytochrome C (Cyt c). Cyt c plays a fundamental role in the intrinsic apoptotic pathway, and there is evidence of the quercetin's role in this cellular event, which is involved in several biological effects of this phytochemical. In this work, we questioned ourselves if something special in quercetin could explain its high reactivity with Cyt c. The reducing potency of quercetin and its reactivity with Cyt C were compared with other antioxidants. Molecular docking and dynamics simulations were performed to explain the results. The product of the reaction was identified, and the pro-oxidant feature of quercetin was demonstrated. Among the antioxidants evaluated, gallic acid was more effective than quercetin as a reducer of the 2,2-diphenyl-1-picrylhydrazyl free-radical and less efficient in the 2,4,6-tri(2-pyridyl)-S-triazine-complexed ferric ion reduction assay. Regarding Cyt c reduction, which is also related to ferric reduction, quercetin was significantly more potent than gallic acid. These findings were explained by molecular docking and dynamics simulations, which indicated that quercetin has more privileged access to the protoporphyrin prosthetic group and more negative binding free energy (-46.4±2.0) than gallic acid (-13.9±6.8) kJ. Over the 35 ns of molecular dynamics, the reduced form of quercetin remained in the binding pocket, while the oxidized form dissociated from the protein after 20 ns. The oxidation of quercetin had as an outcome the formation of a heterodimer. In the reaction course, the transient quercetin free radical was able to oxidize glutathione. This result is an in vitro demonstration of quercetin's pro-oxidant features, an effect that has been reported in the cellular medium. In conclusion, the reaction between Cyt c and quercetin is related to its reduction potential and favorable protein-ligand interaction. This reaction can play a role in apoptosis triggered by quercetin.
Keywords: cytochrome C, quercetin, pro-oxidant. **Supported by:** FAPESP

SP-23.05 - The antioxidant role of the prion protein explained by copper storage in liquid condensates

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Intrinsically disorder protein's ability to form the scaffold of biomolecular condensates through liquid-liquid phase separation (LLPS) has emerged as a new possibility for pharmacological intervention of untreatable neurodegenerative diseases. Condensates contain highly concentrated biomolecules (~10-300 times enriched compared to light phase), functioning as nucleic acid storage/processing hubs and enable cellular spatiotemporal organization. Transition from the dynamic state of condensates to solid-like structures is likely behind the etiology of neurodegenerative processes. The prion protein (PrP) undergoes condensation modulated by DNA/RNA sequences. However, the characterization of LLPS should consider the complexity of cytosolic ionic composition, intracellular protein concentration and the effect of cognate metals. The PrP has six histidine residues that coordinate Cu(II) along its N-terminus. Also, the conserved C-terminal H139 and H176 co-bind Cu(II), tethering both N- and C- terminal domains. Several PrP functions are attributed to its high affinity towards copper ions whereby in vivo, it is believed to sequester excessive Cu(II), which are redox-active. If so, PrP condensates may concentrate Cu(II) to prevent oxidative burden, explaining a key biological function that if disturbed might preclude aggregation. To provide physiological relevance, we characterized PrP phase transitions by using recombinant proteins in the presence of copper-containing buffers mimicking cytosolic environment. Further, we examined the region that mediate copper-driven LLPS by comparing the full-length mature PrP to the C-terminal constructs PrP $^{90\text{-}231}$ and PrP $^{120\text{-}231}$. By microscopy and a range of biophysical techniques, we show that H139 and H176 are potential residues that mediate LLPS. Interestingly, addition of EDTA led to vacuolated intermediates until complete disassembly of droplets. Mammalian cells expressing PrP-YFP showed a subcellular distribution reminiscent of protein condensates. Reversible LLPS might be the molecular basis for PrP fine control of copper homeostasis. If uncontrolled, copper-catalyzed oxidation of PrP can led to aberrant condensates that evolve to solids implicated in prion diseases. **Keywords:** prion protein, liquid-liquid phase separation, biomolecular condensate
Supported by: FAPERJ, CNPq, CAPES

SP-24. Biophysics of immune system

SP-24.01 - Structure and dynamics of signalling complexes in the innate immune response and inflammation.

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The innate immune system is at the frontline of defence against pathogenic microorganisms and viruses. Toll transmembrane receptors are found in both vertebrates and invertebrates and are activated directly or indirectly by pathogen associated molecules such as lipopolysaccharide and peptidoglycan from bacteria. In this talk we will describe the use of CryoEM and single molecule imaging techniques to elucidate the molecular mechanisms of signal transduction in the human and insect Toll pathways and the basis of observed positive and negative cooperativity. In humans oligomers of the signalling adaptor MyD88 are recruited to activated receptors which nucleate the assembly of f Myddosomes that are fixed complexes incorporating the downstream protein kinases IRAK-4, IRAK-2 and IRAK 1 and display positive cooperativity. By contrast in insects the cytokine ligand Spatzle induces dimerization of the Toll receptor ectodomains to form a stable 2:1 complex. However biophysical and cellular analysis shows that the active signalling form has a stoichiometry of 2:2. Interestingly the CryoEM structures suggest that the binding of the second Spatzle ligand is likely to be energetically unfavourable which provides an explanation for the negative cooperativity of insect Toll signalling.
Keywords: CryoEM, Toll receptors, Innate immunity, cooperativity

SP-24.02 - New activators of the innate system: from assembled lipids to amyloids

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Numerous ligands of bacterial,viral origin are implicated as TLRs activators. This promiscuity raises questions concerning the manner in which molecules unrelated to the bonafide microbial ligands might productively engage a signaling. During this talk we will discuss how molecules unrelated to microbial ligands(natural nanoparticles,engineered nanoparticles) might activate innate immunity .These inflammatory reactions can be desired (for vaccine development), unwanted (for delivery applications) or involved in the induction of non-infectious diseases (amyloidoses, prion-related diseases).Development of new molecules targeting or inhibiting these inflammatory responses may lead to therapeutic perspectives largely unintended until now.
Keywords: innate immunity, nanoparticles, amyloids

SP-24.03 - Design of mammalian cell regulatory circuits

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Modularity has been extensively used in engineering for the rapid efficient construction of complex devices and assemblies. Usually, components are harvested from other organisms, such as bacteria or yeast for use in human cells yet rational expands the accessible range.
Our aim was to design orthogonal signalling pathways and information processing circuits for mammalian cells to enable control cellular response to selected input signals.
DNA binding domains and coiled coil modules were designed and encoded into DNA sequence. Plasmids were introduced into human or mouse cells using transfection and response of cells was monitored through luminescence and fluorescence. Designed Transcription activator-like effectors (TALE) were used to displace another TALE protein from DNA in a highly polarized manner, displacing only 5'- but not3' bound overlapping or adjacent TALE. The polarized TALE displacement provides strategies for the specific regulation of gene expression, for construction of Boolean genetic logic circuits, contributing to the understanding of the underlying principles of the facilitated displacement. Designed CC pairs were applied for multiplexing localization and to tune gene transcription strength and amplify the response of light- and small molecule inducible transcription in cell culture as well as in vivo. Further signaling pathways were designed based on proteolysis and designed coiled coils (CC) and implemented in mammalian cells. A set of split proteases with highly specific orthogonal cleavage motifs was constructed and combined with cleavage sites and designed CC domains for competitive displacement after proteolytic cleavage. This enabled implementation of Boolean logic functions and signaling cascades in mammalian cells that respond within minutes rather than hours. We devised new strategies to regulate gene transcription and fast protein-based signalling pathways that accelerated cellular response by an order of magnitude. This type of designed regulation can be used to regulate therapeutic cells.

Keywords: synthetic biology, signal pathways, designed protein fold
Supported by: ERC, Slovenian Research Agency

SP-24.04 - Gliadin proteolytical resistant peptides: the interplay between structure and self-assembly in gluten-related disorders

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Gliadin, a protein present in wheat, has become of great interest due to its role in gluten-related disorders as celiac disease and gluten sensitivity. It is known that this protein is not fully digested by humans, producing large peptides that elicit an immune response in susceptible individuals. In celiac disease, the adaptative immune response has been well characterized; however, the first inflammatory events that trigger the innate response remain elusive. Considering the relation between protein structure and function, combining different biophysical methods with cellular models is of key importance in an integrative understanding of a complex biological problem. In this context, it is hypothesized that gliadin peptides, such as the immunodominant 33-mer gliadin (LQLQPF(PQPQLPY)3PQPQPF) and the toxic p31-43 (LGQQQPFPPQQPY) could elicit an inflammatory response prior to disease due to their structural behavior. Based on that, an extensively biophysical characterization was performed in association with cellular analysis. The 33-mer peptide forms oligomers and large quaternary structures at high concentration with a Polyproline II conformation in equilibrium with parallel β-sheet secondary structure. These nanostructures activate the NFκβ pathway via TLR2 and 4, inducing the expression of proinflammatory biomarkers. The p31-43 has similar conformational behavior as the 33-mer peptide and self-organizes as oligomers and linear arrangements. These assemblies might be responsible for NLRP3 inflammasome activation in the gut, recapitulating the damage observed in patients. These results indicate that a multidisciplinary evaluation of a biological problem could help connect and reveal new pathways that were not explored in the relationship between health and disease.

Keywords: biophysics, gluten related disorders, inmune response
Supported by: CONICET, ANPCYT and DFG.

Graduate Program in Biochemistry - FMRP - USP: 50 years of history and achievement

Proteoliposomes as a mimic model of matrix vesicles and bone mineralization

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Chondrocytes and osteoblasts mineralize the extracellular matrix (ECM) by promoting the synthesis of hydroxyapatite (HA) seed crystals in the inner part of matrix vesicles (MVs) during endochondral bone formation. Several lipids and proteins present in the membrane of the MVs mediate the interactions of these vesicles with the ECM and regulate the initial mineral deposition and further propagation. Among the proteins of MV membranes, ion transporters control the availability of phosphate and calcium needed for initial HA deposition. Phosphatases (orphan phosphatase 1, ectonucleotide pyrophosphatase/ phosphodiesterase 1 and tissue-nonspecific alkaline phosphatase) play a crucial role in controlling the inorganic pyrophosphate/inorganic phosphate ratio that allows MVs mediated initiation of mineralization. The lipidic microenvironment can help in the nucleation process of first crystals and also plays a crucial physiological role in the function of MVs associated enzymes and transporters (type III sodiumdependent phosphate transporters, annexins and Na+,K+-ATPase). The whole process is mediated and regulated by the action of several molecules and steps, which make the process complex and highly regulated. Liposomes and proteoliposomes, as models of biological membranes, facilitate the understanding of lipid–protein interactions with emphasis on physicochemical and biochemical processes. In this presentation, we discuss the use of proteoliposomes as multiple protein carrier systems intended to mimic the various functions of MVs during the initiation and propagation of mineral growth in biomineralization. We focus on studies applying biophysical tools to characterize the biomimetic models in order to improve the understanding of the importance of lipid-protein and lipid-lipid interfaces throughout the process. (Financial Support: FAPESP 2019/08568-2, CNPq 304021/2017-2).

Deletion of AA9 lytic polysaccharide monooxygenases impairs fungal growth on lignocellulose

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Lytic polysaccharide monooxygenases (LPMOs) are oxidative enzymes found in viruses, archaea, bacteria as well as eukaryotes such as fungi, algae and insects, actively contributing to the degradation of different polysaccharides. In Aspergillus nidulans, several LPMOs from family AA9 (AnLPMO9s) as well as one AA3 cellobiose dehydrogenases (AnCDH1) were found to be co-secreted upon exposition to crystalline cellulose and lignocellulosic substrates, suggesting their role in the degradation of plant cell wall components. Functional analysis confirmed three main LPMO9s (AnLPMO9C, AnLPMO9F and AnLPMO9G) corresponded to cellulose-active enzymes with variable regioselectivity. Deletion of AnLPMO9F, abundantly secreted in cultivation with crystalline cellulose and sugarcane straw, had a major impact, decreasing the activity of a secretome (produced under crystalline cellulose induction) against cellulosic substrates by 50-75%, while AnLPMO9G, minority secreted, may have a role in oxidizing crystalline fractions of cellulose. Single or double deletion of these AnLPMO9s partially impaired fungal growth on sugarcane straw but not on crystalline cellulose, demonstrating the importance of these enzymes for saprophytic fungi relies on the degradation of complex lignocellulosic substrates instead of only on the cellulose crystallinity. In turn, deletion of AnCDH1 had a minor impact on the activity against filter paper but not on fungal growth, indicating other players may be acting as electron donors for the LPMOs. Additionally, double or triple knockouts had no accumulative deleterious effect on the cellulolytic activity nor on fungal growth, regardless of the deleted gene.

Unraveling the neurotropic potential of the emergent viruses Oropouche and SARS-CoV-2 using adult human brain slice cultures

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Neurotropic viruses can cause central nervous system (CNS) diseases. Indeed, about 30% of confirmed encephalitis cases are attributed to virus infections. Cellular and molecular mechanisms on CNS viral infections have been obtained using rodent models. Despite these advances, significant biochemical and functional differences between rodent and human brains limit their application as disease models in translational neuroscience. Here we have used slice cultures from adult human brains to investigate whether Oropouche (OROV) and SARS-CoV-2 viruses, reported to cause neurological symptoms in some infected individuals, are capable of infecting human neural cells in a context of preserved brain cytoarchitecture and neural connections. Brain tissue was obtained from patients undergoing temporal lobectomy for the treatment of refractory epilepsy (Ethics Committee approval HCRP 17578/15). Cortical fragments were collected at the surgical room and immediately transported to the laboratory, where the tissue was carefully sliced using a vibratome and cultured in 24-well plates. At days in vitro 1-2, brain slices were infected by OROV or SARS-CoV-2 for 2h. Infected slices were cultivated for 24-48 h post-infection. Our results indicate that both OROV and SARS-CoV-2 infect human neural cells and that these cells support virus replication. Interestingly, while OROV infects mainly microglia, SARS CoV-2 was seen to preferentially infect astrocytes. Both viruses also infected neurons to a lesser extent. OROV infection led to tissue damage and the release of the pro-inflammatory cytokine TNF-α. SARS-CoV-2 infection increased the RNA-expression of pro-inflammatory cytokines such as CCL2, IL-8, and IL-6 by brain slices. We are currently driving efforts to unravel the ultrastructural consequences of OROV and SARS-CoV-2 infections in adult human brain slices using transmission electron microscopy. Given the uncertainties on both acute and long-lasting neurological consequences of neural infection by OROV and SARS-CoV-2, our present work helps to raise awareness about the potential impact of these viruses on the human brain.

Keywords: Human brain slices, Oropouche, SARS-CoV-2

Funding: FAPESP, CNPq, CAPES, FAEPA

Glucocorticoids decrease the thermogenic capacity and increase the triacylglycerol synthesis by glycerokinase activation in brownadipose tissue of rats

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The maintenance of adequate triacylglycerol (TAG) stores is essential for normal brown adipose tissue (BAT) functioning and requires a continuous supply of glycerol-3-phosphate (G3P). This study aimed to investigate the effects of glucocorticoids on thermogenic capacity and in G3P generation pathways for TAG synthesis in interscapular brown adipose tissue (IBAT) of rats. Male Hannover rats received a single daily injection of dexamethasone (DEXA) (1 mg/Kg) or saline 0,9% during 7 days (CEUA protocol 195/2018). The mitochondrial proteins content, the temperature after noradrenaline stimulation, and noradrenaline content were measured in IBAT. The generation of G3P was evaluated by glycolysis, glyceroneogenesis, and direct phosphorylation of glycerol, respectively, by 2-deoxyglucose uptake, phosphoenolpyruvate carboxykinase (PEPCK) activity and pyruvate incorporation into TAG-glycerol, and glycerokinase (Gyk) activity and glycerol incorporation into TAG in IBAT. DEXA increases the IBAT mass and lipid content probably by increasing the *de novo* fatty acid (FA) synthesis, evaluated by increased glucose-6-phosphate dehydrogenase and ATP citrate lyase activities (79% and 48% respectively), compared to control. DEXA increases the content (~55%) and activity (~41%) of Gyk, without affecting the glucose uptake and glyceroneogenesis. DEXA reduces the glycerol incorporation into TAG (~54%), the AQP7 content (~50%), and the rate of basal glycerol release (~54%) in IBAT. In addition, DEXA decreases the thermogenic capacity of IBAT, evidenced by a reduction in the content of mitochondrial proteins, including UCP-1, and respiratory complexes, reduction in the noradrenaline content (53%), and the capacity of IBAT to increase the temperature after noradrenaline stimulation. Our data suggest that direct phosphorylation of glycerol by Gyk may be responsible for maintaining the supply of G3P for the increased esterification of FA and TAG synthesis in IBAT from DEXA-treated rats. The reduction of IBAT thermogenic capacity in these animals could be probably due to reduced sympathetic stimulation of IBAT.

Keywords: Brown adipose tissue, glucocorticoids, glycerokinase Funding: FAPESP and CNPq.

Effects of NT157 on tyrosine kinase signaling pathways in BCR-ABL1 T315I cells

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Chronic myeloid leukemia (CML) is characterized by the presence of the oncoprotein BCR-ABL1, which constitutively activates the tyrosine kinase activity triggering a neoplastic transformation of hematopoietic stem cells. Although, some reports using tyrosine kinase inhibitors have represented an advance in the treatment of CML, up to 20% of patients are resistant to those inhibitors. In this sense, other studies aiming proteins thatbind indirectly to the BCR-ALB1 have identified insulin receptor substrates (IRS) as a potential targets, representing new therapeutic strategies for CML. Evaluation of the proteomic alterations in primary samples from patients carrying T315I mutation undergoing treatment with an IGF1R-IRS1/2 inhibitor, NT157. Peripheral blood mononuclear cells (PBMCs) from a CML patient with a BCR-ABL1 T315I phenotype were treated with 6.4 µM NT157 for 48 hours and submitted to analysis of cell viability and apoptotic molecular markers by flow cytometry. To determine the changes in the protein abundances of the cells, we performed a global proteomics analysis. The proteomic data was acquired using a Q-Exactive-HF LC-MS/MS system and processed using Label-Free-Quantification approach with MaxQuant and Perseus softwares. The 6.4 µM NT157 treatment for 48 hours significantly decreased cell viability and increased apoptosis in PBMCs. Overall 3244 proteins were confidently identified with FDR < 1%. Label-free quantitative analysis highlighted a list of 116 upregulated and 85 downregulated proteins differentially detected among from cells not treated and cells treated with NT157. In particular, the BCR protein, which is directly involved in CML pathogenesis is decreased by the NT157 treatment. Also, several metabolic pathways involved to TCA cycle, angiogenesis, immune response and another's are altered. The pharmacological inhibitor of IGF1R- IRS1/2 NT157 showed antineoplastic effects in primary cells from patients CML BCR-ABL1 T315I, including reduced cell viability and increased apoptosis. Therefore, the inhibition of IGF1R/IRS signaling, using NT157 has revealed a potential therapeutic approach, and maybe an alternative to the tyrosine kinase inhibitors in the context of patient's inhibitor resistance (BCR-ABL1 T315I). **Keywords**: Proteomics, NT157, Chronic myeloid leukemia, BCR-ABL.
Funding: CNPq (Proc. 308561/2014-7, 402697/2016-2 and 305854/2017-8), CTC•CEPID (2013/08135-2)

Keynote Lectures

KLBN-01. **The control of exposure to natural occurring radioactive materials**

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The current radiation protection paradigm was developed in the form of recommendations by the International Commission on Radiological Protection (ICRP) over around a century. These recommendations were originally mainly aimed to the protection of radiologists in their practice. Then they evolved into other activities; initially they would focus on occupational protection, then on the protection of members of the public, then on patients undergoing radio-diagnosis and radiotherapy, and finally on the protection of the environment. From the beginning the ICRP recommendations were focused on what would eventually be termed firstly as 'practices' and then as 'planned exposure situations', namely everyday situations involving the planned operation of radiation sources. The ICRP recommendations confront severe challenges when applied to natural radiation and radioactivity, and they are particularly confusing when applied to the so called naturally occurring radioactive materials (NORMs). There are solutions to the conundrum of regulating natural radiation, and particularly NORMs. One possibility is to change the current radiation protection system; but this could be too ambitious and politically unfeasible. Another, perhaps more feasible approach, would be to undertake clear legislative and regulatory decisions on exclusions and exemptions from the regulatory scope of natural radiation. Under these conceptual limitations, the ICRP recommendations clearly indicate that: (i) for exposure situations involving specified processed materials and by-products containing radionuclides of natural origin, consideration may be given to extending the use of exclusion beyond the case of raw materials, whenever their regulation is unjustified and should the legal national conditions permit; and, (ii) in jurisdictions where the mechanism of exclusion may not be appropriate, the concept of exemption may be applied to these products in order to achieve an equivalent objective. These unspecific recommendations were not sufficient for international intergovernmental organizations to develop a quantitative and universal consensual definition of scope for radiation safety standards dealing with natural radiation in general and with NORM in particular. The suggested solutions are that relevant international and intergovernmental organizations should: (i) promote an international legislative consensus for exclusion, through legislation, of some natural radiation exposures; and (ii) establish, unambiguously, criteria for regulatory exemption, for instance establishing that criteria of no-control as an optimum protection option.

Keywords: ionizing radiation; licensing; NORM

KLBN-02. - The Chernobyl Tissue Bank - a resource for radiation research

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The Chernobyl Nuclear Power Plant accident in 1986 released large amounts of radioiodine into the environment. This resulted in an increase in thyroid cancer in those exposed as children, resident in the contaminated areas of Belarus, Ukraine and Russia. This remains the only radiobiological consequence of the accident for the population at large. he Chernobyl Tissue Bank (CTB) was established in 1998 to ensure that there was co-ordination of studies linking environmental low dose radiation exposure to molecular and clinical phenotype of thyroid cancer. It provides infrastructure in Ukraine and Russia to ensure that there is benefit to both the wider scientific community, and to the patients and local Institutes that were responsible for collection of the samples. The CTB was the first tissue bank of its type, providing multi-format biological samples (frozen tissue, fixed tissue, DNA extracted from both blood and frozen tissue, serum and RNA extracted from frozen tissue) which is pathologically assured and consented for research, to international research groups, together with an infrastructure to track and collate research results from each individual sample. Ultimately, a data repository for studies taking an “integrated biology” approach to understanding the mechanisms that underpin development of thyroid cancer has been established. The CTB currently holds clinical and pathological information from 5283 patients, who have provided 112,661 individual biological samples. 11,977 biosamples have been issued from 858 individuals who were exposed to radiation from Chernobyl and 5399 samples from 308 individuals who were not exposed, to 43 individual research projects, including 651 cases for whole genome sequencing (WGS). Of those 519 cases of papillary carcinoma issued to projects that use WGS or next generation sequencing (NGS), driver mutations have been identified in 96%, with point mutation in the BRAF gene being the most common (42.2%) followed by fusions of the RET oncogene (20%). There was no association with radiation exposure and individual driver mutations, but oncogene fusions were more frequent in those aged under 19 at operation compared with those aged over 19 (61.8% versus 39.3%). The reverse was true for cases with an oncogenic driver with a point mutation (28.9% in under 19s and 57.9% in those aged over 19 at operation), irrespective of radiation exposure. The CTB provides a paradigm for biobanking in the “omics” era and demonstrates the added value of returning research results to a tissue bank to enrich data on any remaining samples from the same patient.

Keywords: Chernobyl, Tissuebank, Biosamples
Supported by: National Cancer Institute of the US and the Sasakawa Foundation, Japan

KLBN-03. - Radiation biodosimetry: New paradigms

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As concerns about the possibility of radiological or nuclear accidents or incidents have grown, many countries have invested in science to support preparedness for such events. Much effort has gone into the development of drugs to mitigate the effects of acute or delayed radiation syndromes. At the same time, we must be able to identify those individuals within potentially exposed populations who could best benefit from mitigator treatments. This presentation will overview biodosimetry needs, and then focus on the development of gene expression based approaches to provide rapid radiation biodosimetry. Most work to date has focused on dose reconstruction for acute external gamma-ray exposures, or on detection of thresholds for triage. Here, we will consider more complex exposures, such as those including neutrons or internal emitters, and some of the challenges of extrapolating from model systems to a human population.

Keywords: biodosimetry, radiobiology, RNA
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KLBN-04. - Particle Radiation Therapy: developments, studies and applications
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In this presentation the science, developments and applications of hadron therapies, especially Boron Neutron Capture Therapy (BNCT) and Proton Therapy will be presented, with special focus on the activities performed in Argentina in this field of scientific research. The rationale for using hadrons (protons, neutrons and light nuclei) for the treatment of cancer has been substantiated by their dosimetric as well as radiobiological properties. The former is achieved by the technological improvements that allow controlling very precisely the charged particle beams and, in the case of BNCT, by expanding further the properties of new boron compounds that deliver highly localized doses to tumor cells. The latter are more related with the intrinsic properties of high ionization density particles, that create complex chromosomal damage and inhibits proliferation in a very effective way. Both modalities also benefit from the use of concomitant applications and procedures, which together with irradiation increase the tumor control and minimize the toxicity of the treatment. During this lecture, examples of developments and applications will be shown, and the importance of expanding regionally these options in pursuit of achieving a benefit to patients and, at the same time, increasing the scientific and technological capacities of the countries of the region.
Keywords: BNCT, Proton, Therapy

KLBN-05. - Women in the nuclear field promoting Latin American integration
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Nuclear energy is used for the generation of electricity, but also for the production of radioisotopes, desalination of sea water and also for the production of hydrogen. Activities in the nuclear field are in the area of science, technology and innovation that has long belonged to an essentially male domain, in which the contributions of women were neglected or underestimated. The central idea for the creation of Women in Nuclear, WiN Global, was to support and encourage women working in nuclear science and technology and encourage the promotion of understanding and knowledge of the benefits of the peaceful use of nuclear energy by the public. WiN Global currently has predominantly female members coming from 129 different countries, belonging to chapters or individually. Today, WiN Global is integrated by 53 WiN Global chapters. Forty-nine countries have their own chapters and there are also regional and international ones. The history of Latin American integration started during the political independence movement of the countries of the New Continent. Since then, up and downs were overcome in order to keep a regional ambiance of good relationship. In the present study, a new form of integration is presented by the efforts of the women working in the nuclear ambit. This important movement involves Latin American WiN chapters (such as WiN Argentina, WiN Brazil, WiN ARCAL) promoting activities for the integration of our region. In order to quantify, to some extent, the participation of Latin American women, this paper presents a survey crossing data of the number of related publications to help to address an objective analysis of the trend of this integration
Keywords: Women in Nuclear, WiN, nuclear energy, Latin American integration

Symposia

SPBN-01- The nuclear technologies: innovations for minimizing the environmental impact

SPBN-01.01 - Future of Nuclear Energy Beyond Electricity

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Nuclear energy will continue to play a key role in the world's low-carbon energy mix, with global nuclear electrical capacity projected to double by 2050. The world's nuclear power industry has not only proven that it can be flexible even during a pandemic, but it also continues to serve a vital role in sustainable climate change mitigation. Non-electric applications powered by nuclear energy could present sustainable solutions for a number of energy challenges current and future generations will have to face. There is growing interest around the world in using nuclear energy for such applications as seawater desalination, hydrogen production, district heating and various industrial applications. Industrial applications and nuclear cogeneration involve the integration of nuclear power plants with other systems. The heat generated by the nuclear power plants can be used to produce a vast range of products such as cooling, heating, process heat, desalination and hydrogen. The use of nuclear energy for cogeneration provides many economic, environmental and efficiency-related benefits. Most of the world's energy consumption is for heat and transportation. Potential is in penetrating Transportation sector (Nuclear Hydrogen Production for H2-FCEV) and Heat sector (Desalination, district heating/cooling, heat for industry). The nature of industrial heat market is highly fragmented, hence very much suitable for Small Modular Reactors (SMR). Individual large users with energy intensive industrial processes (desalination, petrochemical, district heating…etc) cover the remaining portion of the industrial heat market with requirements up to 1000 MWth, and exceptionally even more. Large reactors for cogeneration could fit in industrial parks. But there are a number of Challenges for Cogeneration: Public acceptance; National position (political will, Government commitment); National Regulations including licensing issues; Availability of qualified human resources; Selecting the most appropriate NPP based on demand and grid capacity; Disparity between characteristics of nuclear reactors & heat markets; Industry trends (Require small amount of heat); Buy energy but not risk build it; Demonstrate newly NPPs tailored for industry (HTR); Economics; Licenseability of tailored cogeneration NPPs with ensured safety and Siting.

Keywords: nuclear energy, heat transport applications , energy
Supported by: ELETRONUCLEAR

SPBN-01.02 - Deployment of Small Modular Reactors (SMR) and Transportable Nuclear Power Plants (TNPP)

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Nuclear power has the capability to play a vital role in our societies in the future. The pace of the development of Small Modular Reactors (SMR) and Transportable Nuclear Power Plants (TNPP) concepts, has increased in recent years and the success of this sector will be dependent on an appropriate transport safety infrastructure and public acceptance. To achieve a revised transport safety infrastructure will require cooperation and collaboration by all parties involved. A requirement that also extends to the necessary review and revision of the other aspects involved namely, security, nuclear safety, safeguards, environmental and liability, regulations, and conventions. The increased use of nuclear power in electric and non-electric applications will have a significant beneficial effect of increasing the decarbonization of our future societies.

Keywords: transport, SMR, TNPP, Decarbonization

SPBN-01.03 - Natural occurrence of radioactive materials (NORM) in mining and oil industry

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Natural radioactivity comes from several sources: From the extra planetary space in the form of cosmic rays, from the soil where radionuclides are widespread and from Earth's atmosphere. Whenever we act on the environment, we produce some impact. Mining, for instance, mobilizes materials naturally present in nature, some of which are the radioactive isotopes of the uranium and thorium decay series. here are natural anomalies which potentiate even greater risks. However, with the available technological knowledge, these impacts can be managed, and their risks controlled. NORM in oil reservoirs can be dissolved during contact with water and/or oil with rock. The fluids coexisting in the reservoir may have high concentrations of radionuclides that have accumulated during quite extensive periods of contact. During oil exploration most of the NORM accumulate either as hard scales or sludges. Several deleterious occurrences are produced by the presence of NORM, such as plugged piping and pumps, workers exposure and contamination, contaminated soils and water, and voluminous waste generation. Exposure to NORM scenarios demand monitoring the amount of alfa, beta, and gamma radiation and exposure levels in air, soil, and water environment, as well as scale, sludge and scrap. Consecutive to sorting and volume reduction NORM can be safely isolated in three types of radioactive waste repositories. Occasionally, in case of accidents or other peculiar situations, interim repositories receive waste from nuclear or radiological accidents. Hence, in case a final repository authorized to receive NORM waste is missing, the mining and oil industry production cycles remain open. The interim solution - initial storage – affords neither financial nor environmental sustainability. Waste Management is a set administrative and technical activities related to waste, from their origin to their disposal. Its fundamental principle is based in not generating - or reducing to a minimum - both the generated volume and the volume to be disposed of. Accordingly, every radioactive, nuclear, metallurgical, mining, or oil extraction activity must have a radioactive waste management plan. All that can be done is conditioning and/or processing, aiming at amending the radwaste characteristics, such as changing its composition, removing radionuclides or reducing radwaste volume opens up vast opportunities for R&D.

Keywords: NORM, Natural radioactivity, radioactive isotopes

SPBN-02- Biochemistry and biotechnology for radiopharmaceuticals development

SPBN-02.01 - New Developments in Imaging Cell-Based Therapy

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The development of cell-based therapies is an exciting field which harnesses the immune system to kill cancer cells. This involves injecting living cells into a patient, which then target tumour cells within the body. Although this approach has seen some success in treating haematological malignancies, solid tumours are much more challenging and questions remain around where the administered cells go in the body, whether or not they reach the site of the disease, how many cells are needed to effect a response and what is the timescale? The ability to visualise and quantify cell therapies in living subjects both on initial administration and over time is immensely valuable to development programmes and for monitoring patient response. This presentation will cover nuclear imaging approaches for visualising cell therapies in living subjects, including both by direct cell labelling and reporter gene imaging approaches, and detail the advantages and disadvantages of each.

SPBN-02.02 - Global initiatives for diagnosis and therapy (theranostics) radiopharmaceuticals availability

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The production and application of theranostic radiopharmaceuticals has opened a new gateway to diagnostic/therapeutic nuclear medicine for critical human diseases. Advances in development of peptide/small targeting molecules for various human malignant molecular targets such as somatostatin receptors, PSMA, GPR etc. in combination with a large list of theranostic radioisotopes including but not limited to Lu-177, Y-90, Ac-225, Cu-series, Sc-series etc. has provided a powerful toolbox for clinicians. The International Atomic Energy Agency (IAEA), a source of service to the Member States on nuclear science and technology, is observing and monitoring worldwide developments in the field of medical radioisotope and radiopharmaceutical production together with professional societies and private companies. The agency promotes the production and application routes including research reactors, cyclotrons, linear accelerators, and other cutting-edge methods, and not only designs and promotes activities such as Coordinated Research Projects (CRPs), Technical Meetings (TMs), national/regional training courses and conferences, but also supports and joins forces with international professional societies to support and promote radiopharmaceutical sciences. Various IAEA CRPs on the production and application of theranostic radiopharmaceuticals have been proposed, finalized, or planned during the last decade, with exemplary outcomes and outputs with participation of major role players, industries, and Member States research teams with focus on local, regional, and international production and sharing networks.

Keywords: radiopharmaceuticals, molecular imaging, therapy

SPBN-04- Patients radiation exposures and epidemiological surveys

SPBN-04.02 - The EPI-CT - a European cohort study to quantify cancer risks in paediatric and young adult patients from CT radiation

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The use of computed tomography (CT) has increased dramatically during the last decades and raised concerns regarding potential of its iatrogenic effects, particularly in children who are more sensitive to the effects of ionizing radiation. EPI-CT aims at evaluating cancer risk potentially related to radiation doses from CT in childhood and adolescence. Based on a common protocol, national cohorts were assembled in 9 European countries both retrospectively and prospectively, by identifying eligible participants from radiology department records. Patients were linked with national/regional registries of cancer, vital status and migration. The EPI-CT addresses three major outcomes of interest: brain, haematological and other solid cancers. A complex individual organ dose and uncertainty estimation based on the Two-Dimensional Monte-Carlo simulation method was developed to reconstruct the absorbed radiation dose from each CT scan using the National Cancer Institute Dosimetry System for CT (NCICT) software. The EPI-CT study includes 658,752 patients who were still alive and cancer-free before and five years after their first CT. Overall,165 brain cancers occurred, including 121 glioma. Mean cumulative brain dose was estimated to be 49.3 mGy. A statistically significant linear dose-response relationship was observed for all types of brain cancers combined, as well as for gliomas separately. The excess relative risk (ERR) for all brain cancers at 100 mGy of absorbed brain dose was 1.27 (95 % CI 0.51, 2.69) and for gliomas 1.11 (95 % CI 0.36, 2.59). This is the first study with a complex individual organ dose and uncertainty estimation. The observed dose-response relationship is unlikely to be fully explained by indication bias based on the results of sensitivity analyses and external evidence. Further follow-up is needed as numbers of solid cancers will increase with age.

Keywords: Cancer, Computed tomography, Epidemiology
Supported by: European Union

SPBN-04.03 - Global surveillance of trends in cancer survival

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The lecture is an overview of cancer survival worldwide on population-based cancer registries incidence. The Concord -3 study is monitoring selected cancer and lately besides the selected solid tumors, childhood lymphoblastic leukemia and brain is now included. More data will be available soon about morphological classification and survival to refine biological and epidemiological association linked with also biomarkers. I am going to present survival for selected solid tumors and lymphoblastic leukemia and brain in children. There is a disparity in relative survival between the populations worldwide high-income countries with highest survival while upper and low-income countries with lower. access to diagnosis and treatment are main limitation associated with poor survival. Continuous monitoring in cancer incidence, mortality and survival can improve life quality hence better understand cancer trends and biological differences among populations.

Keywords: cancer, survival, population based

SPBN-05 - *Development of models for tumoral and inflammatory imaging*

SPBN-05.01 - Three-dimensional cellular culture system for testing of biological effects of radiations in tumoral and non-tumoral models

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In vitro cell cultures are a well-known controlled test system used to analyze tumor physiologic responses upon negative stimuli. Updated techniques, us-ing three-dimensional organization of cells in cultures, are being increasingly used to this purpose. Research organizations and industry are striving to pro-duce in vitro tumor surrogates that could be better test systems to antitumor agents as new compounds or to study radiation effects on cancers. The presentation will show some techniques currently used to build and maintain these specific cell cultures, and how experiments are evolving towards the production of tumoroids, or tumoral organoids, which will include various cell types and additive manufacturing
Keywords: 3D cell culture, tumoroids, radiations
Supported by: FAPESP (2017/50332-0), FINEP (23784-17) & IPEN/CNEN-SP

SPBN-05.02 - 131I-Ixolaris development as a theragnostic agent: metastatic melanoma pre-clinical studies

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Metastatic melanoma is a very aggressive neoplasm presenting high mortality rates in a few months and resistance to therapeutic interventions. Previous studies have shown that tissue factor expression (TF), a blood coagulation initiator protein, correlates with the histological grade of malignancy and vascularity, playing a fundamental role in tumor invasion, tumor growth, angiogenesis and metastasis. Ixolaris, a non-immunogenic molecule that specifically binds to TF, has already demonstrated in vivo reduced growth of melanoma tumor metastatic nodules (B16-F10). Thus, the main objectives of this work were: I) To develop an efficient and stable labeling technique of Ixolaris with Iodine-131(131I) which could also maintain its biological activity; II) To study and compare in healthy and melanoma-induced mice, the biodistribution of 131I and 131I-Ixolaris; and III) to evaluate whether 131I-Ixolaris could serve as a metastatic melanoma agent. Ixolaris radioiodination was done using iodogen at room temperature. Quality control was made with paper and liquid chromatography (sephadex G-75). Labeling stability was accessed for 24h and the anticoagulant activity of 131I-Ixolaris was measured using a coagulometer. Planar and SPECT imaging and biodistribution studies were performed after intravenous administration (iv) of 131I or 131I-Ixolaris in a murine melanoma model (B16-F10) divided in 3 groups: I-D0 of induction; II-D15; and III-D1 and D15 (double treatment). Animals were sacrificed at D18. In vitro studies have demonstrated that 131I-Ixolaris is stable at plasma and saline for at least 24h and maintains its inhibitory activity on blood coagulation. Biodistribution studies and lung nodules counts showed that the fractionated use of 9MBq of 131I-Ixolaris (D1/D15) reached better results showing a decrease in lung metastatic nodules. Scintigraphy 90 minutes after iv of 131IIxolaris demonstrated uptake in pulmonary topography. These results suggest that 131I-Ixolaris has a promising future as a theragnostic agent and could serve as a new tool for the management and treatment of metastatic melanoma.
Keywords: Ixolaris, Melanoma, Theragnostic
Supported by: CNPq, FAPERJ

SPBN-05.03 - Derivative from the antimicrobial peptide LyeTx I as potential positron emission tomography (PET) radiopharmaceutical

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Current diagnostic methods and imaging techniques are not able to differentiate infection and sterile inflammation. Thus, reliable methods are sought to provide this distinction and molecular imaging techniques are interesting options, since they are based on physiological changes. In this context, radiolabeled antimicrobial peptides have been investigated as they accumulate in infectious sites instead of aseptic inflammation, due to their selectivity for interaction with microorganism cells rather than with mammalian cells. Previously, the antimicrobial peptide LyeTx I was isolated from the venom of the spider *Lycosa erythrognatha*. In this lecture, it will be described the development of a ^{68} Ga-labeled derivative from LyeTx I as a potential radiopharmaceutical for infection imaging using the PET/CT technique. Three novel shortened derivatives (LyeTx I mn; LyeTx I mn∆K; LyeTx I mn∆KAc) were synthetized and evaluated for their toxicity and biological activity. Among them, LyeTx I mn∆K presents the best score between antimicrobial (↓MIC) and hemolytic (↑EC50) activities, and LUHMES cell-based NeuriTox test showed that it is less neurotoxic than the original LyeTx I (EC_{50} [LyeTx I mn∆K] < EC_{50} [LyeTx I]). Data obtained in a mouse model of septic arthritis (*S. aureus*), showed that LyeTx I mn∆K is able to reduce infection and, then, the inflammatory process and pain. Next, LyeTx I mn∆K was synthetized with the chelating agent DOTA attached to its C-terminal portion, aiming ^{68} Ga-labelling. The radiopeptide presents high radiochemical stability in saline and serum. *In vitro* assay showed correlation between the amount of bacterial cells (*S. aureus*) and the percentage of radiopeptide binding. PET/CT images, obtained in animal infection (*S. aureus*) and sterile inflammation models, revealed the ability of ^{68} Ga-DOTA-LyeTx I mn∆K to identify infection focus (target/non-target = 4.9) and differentiate it from sterile inflammation (target/non-target = 1.3). Therefore, it is a promising radiopharmaceutical for infection imaging using the PET/CT technique.

Keywords: gallium-68, PET/CT, radiolabeled antimicrobial peptides
Supported by: Associação PROUNIEMP / HIAE, FAPEMIG, CNPq and CAPES

SPBN-05.04 - Use of 99mTc-anti-TNF-alpha as a marker of inflammatory disease activity

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Graves' ophthalmopathy (GO), also called Graves' orbitopathy,is characterized by an initial inflammatory stage of active disease that has a variable course (duration range, 6–24 months), after which there is an inactive disease stage characterized by predominant fibrosis. Active-stage GO involves a multifactorial inflammatory process that enlarges extra ocular muscles, During the initial inflammatory stage, there are primarily increases in interferon-a and tumour necrosis factor alpha (TNF-α). It has been suggested that 99mTc-anti-TNF-α scintigraphy may be a useful diagnostic tool in GO. Here we present our experience regarding the use of this radiopharmaceutical in the evaluation of different diseases, such as Rheumatoid Arthritis, Graves Ophtalmopathy, Psoriatic Arthritis, Ankylosing Spondylitis, Autoimmune Enteropathy, and Inflammatory Bowel Disease. Our experience suggests that 99mTc-anti-TNF-α scintigraphy is a good complementary tool for the diagnosis of disease activity where TNF-α has a role in the physiopathogenesis of the disease.

Keywords: TNF-alpha, Disease activity, Scintigraphy
Supported by: CNPq, FAPERJ

SPBN-06- Radiotracers as signatures evaluating water quality and the biodiversity protection

SPBN-06.01 - High Uranium Concentrations in the Groundwater of the Rio de Janeiro State, Brazil, Mountainous Region

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Unexpectedly high uranium concentrations, up to 930 µg L-1, approximately thirty times higher than the World Health Organization (WHO) guidance level, were observed in groundwater samples from the mountainous region near Rio de Janeiro City, the so-called “Região Serrana”, approximately 60 km from the city. This region is characterized by a large number of tourist activities and water-related industries, such as mineral water and breweries that can be impacted by these findings. In addition, the water supplies in small communities of this region are partially or entirely based on groundwater sources. Uranium contamination was observed in 7 of the 16 counties in this region. Based on these data, this study concluded that the probability of obtaining uranium contaminated groundwater is high in some specific areas of this region. In addition, high 222Rn concentrations were verified, with levels reaching 1570 Bq L-1. Furthermore, a maximum level of 4.6 Bq L-1 210Pb was also measured, which has a WHO guidance level of 0.1 Bq L-1. Based on the present findings, it is suggested that any artesian well deeper than 80 m in this region should be tested for uranium and 222Rn.
Keywords: radon, groundwater, uranium

SPBN-06.02 - Removal of Zn and Cd from overlying water by mangrove sediments: testing the effects of sediment resuspension

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Radiotracer experiments have been useful to improve our comprehension on biogeochemical processes involving trace metal pollutants. This study tests the hypothesis that coastal sediments redeposition after resuspension events in tidal water may change the sediment capacity to sequester pollutants from overlying water after formation of new sediment-water interfaces. Microcosm experiments were performed with mangrove sediments from the Itacuruçá mangrove forest, located at Sepetiba Bay (Brazil) to evaluate 65Zn and 109Cd removal kinetics by redeposited mangrove sediments, in a region in which these trace metals are the major industrial pollutants affecting the coastal zone. Water columns that overlaid redeposited and control sediments were spiked with artificial radiotracers Overlying water was sampled at 10 min, 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 19 h, and 24 h. After 24 h, sediment cores were sectioned in 1-cm intervals. The determination of radionuclide activities in these samples was performed by gamma-ray spectrometry with a high-purity Ge detector. Metal retention within redeposited sediments were approximately 20% lower than in control sediments. Average decreases of 41% (109Cd) and 27% (65Zn) in the half-removal times (t1/2) from overlying water were promoted by redeposited sediments in comparison with control sediments (without statistically significant differences). More limited depth diffusion of metals was observed within redeposited sediments, limited to the uppermost centimeter. This experimental approach indicates that frequent disturbances that cause sediment resuspension-redeposition events are not able to change the ability of mangrove sediments to trap metal pollutants. Mangrove vegetation cover acts stabilizing and retaining coastal sediments and associated pollutants more than occur in unvegetated sites, which may compensate a higher metal remobilization susceptibility due to physical and biological disturbances when retained within upper layers, as observed for redeposited sediments.
Keywords: Metal radiotracers, Sediments, Mangroves.
Supported by: FAPERJ e CAPES

SPBN-06.03 - Chemical diversity in tree species from Caatinga

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Of a biodiversity under discovering at diverse trophic levels (microorganisms, plants, animals), Caatinga ecosystems are exclusively present in the Northeast Region of Brazil. Its typical plant species are adapted to hot dry environments, to soils of low water and nutrient contents, mainly phosphorus, and, sometimes, under influence of uraniferous areas containing high activity concentrations of natural radionuclides. Despite the high adaptability of plant species, these ecosystems are subjected to strong anthropogenic impacts like firing, agriculture and pasture. Particularly, the mineral cycling is quite intricate for Caatinga species, in which little is known about the diversity of chemical elements in tree species. This lecture compiles the first results on the distribution of trace elements such antimony, cadmium, molybdenum, thorium, uranium, lanthanum and lanthanoids on Caatinga tree species. Compared to the Atlantic Forest species, leaves from Caatinga presented the lowest concentrations, including nutrients such as calcium; however, some accumulation was noted in leaves for all chemical elements studied compared to the expected range for plants. As noted for other worldwide natural forests, bioaccumulation was mostly consistent for trees from the same plant species even growing in the same area. The independence of soil contents indicates the needs for increasing the knowledge on the trace element distribution in Caatinga plants aiming at biodiversity conservation and sustainable use.

Keywords: biodiversity, rare earth elements, bioaccumulation

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SPBN-07 - Radiation occupational exposures

SPBN-07.01 - Mathematical tools for radiological protection and dosimetry

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In radiological protection, calculations are usually too complex to be ex-actly solved by means of mathematical analytical techniques. Computational tools are then needed in order to evaluate the quantities used in the area. Some physical quantities, like the kerma in a radiation field, can be directly measured but others, like human organs equivalent doses and the effective dose, cannot be directly measured by their own definition. The connection be-tween those measurable quantities and the unmeasurable ones is then made by computer radiation transportation codes, usually by means of Monte Carlo simulations, acting upon mathematical anthropomorphic models that represent a standard human being. In external radiation dosimetry, auxiliary operational quantities are defined that can be measured and should overestimate the pro-tection quantities. In internal dosimetry, biokinetic models of the intake and re-tention of radionuclides in the human body are established. Those biokinetic models, together with radiation transportation codes are then used to estimate organ doses during a fixed time. Monte Carlo simulations are also used to evaluate air crew doses coming from cosmic rays exposures and in dosemeters development, once the complete device structure is known and can be imple-mented in the simulation code. This lecture will give an overview of the role that these computer calculations play in radiological protection. **Keywords:** Monte Carlo, Radiological protection, Dosimetry

SPBN-07.02 - Occupational Exposure to Ionizing Radiation

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According to the International Commission on Radiological Protection (ICRP) and the International Atomic Energy Agency (IAEA), the definition for Occupational exposure is the radiation exposed workers in the course of their work, with the exception of: (a) exposure to the normal local natural background radiation; (b) exposure from exempt activities involving radiation or exempt sources; and (c) any medical exposure of patients. Occupational radiation exposed workers are subject to controls established by the national regulatory authorities. The workers can be exposed to natural sources or human-made sources, the exposure to either result in radiation doses. The natural sources of radiation are cosmic radiation and naturally occurring radionuclides belonging to the 238U, 228Th and 232Th decay series. This situation is called NORM (Naturally Occuring Radioactive Material) and TENORM (Technologically Enhanced Naturally Occurring Radioactive Material). The sectors involved in occupational exposure to NORM and TENORM are extraction and processing industries (minerals, oil and gas), workplaces with high concentrations of radon (drink water treatment, thermal spas, show-caves, wine cellars), consumer products (fertilizers and fertilizer production wastes, cigarettes, granite countertops). Some countries do not regulate the sectors involving exposure to natural sources of radiation, as a result the occupational exposure can exceed the dose limits. The sectors that include human-made sources of radiation are regulated, the workers are monitored on a routine basis. It includes the sectors of the nuclear fuel cycle, medical uses of radiation, industrial uses, educational uses, military uses. In general, the average annual effective doses are below the investigation level for all sectors. The exception is for workers involved in interventional radiology, in nuclear medicine and in industrial radiography; which the average annual effective doses may exceed the dose limits if the radiological protection measures are not implemented properly. According to the UNSCEAR 2008 Report, for the period 2000-2002, the worldwide number of workers exposed to natural sources was 13 million, the average annual effective dose was 2.9 mSv. On the other hand, the worldwide number of workers exposed to human-made sources was about 10 million, the average annual effective dose was 0.4 mSv. The evaluation shows a decline of radiation exposure in all sectors involving exposure to human-made souces. **Keywords:** occupational exposure, radiation exposure, natural sources, human-made sources

SPBN-07.03 - Radiation protection from a personal dosimetry service perspective

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Personal Dosimetry is the ultimate measurements of the effectiveness of the radiation protection culture implemented on any institution that makes use of ionizing radiation. In Brazil since 1995, CASEC/IRD/CNEN implemented a very modern certification process for all personal dosimetry services. Also, the Health Ministry, through ANVISA established the mandatory use of personal dosimetry for all Occupational Exposed Individual. Sapra Landauer is a private service offering personal dosimetry since 1979 with marked presence of about 40% of all personal dosimetry evaluations in Brazil. Data analysis of these large number of records is a important indication of the current situation of Radiation Protection in Brazil. Time evolution of the last 4 years is presented showing the increase in total number of occupational Exposed Individual in Brazil as well as the distribution of dose occurrence in some of the significant growing sectors like Nuclear Medicine and Veterinary. This analysis shows which sectors presents growth above overall average as well as tendencies of the personal dose records. The result of this analysis is a guide to the need to implement training programs aiming to the improvement of the radiation protection culture of specific applications.
Keywords: personal dosimetry, radiation protection, dose distribution

SPBN-08- External radiation cancer therapy

SPBN-08.01 - Dose fractionation in Radiotherapy

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The present lecture covers conventional and modified dose fractionation protocols used in radiotherapy, as well as the radiobiological basis for dose fractionation. The modified fractionations addressed are: hyperfractionation, accelerated fractionation and hyperfractionated accelerated treatment. Concerning the radiobiological bases, DNA and cell cycle response to radiation, tolerance dose and the 5 Rs of radiotherapy are addressed.

Keywords: Radiobiology, Dose fractionation, Radiotherapy

SPBN-08.02 - Developments in proton therapy to treat pediatric patients

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Around 60% of all malignant neoplasm are treated with radiation therapy alone or combined with other modalities. While most cancers appear later in adult life when critical organs such as brain and heart are fully developed and body is fully grown, survivors of pediatric cancer patients are left with long lasting and crippling late effects of radiation treatments. In early 2019 pediatric cancer treatments was a theme in the scientific journal Science alongside the most common side effects of childhood cancer such as memory problems, hearing and visual problems, heart conditions, hormonal and infertility problems, among others. Proton therapy has been identified as one of the best radiation therapy for most pediatric solid cancers due to its lack of exit dose and target high precision. Traditional passive scattering beams use components along the beams-eye- view to scatter and modulate the proton beam, producing a large amount of neutron radiation that showers over the patients' body. Pencil beam scanning uses magnetic steering to spread the proton beam along a large area, while the energy modulation is done in the acceleration vault, far away from the treatment room. This technique produces no neutron outside the patient's body. Eliminating the source of neutrons inside the treatment room is a step forward, there are still more challenges. Normal tissues in the entrance of large spread- out-of-Bragg-peak (SOBP) do receive a reasonable amount of dose. Which is especially concerning when tumors are located deep in the brain tissue. Techniques that distribute multiple beams like intensity modulated proton therapy (IMPT) and rotating proton therapy arc (Sparc) can help reduce such burden. Another potential candidate is the mini-beam, which splits the beam into small beamlets and has the potential to further spare tissue in the entrance dose region.
Present the current developments in proton therapy for pediatric cancers

Keywords: pediatric cancer, proton therapy, pencil beam scanning
Supported by: N/A

SPBN-08.03 - Postmastectomy radiation therapy: challenges on the ESTRO-ACROP consensus guideline

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Treatment planning of post-mastectomy radiotherapy (PMRT) plans for patients with mammary prostheses are still based on the field and not on irradiated volume, which would consider the target volume with the inclusion of the prosthesis or the reconstructed breast itself. In treatment planning techniques based in field, the radiation dose absorbed in the skin is relatively high, which causes complications for the patient, such as erythema and edema in the treated region. Aiming to reduce treatment-related toxicity without compromising target coverage, in 2019 a new consensus guideline from the European Society of Radiotherapy and Oncology - Advisory Committee on Radiation Oncology Practice (ESTRO-ACROP) for target volume delineation in the post-radiotherapy setting mastectomy was published. According to the guideline, the permanent silicone implant and the contralateral breast must be delineated in the planning tomography, but the transplanted tissues (skin, fat, muscle) and synthetic materials (silicone implants and tissue expander) are not part of the clinical target volume (CTV). Although ESTRO-ACROP presents detailed instructions on target volume definitions for Breast Radiotherapy in the setting of immediate breast reconstruction, this document does not instruct on important aspects of treatment planning and delivery, such as the use of planning target volume (PTV) and the dose limit values for organs at risk (OAR) expected with the change in treatment volume. Considering the importance of evaluating the aspects related to the process of implementing the new mode of target volume delineation proposed by ESTRO-ACROP, this work presents an experience of evaluation of these aspects in one of the largest radiotherapy centers in Brazil. The goal is that this experience will be useful to professionals in other radiotherapy centers who intend to implement the new consensus guideline in breast delineation in their clinical practice. **Keywords:** post-mastectomy, radiotherapy, new consensus guideline.
Supported by: CNPq and CAPES

SPBN-08.04 - Biological dosimetry for predicting acute side effects in radiotherapy

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Radiotherapy (RT) induces lethal lesions in malignant neoplasias, through the interaction of ionizing radiation (IR), generally without causing severe reactions to healthy (normal) tissue adjacent to the tumor. However, the occurrence of acute side effects may compromise the RT outcomes. This phenomenon was first described in patients with genetic syndromes like Fanconi Anemia and Ataxia-Telangiectasia since patients with these diseases have shown severe adverse effects due to their difficulty in repairing DNA radioinduced damages. Additionally, studies have pointed out cases of significant individual radiosensitivity that were not associated with any known genetic syndromes. Although promising, no pre-therapeutic radiosensitivity test is commonly used in oncology practices. In this sense, several studies have proposed potential biomarkers for assessing individual radiosensitivity based on cellular or molecular techniques, and most of them are either laborious or high-cost techniques. On the other hand, biological dosimetry encompasses individual dose evaluation based on biological endpoints induced by ionizing radiation (so-called biomarkers). Although the relationship between IR-induced biomarkers and the absorbed dose is not always straightforward, using biological dosimetry as a reference in investigating individual radiosensitivity would contribute to protocol individualization in radiotherapy. This study was designed to examine in vitro irradiated peripheral blood mononuclear cells (PBMC) as a pre-therapeutic test for predicting the individual degree of these acute side effects in head and neck cancer radiotherapy. For this, blood samples were collected and irradiated using a 6 MV linear accelerator. Based on the International Atomic Energy Agency manual for Cytogenetic Dosimetry (2011), the viability of PBMC, frequency of unstable chromosome aberrations, and micronuclei were compared to the intensity of acute side effects experienced by the studied patients. Although further studies in larger cohorts are needed to ensure the validity and reliability of the methodologies, our initial results point to a practical and affordable pre-radiotherapeutic test for predicting patient radiosensitivity.
Keywords: Biodosimetry, Radiosensitivity, Radiotherapy.
Funding: IAEA, CNPq and FACEPE

SPBN-09- Androgen receptors signaling and clinical studies for prostate cancer

SPBN-09.01 - Androgen receptor and prostate cancer therapy

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Prostate cancer is a highly prevalent disease. It has devastating effects in patients and their families, summing up enormous costs to both society and economy. Androgen blockade has been a primary therapy for prostate cancer for more the fifty years. The remarkable effects on tumor size are, however, followed by biochemical recurrence and, eventually, death. The androgens testosterone and dihydrotestosterone act via the androgen receptor, a 110kDa protein, member of the nuclear receptor superfamily. Upon activation by androgens, the activated androgen receptor is translocated to the cell nucleus where it exerts its transcription factor function, regulating proliferation and differentiation related genes. The expression of PSA and PSMA is tightly regulated by androgen levels. Important transitions in prostate development are regulated by androgen level variations. Castrated men never develop prostate cancer, suggesting the importance of androgen stimulation for prostate function and tumor development. It has become evident that changes associated with the androgen receptor are associated with biochemical recurrence. Gene point mutations, gene amplification, receptor promiscuity and cross talk to other signaling pathways have been shown to contribute to the progression of the so-called castration-resistant prostate cancer. In this talk I will summarize aspects of prostate development, prostate cancer initiation and progression, and treatment in relation to androgen stimulation and deprivation, using the literature and data from the laboratory. I will introduce the participation of macrophages as important factors in inducing prostate epithelial cell death in response to castration. Finally, I will comment on the specificity of PSMA as an excellent target for prostate cancer radiotherapy.

SPBN-09.02 - Clinical trials with 18F-PSMA1007

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Proper assessment of prostate cancer in both initial staging and biochemical recurrence of prostate cancer remains a challenge for the attending physician and for conventional imaging methods in the evaluation of these patients. The aim of the present study is to demonstrate the success of the identification of extra-prostatic disease in the inicial staging or the localization of disease in patients with biochemical recurrence using 18F-PSMA1007 PET/CT, in our experience. The aim of the present study is to demonstrate the success of the identification of extra-prostatic disease in the inicial staging or the localization of disease in patients with biochemical recurrence using 18F-PSMA1007 PET/CT, in our experience. Methods: this is a prospective cross-sectional study. 18F-PSMA1007 PET/CT were performed between October/2019 and June/2021. The scans were analyzed by four independent specialist physicians. Positive findings for recurrence of prostate cancer were classified according to their location, with recurrence at the prostate, lymph node or metastasis sites. Results: More than 200 patients underwent 18F-PSMA1007 PET-CT, mostly for the evaluation of biochemical recurrence. The identification of metastases in patients at an inicial stae was more common in those classified as high risk prostate cancer. In patiens who were evaluating biochemical recurrence, negative tests occurred in patients with very low PSA levels. Conclusion: 18F-PSMA1007 PET/CT is very useful in the initial assessment of high risk patients and plays a fundamental role in the assessment of high risk patients and plays a fundamental role in the assessment of biochemical recurrence, allowing for a personalized and adequate treatment for each patient.

Keywords: 18F-PSMA1007, PET-CT, Prostate cancer

SPBN-09.03 - Clinical applications of positron emission tomography (PET) with 68Ga-PSMA
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Prostate-specific membrane antigen (PSMA) has become one of the most promising molecular targets in the clinical practice. As there is an increased expression of PSMA on the membrane of prostate cancer (PCa) cells, radiolabeled PSMA inhibitors have been developed to provide PCa imaging and therapy. 68Ga-PSMA positron emission tomography/computed tomography (PET/CT) plays a key role in the majority of clinical setting of PCa, in particular during staging of disease, biochemical recurrence detection and evaluation of castration-resistant PCa patients. It is demonstrated the high sensitivity of 68Ga-PSMA PET/CT in the setting of initial staging and in biochemical recurrence, even in patients with low PSA levels, allowing early detection of disease. 68Ga-PSMA PET/CT is superior to conventional imaging and choline-based PET/CT in the evaluation of biochemical relapse. PET Imaging findings has influenced in the management of these patients impacting on the choice of therapeutic strategy. 68Ga-PSMA PET is useful for monitoring systemic therapy and is mandatory for selecting patients with metastatic PCa who most likely will benefit from PSMA-directed therapy. Positron emission tomography/magnetic resonance (PET/MR) is a new tool for the evaluation of PCa allowing the acquisition of detailed anatomic data in conjunction with molecular information. There is increasing evidence supporting the improved accuracy of 68Ga-PSMA PET/MR for imaging PCa.
Keywords: PSMA, PET/CT, PET/MR

SPBN-09.04 - Radionuclide therapy with 177Lu PSMA for prostate cancer
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Prostate cancer is one of the most common cancer in men with an incidence around 1,5 million new cases worldwide every year. The first-line therapy for metastatic prostate cancer is androgen deprivation therapy(ADT) which may be combined with androgen receptor(AR) based therapy or chemotherapy. However, disease eventually progresses to a castration-resistant form and other strategies must be used in order to improve survival. Theranostic agents that target disease-specific structures in cancer patients has been under investigation for a while. PSMA – prostate-specific membrane antigen was first discovered in the early 90s. This transmembrane protein was found to be highly upregulated in the majority, more than 90%, of prostate carcinoma. Positron emission tomography(PET) combined with computer tomography(CT) or magnetic ressonance imaging(MRI) is essential for better anatomical information and for selection of those who will benefit from the radioligand therapy(RLT). The first studies perfomed with 177Lu-PSMA have shown a profile of safety and therapeutic response with minimal side effects, and improvement in survival and quality of life. It is important to reinforce that the studies have added information to cement the role of PSMA as a successful theranostics for prostate cancer.
Keywords: Lutetium 177, PSMA, PET, radioligand therapy, prostate cancer; ADT

SPBN-10. Molecular imaging in Neurosciences

SPBN-10.01 - Peripheral Nervous System in the War Against Cancer

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The tumour mass is composed not only of heterogeneous neoplastic cells, but also a variety of other components that may affect cancer cells behaviour. The lack of detailed knowledge about all the constituents of the tumour microenvironment restricts the design of effective treatments. Nerves have been reported to contribute to the growth and maintenance of numerous tissues. The roles of peripheral nervous system on tumour growth remain unclear. Here, by using state-of-the-art techniques, including Cre/loxP technologies, confocal microscopy, in vivo-tracing and chemical denervation, we revealed the presence of sensory nerves infiltrating within the melanoma microenvironment, and affecting cancer progression. Strikingly, melanoma growth in vivo was accelerated following genetic ablation or chemical denervation of sensory nerves. In humans, a retrospective analysis of melanoma patients revealed that increased expression of genes related to sensory nerves in tumours was associated with better clinical outcomes. These findings suggest that sensory innervations counteract melanoma progression. The emerging knowledge from this research provides a novel target in the tumour microenvironment for therapeutic benefit in cancer patients. **Keywords:** sensory neurons, tumor microenvironment, transgenic mouse models; **Supported by:** Instituto Serrapilheira, FAPEMIG, CNPq and CAPES

SPBN-10.02 - New trends in PET radiopharmaceuticals for neurological diseases: preclinical research in astrocytocis in Alzheimer Disease

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Neurodegenerative diseases have mainly been associated with neuronal death. Neuroinflammatory changes, characterized by reactive astrocytes and activated microglia, contribute greatly to neurodegeneration throughout the course of Alzheimer's diseases (AD). Reactive astrocytes overexpress monoamine oxidase-B (MAO-B) in the outer mitochondrial membrane. [^{11}C]Deuterodeprenyl is a tracer that has been used for reactive astrocyte detection in AD, Creutzfeldt–Jakob disease and amyotrophic lateral sclerosis, among others, with some limitations. For imaging astrogliosis in the human brain, we developed the novel MAO-B PET tracer named [^{18}F] 2B-SRF101. We reported the synthesis of a sulfonamide derivative of Sulforhodamine 101 (SR101), labeled with 18F, as well as toxicity and prelimininar molecular imaging studies. A preclinical assessment by functional multimodal images and cell cultures in astrocytocis process in AD is being performed. The objectives are: i) to elucidate the cellular specificity of the radiotracer in the CNS, ii) to establish pharmacokinetics parameters and iii) to assess the contribution of multimodal imaging (PET and functional resonance) in the monitoring of neurodegenerative processes in AD. At the same time we are searching for new therapeutic strategies and targets, as well as early diagnosis of AD. In a triple transgenic mice model (3xTg) it was isolated a subtype of astrocytes derived from old 3xTg-AD mice with neurotoxic effects. The generation of 3xTgv astrocytes-derived conditioned medium was achieved, with neurotoxic properties. We have been studying the underlying mechanisms of 3xTgv astrocytes neurotoxicity, their role in the pathogenesis of AD (metabolomic and transcriptomic characterization of 3xTgv astrocytes) and their role in neuroinflammation (study of involved cytokines and inflamatory pathways). Besides that, at the present we have started a longitudinal study using 3xTg-AD mouse model, with a histological, behavioral and imagenological evaluation. We expect to characterize the role of these neurotoxic astrocytes throughout disease progression in the 3xTg-AD model, with the aim of supporting the development of diagnostic and therapeutic approaches for AD. Keywords: astrocytocis, Alzheimer's disease, MAO-B, sulforhodamine 101, 3xTg-AD transgenic mice. Funding: ANII, FMV_3_2020_1_162870

SPBN-10.03 - Temporal and Spatial Changes In Cerebral Blood Flow In Neuropsychiatric Systemic Lupus Erythematosus: A Subtraction Brain Spect Study.

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This lecture will address to discuss the temporal and spatial changes in regional cerebral blood flow (rCBF) of patients with neuropsychiatric systemic lupus erythematosus (NPSLE). We correlated the subtracted SPECT coregistered to MRI features (SISCOM) with demographic, clinical, and laboratory findings to shed light upon the pathophysiological evolution of the NPSLE. Twenty-six NPSLE patients with MRI and pre and post-treatment brain SPECT with [99mTc]Tc-ECD. SISCOM features were categorized as improvement, worsening, activation, and/or deactivation of rCBF findings. Patients' mean age of 43.19 years and 65.38% white were evaluated. The patient's mean age at onset of SLE was 26.05 and 42.29 for NPSLE. The mean time between the onset of SLE and the first NPSLE symptoms was 5.57 years. The disease has already been initiated as NPSLE in 4 patients. The SLEDAI average score was 31.69 and the SLICC/ACR-DI score was 6.96. The patients underwent an average of 9.23 cyclophosphamide. The SISCOM findings showed functional and pathological states on different brain regions. The rCBF changes were not associated with index scores. There was, however, a trend towards an association between lower SLEDAI scores with improvement and higher SLEDAI with worsening in SISCOM. Also, a trend of association between lower SLICC score with improvement, and higher SLICC with worsening. The female gender was predictive of activation and worsening, separately, and deactivation and worsening in a set. Non-white patients were predictive of worsening. The seizure was predictive of deactivation separately, and deactivation and worsening in a set. Finally, normal C3 was a predictor of improvement. We showed dynamic brain changes in NPSLE patients. SISCOM technique showed improved rCBF in some brain areas and worsening, activation and deactivation in anothers. There were associations between rCBF changes and gender, skin color, and complement C3, and association trends with SLEDAI and SLICC scores.

SBPN-11. *Perspectives for innovations and the intersection between research and the health public attention*

SPBN-11.01 - Legal framework, scenarios, and perspectives for technology innovations in Brazil

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Created in 2008, the Entrepreneurial Mobilization for Innovation (MEI), coordinated by the Brazilian National Confederation of Industry (CNI), works to make innovation recognized as indispensable for Brazil to achieve economic growth, competitiveness, and social well-being. With a complete agenda, MEI has become a protagonist in collaboration and engagement among the private, public, and academic sectors, acts in the proposition of public policies for improvement and strengthening of the science, technology, and innovation ecosystem in the country. It is currently the best consolidated private-public dialogue environment in the country, with regular meetings and the participation of 400 business leaders, representatives from the Executive and Legislative powers and from academy for the construction of initiatives and measures to stimulate innovation. Initiatives as immersion program in innovation ecosystems, partnership CNI+SOSA, Brazilian Industry Innovation Summit, National Innovation Award, MEI Working Group, MEI Tools, InforMEI News Letter and many others to contribute to innovation ecosystem. Keywords: Entrepreneurial Mobilization for Innovation (MEI), private-public dialogue, innovation initiatives Funding: CNI

Keywords: Inovação, Mobilização empresarial pela inovação, Politicas publicas
Supported by: Confederação Nacional da Industria - CNI

SPBN-11.03 - Cyclotron Products Certified by a Public Hospital in São Paulo

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In July of 2021, the Cyclotron at University of São Paulo's Hospital das Clínicas Radiology Institute got the approval for three radiopharmaceuticals: Pittsburgh Compound B (PIB-11C), Prostate Specific Membrane Antigen (PSMA-68Ga), and DOTATATE (68Ga). Hospital das Clínicas is a public institution that has a cyclotron and all the facilities required to produce radiopharmaceuticals under all the Good Manufacturing Practices terms of compliance. It does not receive any public funding to maintain its cyclotron facilities, because the main Hospital's goal is to provide health care services, instead of producing pharmaceuticals. Innovation regarding pharmaceuticals at University of São Paulo happens in two ways: 1. the university discovers a new tracer, then it synthesizes the new tracer, validates all procedures, and runs some nonclinical tests; 2. the university carries out a 3-phase clinical trial or clinical research projects. The first way is truly innovative: the university can plan ahead, synthesize the tracer, and run some nonclinical studies, even if these are not all the nonclinical tests recommended by health regulatory agencies. Considering that the tracer has good potential, the product could be transferred to the drug industry in return for royalties, given that the pharma industry does run all nonclinical tests and phases 1, 2, and 3 of clinical trials. The second way is when the pharmaceutical industry or universities abroad have performed the nonclinical studies and some clinical trials. Therefore, the tracer's safety and efficacy have already been proved. The university can then participate in the 3-phase clinical trial as well as carry out other clinical studies. The main challenges for public universities is to figure out how to fully implement innovative processes: where does the funding come from? How do you comply with the legal framework recommended by the regulatory agency? Whose intellectual property is it? The Brazilian Health Regulatory Agency ANVISA approved three new radiopharmaceuticals: PIB (11C), PSMA (68Ga), and DOTATATE (68Ga); many research projects have been developed with them, so Hospital das Clínicas has received funding from São Paulo Research Foundation FAPESP, in order to study other clinical uses.

Keywords: cyclotron, innovation, radiopharmaceuticals. **Supported by:** FAPESP, FINEP

SPBN-11.02 - Growth and strengthening of the private sector in the Brazilian radiopharmaceutical market: the case of R2IBF

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The private sector has only recently been able to present itself to Brazilian Nuclear Medicine and to patients who depend on this technology for diagnosis and treatment. Until mid-2000 there was a federal monopoly in the production of radiopharmaceuticals. With the flexibilization of the monopoly specifically for short half-life radioisotopes, up to 110 minutes, the private sector began, in 2010, to supply FDG (18F), the only radioisotope for PET/CT available in Brazil until 2020. With the entry from the private sector and the implantation of new cyclotron plants in several cities, such as Porto Alegre, Curitiba, Campinas, Sao Jose do Rio Preto, Brasília and Fortaleza, Brazilian Nuclear Medicine grew rapidly from 30 PET/CT in 2010 to about 160 PET/CT currently. The company R2IBF emerged at that time and, in 10 years of existence, became a group of companies with four installed plants and two more in the construction and licensing phase, supplying around 50% of the national demand for radiopharmaceuticals for PETCT. Importantly, R2IBF has been investing heavily in R&D&I which enabled the launch of the new product PSMA1007 (18F) in 2020 and has four more innovative radioisotopes under development and clinical studies in the country. Comparing Brazil to Argentina, there is still a lot to be done in Nuclear Medicine so that Brazilians can have a service, in this technology, close to what is done in that country and, still, very far from what is practiced in the USA. For this, the private initiative should occupy more and more space, supporting the growth of Brazilian Nuclear Medicine. At this symposium, R2IBF presents its plants, products, mentoring program, and R&D&I strategies to serve the Brazilian market of radiopharmaceuticals for PET/CT diagnostics.

Keywords: radiopharmaceuticals, positron tomography, diagnostics

SPBN-11.04 - A public facility for alpha emitters production: the intersection between reaserch and the healph public attention

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The main equipment we have at DIRAD-IEN is a CV-28 cyclotron accelerator. Installed in IEN in 1974 it is capable to produce 24 MeV energy proton beams. This equipment is used in the production of iodine 123. The Instituto Nacional do Câncer - INCA is our principal client. It is evident that our facilities are obsolete and don't meet the requirements of Good Manufacturing Practices. So, we are proposing the installation of a new radiopharmaceutical production plant in IEN, equipped with a new cyclotron accelerator capable of producing proton beams of 30 MeV. In Brazil, only IPEN and IEN are able to produce iodine-123. The main reason is because this production is only possible with the use of accelerators capable of generating protons of energies greater than 20 MeV. It is also remarkable the reduced commercial demand for iodine 123. These two aspects make the interest of private companies in produce it in Brazil less attractive. As iodine 123 has an enormous social application for diagnosis of pediatric diseases, it is very important to have redundancy in its production. To innovate in the production of radiopharmaceuticals in Brazil, it is convenient also to enable the possibility to produce alpha particle beams. The IBA 30XP cyclotron is currently the best choice for that. Capable of generating alpha beams up to 30 MeV, as well as proton beams, it can produce Astatine 211. There are dozens of alpha particle-emitting radionuclides that can be produced with such an accelerator. However, only few fulfill the criteria for nuclear medicine application. Its characteristics make 211-At interesting for research and development of nuclear techniques for therapy and theranostic. The advantages of using 211-At, clinical studies carried out and research possibilities are pointed out, as well as some details of the proposed radiopharmaceutical production plant. **Keywords:** cyclotron, iodine-123, astatine-211

SPBN-12. Synchrotron Radiation in Biology and Medicine

SPBN-12.01 - Synchrotron-based x-ray imaging and microspectroscopy applied to life sciences

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The use of a synchrotron radiation source allows constructing effective x-ray microprobes for studying trace elements in small (nanogram) samples or their distributions with high spatial resolution. The significant increase in source brightness offered by diffraction-limited storage rings, like the new fourth generation Brazilian synchrotron light source - Sirius, translates directly into coherent flux. Therefore, x-ray imaging and spatially resolved spectroscopic techniques are benefit from this main feature, giving rise to an increase of spatial resolution (reaching the nanometer scales), detection limits and staggering improvement in spatial resolution of x-ray images when collecting the coherence diffraction patterns from samples. In my presentation, an overview of synchrotron-based x-ray imaging as well as microspectroscopy techniques such as SR-XRF imaging, XRF tomography, and spatially resolved XANES analysis, will be illustrated with some examples within several branches of life science. A brief introduction to the Coherence X-Ray Nanofocus (CARNAÚBA) beamline will also be given, highlighting the capability for performing x-ray imaging and spectroscopic measurements on samples coming from those fields of science.

Keywords: synchrotron radiation, x-ray imaging, spectroscopy
Supported by: FAPESP, CNPq, CAPES, MCTI

SPBN-12.02 - Manganese as a central element in tumor progression

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Manganese is a key element in cell proliferation and migration, two relevant aspects of tumor progression. Our work investigates the role of manganese in tumor progression in an animal model of tumor growth and in an in vitro cell model. High resolution X-Ray fluorescence analyses revealed that manganese accumulates within primary tumors and secondary organs as manganese-rich niches. Consequences of such phenomenon were investigated in vitro, and we verified that short-term changes in manganese alter cell surface molecules syndecan-1 and β1-integrin, enhance collective cell migration and invasive behavior. Long-term increased levels of manganese do not affect cell growth and viability but enhance cell migration. We also observed that manganese is secreted from tumor cells in extracellular vesicles, rather than in soluble form. Finally, we describe exogenous glycosaminoglycans that counteract manganese effects on tumor cell behavior. In conclusion, our analyses describe manganese as a central element in tumor progression by accumulating in Mn-rich niches in vivo, as well as in vitro, affecting migration and extracellular vesicle secretion in vitro. Manganese accumulation in specific regions of the organism may not be a common ground for all cancers, nevertheless, it represents a new aspect of tumor progression that deserves special attention.

Keywords: manganese, cancer, cell migration, integrins, syndecan
Supported by: FAPERJ, CNPq, CAPES, Fundação do Câncer, IFRJ and LNLS

SPBN-12.03 - Synchrotron X-ray biosample imaging: opportunities and challenges

Gabriela Sena[1,2], Gabriel Fidalgo[1], Geicilene Katrine de Paiva Soares[1], Renan Barcelos[1,2], Liebert Parreiras Nogueira[3], Marcos Vinícius Colaço[1], **Regina Cély Barroso [1]**
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Synchrotron radiation phase-contrast microtomography is often particularly sensitive to high spatial frequency features, giving an alternative view of the sample and being useful for investigating microstructure inside biological specimens, without staining them with a contrast medium. The phase-contrast technique has been widely used in the scientific community, as it is a technique associated with radiography and microscopy and that enhances contrast in soft tissues, specifically at the edges, showing details that could not be seen by the absorption technique. This presentation aims to show the ability of phase-contrast micro-CT for visualization of soft tissues and hard internal structures of millimetre-sized biological organisms. Case studies of the anatomy of *Rhodnius prolixus* head (Sena et al. 2016: 10.1016/j.ejmp.2016.05.051; Sena et al. 2018: 10.1088/1748-0221/13/05/C05007) and *Thoropa miliaris* tadpole (Fidalgo et al. 2018: 10.1088/1748-0221/13/05/C05012; Fidalgo et al. 2020: 10.1038/s41598-020-75993-8) are presented to illustrate the imaging technique. The X-ray phase-contrast microCT scans were performed at the microtomography beamline (IMX) at the Brazilian Synchrotron Light Laboratory (LNLS) and at the SYnchrotron Radiation on MEdical Physics (SYRMEP) beamline of the Elettra Sincrotrone Trieste, Italy.
Keywords: synchrotron radiation, microtomography, phase contrast
Supported by: CNPq and FAPERJ

SPBN-12.04 - Opportunities and challenges for achieving high-resolution in vivo tomographic images in animal systems

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The nature of dynamic processes in animals will be discussed. This is an essentially multidisciplinary field to study events which are hierarchically organized across a wide range of spatial and temporal scales. Although several techniques can be used, in this presentation I will talk about synchrotron applications in experiments to address dynamics in animal systems from cells to tissues and whole small animals. The Sirius micro and nano tomography beamline (MOGNO) is planned to have a permanent in vivo experimental setup for small rodents. We will present the challenges and progress of this project from development of electronics to measuring signals from samples (e.g. electrocardiogram) in real-time, to sample environments and radiation impact. Altogether it should be possible to produce prospective and retrospective X-ray computed tomographic imaging with compatible radiation dose and gain in temporal and spatial resolution compared to pre-clinical equipment.
Keywords: bioimaging, synchrotron tomography, radiation damage
Supported by: FAPESP, CNPq, CAPES, MCTI

SPBN-13. Multidisciplinar education and the employment perspectives

SPBN-13.01 - Multidisciplinary education: the case of the Radiology Technology Course of the Federal University of Minas Gerais

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The School of Medicine of UFMG hosts three undergraduate courses: Medicine, Speech Therapy and Radiology Technology, all of them with a multidisciplinary approach. The Radiology Technology is one of the courses created by REUNI – Support Program for the Restructuring and Expansion Plan of Federal Universities – and its first class joined in 2010, inaugurating UFMG's performance in the field of technological graduations. The graduate student in the Radiology Technology course at UFMG will be able to enter the join market, in addition to taking lato sensu and stricto sensu postgraduate courses. The degree admits 80 students per year, 40 per semestre, with the objective of training professionals able to work in all areas of peaceful use of ionizing radiation. Therefore, this professional will be able to exercise the technical/practical principles, management, implementation and entrepreneurship in radiological diagnostic imaging services and therapy, in addition to industrial radiology services that make use of radiation emitting equipment. The Radiology Technologist will be competence to implement and apply national and international principles of radiological protection in medical and industrial services.The graduation in Radiology Technology requires affinities with the exact areas, anatomy, informatics and health. The course's faculty team is made up of professionals from different areas such as Technologists in Radiology, Physicists, Pharmaceuticals, Computer Scientists, Radiologists and Nuclear Physicians. In specific subjects of the course, these professionals teach together, each contributing their expertise so that the student can associate the physical concepts with biological concepts, facilitating the understanding of the formation and interpretation of medical images. This characteristic gives an important interdisciplinar character to the course. Our graduates have been working in radiotherapy services, imaging diagnosis, as radiation protection supervisors and also inserted in stricto sensu postgraduate courses, increasingly strengthening and highlighting more and more the profession of Technologist in Radiology.

SPBN-13.02 - Radiation Technology in Health Sciences at IPEN: A multidisciplinary and interdisciplinary Professional Master Degree

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The Professional Master Program in Radiation Technology in Health Sciences (MP-TRCS) of the Nuclear and Energy Research Institute- IPEN/CNEN is a new program, started in August 2019. It is the only graduation program in the country to offer two nuclear reactors for educational purposes, for the development of dissertations, in addition to providing radiopharmaceuticals production in a nuclear reactor, in linear accelerator for radioisotope production, as well light and lasers applications. In addition to the infrastructure, the program has multidisciplinary training advisors working in an interdisciplinary manner who use their vast experience in radiation applied to medicine to guide students in a productive manner with a high degree of excellence. The MP-TRCS aims to fulfil a growing demand at IPEN/CNEN from professionals working in hospitals and clinics, using ionizing and non-ionizing radiation. These students need a more dynamic course directed to the practical professional activities. We have students from the most diverse areas, such as medical doctors, biomedical doctors working in clinical analyses, radiotherapy physicists, physiotherapists, dentists specializing in imaging diagnosis and laser, among others, participating in the front line, who use radiation or assess its impact on their day-to-day routine. The first students have already begin to present their dissertation. The employability has increased among students enrolled in the program. These professionals bring their experience to the program, which together with IPEN's academic structure and advisors, result in skilled students who are finding numerous career opportunities in the job market. **Keywords:** Professional Master degree, interdicisplinarity, Radiation. **Supported by:** IPEN/CNEN

SPBN-13.03 - The importance of multidisciplinarity in Biology inserted at UFSM

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Multidisciplinarity is the process of connecting disciplines. Multidisciplinary work enables dialogue between different areas and their concepts, in order to integrate different knowledge and with the aim of giving meaning to them. From these words we can understand the importance of multi and interdisciplinarity at the various levels of education. In order to discuss and open new horizons about the importance of the theme proposed in the title, the personal professional experience of the biologist teacher will be presented in the different areas of science. For this also, a literature review was carried out to support the discussion. The terms used were: teaching, interdisciplinarity, purpose areas, biological sciences and curriculum in English and Spanish. Among these themes, modern biology is linked to multi and interdisciplinarity, which in turn is interconnected with intersection, interaction and these with other domains of knowledge. When the practice of multi and interdisciplinarity results in a break with traditional patterns, leading to the prioritization of knowledge construction in a fragmented way, revealing commonalities and thus favoring critical analyzes regarding the different approaches to the same subject. We can conclude that multi and interdisciplinarity is essential for the growth of knowledge in general.

Keywords: study, discipline, physical and biological sciences
Supported by: CAPES

SPBN-13.04 - Multidisciplinary education in a private university: the University Center of Hermínio Ometto Foundation case

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The University Center of Hermínio Ometto Foundation is maintained by the Hermínio Ometto Foundation, a non-profit foundation that has 23 undergraduate courses, more than 30 specialization courses and two master's degree programs (Biomedical Sciences and Dentistry), being Biomedical Sciences and Biology courses are pioneers at FHO with more than 40 years. About the undergraduate courses, nine are in the health area and six have in their curriculum subjects such as biophysics, physiology and chemistry, in addition to specific subjects such as the Biomedical Sciences course. The specific disciplines are biotechnology, imaging diagnosis, molecular diagnosis, among others. The courses of health area maintained by FHO have preserved the tradition to promotes training of professionals in general field related to insert in job market and also by encouraging scientific research, which results in high rates of admission to postgraduate programs, master's or doctoral degrees programs. Education in private universities has gone through several challenges over the years, including the COVID-19 pandemic period. Educational models in health area should emphasize the importance of basic health disciplines such as chemistry, biophysics and physiology, as well as during post-graduate courses. When thinking about educational context, the training of human resources is a challenge given the heterogeneous characteristics of incoming students and there is a need to keep the same level of our students. Demands related to disciplines that are part of the health area courses need to prepare all students to be homogeneously in academic training and continue to be a challenge in education.

Keywords: education, professional, biomedical sciences
Supported by: PROPESQ-FHO

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AA - Biomimetic systems and membrane biophysics

AA.01 - Effects of phase state and charge on membrane fusion using a novel fusogenic system composition

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Cell membranes are believed to be heterogeneous and to exhibit lipid domains (or rafts) of coexisting phases, which can govern cellular functions and processes. As previously reported by us, membrane fusion efficiency was modulated by controlling membrane charge and phase state in giant unilamellar vesicles (GUVs, 10-50 μm) used as biomimetic membranes. In this work, we proposed a new composition of the fusogenic system preferentially in the gel phase at room temperature but fluid in physiological temperatures; additionally, membrane fusion efficiency was also investigated. The fusogenic system was based on large unilamellar vesicles (LUVs, 100-150 nm) and composed of an equimolar mixture of neutral and positively charged gel lipids, whereas the GUVs were composed of a mixture of zwitterionic and/or negatively charged fluid lipids. Initially, the fusogenic system was characterized using dynamic light scattering (DLS), zeta potential, anisotropy and differential scanning calorimetry (DSC). Then, fusion efficiency was characterized using microscopy-based fluorescence assays (lipid and content mixing). The DLS and zeta potential measurements exhibited a size average of ≈ 120 nm and a surface charge of ≈ 42 mV, respectively. The anisotropy and DSC showed that the lipid mixture was in the gel phase at room temperature. The incubation of the fusogenic system with neutral GUVs did not lead to significant lipid mixing and therefore no/low membrane fusion. However, negatively charged GUVs exhibited high lipid and content mixing, as expected in systems with efficient membrane fusion (Lira et al, in preparation). Interestingly, the highly membrane fusion efficiency induced phase-separation in the GUVs. We demonstrated that membrane fusion can be controlled by modulating the membrane charge of the system in spite of the membrane phase state of the fusogenic system. These findings can play an important role in regulating the interaction between cells and liposomes used in drug delivery systems.
Keywords: fusogenic liposomes, biomimetic membranes, phase separation
Supported by: FAPESP

AA.02 - Reactivity and pH mapping with Catalytic Peptides

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The sulfhydryl group (-SH) of cysteine (Cys) is essential for the activity of several enzymes, and its apparent p K_a determines the reactivity. Amphiphilic aggregates are useful models for studying chemical reactivity in dimensionally restricted environments, such as enzyme´s active sites. The acid dissociation, and the ensuing reactivity, of weak acids, such as RSH is determined by the local proton/hydroxide (H^+/OH^-) distribution. Characterize H^+/OH^- radial distribution in vesicles and micelles as a function of the distance from the surface using the following Cys-containing peptides: Hexadecyl-Arg-(Gly)n-Cys-His-NH_2 and Hexadecyl-Lys-(Gly)n-Cys-NH_2, where **n** is the number of glycine residues. The pKap of -SH groups and rate constants (kobs) of the thiolysis of p-nitrophenyl-octanoate (NPO), in micelles of N,N,N-trimethyl-N-hexadecyl ammonium chloride (CTAC), and N,N-dimethyl-N,N-dihexadecylammonium chloride (DHDAC), vesicles were determined spectrophotometrically. The p Ka of -SH in water was 8.9 for all peptides; in 20 mM CTAC the values were 7.5, 7.8 and 8.3, for n = 3, 6 or 9 Gly, respectively. The p Kap change, as a function of n (1/2/3/4/5/6 or 9) shows that the SH groups are located at different distances from the interface. The kobs of NPO thiolysis by the same peptide series decreased with Gly content. The kobs were 18 times higher in DHDAC and 10 times higher in CTAC at pH 6, for probes with one Gly, compared to the reaction in water at pH 10. The cationic aggregates surfaces have high OH^- concentration, a microenvironment in which the sulfhydryl is deprotonated, thus more available for the reaction with NPO. The kobs were not significantly different between probes containing histidine. These results will lead to the first experimental mapping of the H^+/OH^- distribution on the aggregate's surfaces as a function of distance. **Keywords**: micelle, pH probe, catalytic peptides

AA.03 - Solubilization of Biomimetic Membranes by Detergents with Different Physical-Chemical Characteristics

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Detergents are used to solubilize biomembranes and extract their components. Previously, our group has shown that, in giant unilamellar vesicles (GUVs) of ternary biomimetic compositions, the detergent Triton X-100 promotes Lo/Ld phase separation followed by solubilization of the Ld phase only, while Lo phase remains unimpaired. The present work extends the study to detergents with different physical-chemical characteristics: Triton X-165, Dodecyl Maltoside (DDM), C10E5, Octyl Glucopyranoside (OG), Tween 20, CTAB, SDS and Chaps. The membrane compositions studied were Ld phase (pure POPC), Lo phase (SM:cholesterol 7:3), and the ternary mixture POPC:SM:cholesterol 2:1:2. We aim to study different composition vesicles solubilization by different detergents and analyze their interactions. The solubilization profile of each detergent was followed by turbidity measurements on large unilamellar vesicles (LUVs). Then, optical microscopy was used to observe GUVs in the presence of the detergents. Except for CTAB and SDS, all detergents could completely solubilize vesicles in the Ld phase. On the other hand, Lo vesicles were completely or partially insoluble to all detergents. Two different interactions with Ld phase were determined: increase in GUV surface area and turbidity before solubilization (C10E5, TX165, Tween 20 and OG) and vesicle rupturing/bursting (DDM, Chaps, CTAB and SDS) that pairs with no increase on solubilization profile acquired by turbidity measurements. Detergents that promote increase in GUV surface area and turbidity before solubilization apparently can insert on Ld phase and make flip-flop across the membrane. The other detergents are not able to make flip-flop, so their insertion on Ld phase causes tension on the membrane curvature, which is alleviate by bursting. This might be due to polar head size and charge. **Keywords:** Biomimetic Membrane, Detergents, Solubilization. **Supported by:** CNPq

AA.04 - A biomimetic device combining microfluidics with nanotechnology allows studying the adhesion of erythrocytes to blood vessels.

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Erythrocytes under pathological conditions undergo eryptosis, a process characterized by biochemical and morphological changes such as phosphatidylserine (PS) exposure to the plasma membrane external layer. Eryptotic erythrocytes adhere to the endothelial cells (ECs), which may be important in the pathology of bacterial infections and congenital diseases like sickle cell disease. Externalized PS can bind to receptors expressed on ECs under pathological conditions. To understand the adhesion mechanism, we designed a device combining microfluidics with nanostructured surfaces to mimic the capillary architecture and the expression of adhesive molecules by the activated ECs. Microfluidic chips were prepared in PDMS using molds fabricated by photolithography. Nanostructured surfaces were synthesized by block copolymer lithography and consisted of a glass surface covered with 7 nm diameter gold nanoparticles (AuNPs), arranged in a quasi-hexagonal array. The microfluidic chip was adhered to the nanostructured surface by an O_2 plasma treatment. The AuNPs were functionalized with a polyethylene glycol chain (PEG) that binds to the AuNPs by a thiol at one end and has a nitriloacetic group (NTA) at the other end. The NTA binds proteins expressing a His-Tag. The surface not occupied by AuNPs was covered with PLL-g-PEG. We corroborated the specific AuNPs functionalization by quartz microbalance and fluorescence microscopy using a GFP-His Tag. Then, we studied the erythrocytes adhesion to a device functionalized with Annexin V-His Tag at different flows. Two conditions that promote eryptosis, incubation at 50° C or treatment with ionomycin, significantly increased the adhesion of erythrocytes at flows up to 1.5 dyn/cm^2, in comparison with untreated erythrocytes. However, eryptotic erythrocytes also showed adherence to a device functionalized with a PEG lacking NTA groups. This indicates that erythrocytes adhesion may not be mediated only by PS receptors. Future experiments will test the erythrocytes adhesion elicited by proteins usually expressed by the activated ECs.
Keywords: endothelium, nanostructured, biomimetic. **Supported by:** FONCYT (PICT-2019 03218), CONICET, The Company of Biologists (travel grant), UBACYT 20820160401330BA.

AA.05 - From Langmuir monolayers to miniemulsions: an approach to understand the liquid/liquid interfaces

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Lipid miniemulsions (ME) are oil in water (O/W) dispersions stabilized by an interfacial layer of a surfactant and are systems commonly used to encapsulate, maintain, and release molecules of pharmacological interest. In this context, Langmuir monomolecular films at the liquid-liquid interface can be used as experimental models to investigate the dynamic behavior of surfactants at the oil/water (O/W) interface in ME. We have used this technique to characterize the composition and thermal behavior of monomolecular layers of L-α-phosphatidylcholine (EPC) and 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) at the Vaseline/water interface (VAS/W) by using a homemade Langmuir interfacial trough. Besides, we have studied the interfacial behavior, at the air/water interface (A/W), of VAS/DPPC pseudo-binary mixtures with different VAS/PC molar ratios. This combined analysis was carried out with the aim of theoretically predict and design stable formulations of ME. From our study of adsorption isotherms of DPPC at the VAS/W interface, we obtained the saturation equilibrium pressure of DPPC (πeq, sat $\sim$ 27.3 $\pm$ 0.1 mN / m), which could be interpolated in the π-MMA isotherm at the VAS/W interface to calculate the minimum area of DPPC (MMAmin) corresponding to the equilibrium pressure of DPPC at this interface. With the aid of geometric calculations, the MMAmin could be used as a starting point to estimate a suitable DPPC:VAS ratio to formulate stable miniemulsions particles according to the desired size and concentration. Controlling the VAS:DPPC molar ratio, the theoretical predictions made from the Langmuir film model were satisfactory for the synthesis of particles with average diameters in the range of 100 nm to 400 nm and with low DPPC concentrations (0.01mM) in the global system. The ME obtained were characterized by DLS and were stable at least for one month in suspension. **Keywords:** Langmuir films, nanoemulsions, liquid/liquid interface. **Supported by:** CONICET, FONCYT, SeCyT-UNC.

AA.06 - Impact of macromolecular crowding on the mesomorphic behaviour of lipid self-assemblies

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We established that intracellular water dynamics in yeast displaying oscillatory glycolysis, is coupled to, and oscillates synchronously with, the concentration of ATP. These cytosolic water oscillations propagate to the membrane interface. According to the Association Induction Hypothesis (AIH), this phenomenon involves changes in the structure of intracellular proteins. We hypothesize that metabolic changes may regulate mesomorphic changes in lipid self-assemblies via changes in the activity of intracellular water. We attempt to test *in vitro*, whether structural features of polymers may influence the structure of lipid self-assemblies via changes in water activity. We used LAURDAN fluorescence and Raman measurements. In our experiments the polymers are not in direct molecular contact with the membranes (using a dialysis bag). Water dynamics seeing by LAURDAN in DOPC bilayers is differentially regulated by the presence of crowded suspensions of different proteins (HSA, IgG, Gelatin) and PEG. Specifically, we found that the extent of water dynamics correlates with an increased fraction of randomly oriented configurations in the polymers, as Gelatin>PEG>IgG>HSA. Also we found that structural transitions from globular to extended conformations in proteins induced lamellar to non-lamellar phase transitions in mixtures of DOPC and monoolein. In addition, Raman experiments show that high proportions of randomly oriented conformations display increased fractions of tetracoordinated water, a configuration that is dominant in ice. This indicates a greater capacity of this extended polymers for polarizing water and consequently reducing its chemical activity. This effect is in line with the tenets of the AIH, which predicts a long-range dynamic structuring of water molecules via their interactions with proteins showing extended conformations. Our results suggest a crucial role of water in promoting couplings between structural changes in macromolecules and supramolecular arrangements of lipids. This mechanism may be of relevance to cell structure/function when the crowded nature of the intracellular milieu is considered. **Keywords:** lipid polymorphism, macromolecular crowding, membrane hydration

AA.07 - Micellar catalysis of the reaction of 4-nitro-naphthalimides with thiols. Quantitative analysis and analytical applications in biology

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Cysteine (Cys), a reactive amino acid, is essential in proteases, phosphatases, in peptides such as glutathione (GSH) and may also maintain protein structures through disulfide bonds. Thiols react with 4-Nitro-Naphthalimides displacing the nitro group and producing fluorescent compounds. Micelles can accelerate different reactions and are a convenient alternative to organic solvents for the solubilization of hydrophobic compounds. Here we describe the aromatic nucleophilic substitution reaction of the -NO2 group of 4-nitro-N-ethylene-N, N´-dimethyl, N´´-hexadecyl ammonium-1,8-naphthalimide bromide, 4-nitro-NEHN, and 4-nitro-n-butyl-naphthalimide, 4-nitro-NBN, by Cys, GSH, hexadecyl-cysteinamide (HCys), and the enzyme peroxiredoxin 2, PRDX2, in the presence of N, N, N-trimethyl-N-hexadecyl ammonium chloride, CTAC, micelles and analyze the effect of micelles on the reaction rates (kψ) and the dissociation constant of the thiols (pKa). Reaction rates, quantification of SH groups and pKas were determined by UV and fluorescence. The pKa of all thiols decreased as a function of [CTAC] to a minimum and increased at higher detergent concentration. CTAC micelles accelerated all reactions to a maximum in kψ. Micellar rate enhancement reached ca. 2.5×10^{7} for the reaction of HCys with 4-nitro-NEHN at pH 5.5 and 2.5×10^{6} with 4-NBN at the same pH. The main factors leading to the observed effects were pKas changes, substrates concentrations, and changes in the intrinsic micellar rate constant (km). Product fluorescence allowed SH´s quantification. The thiol detection limit was 1×10^{-6} M at pH 7.4. The addition of H2O2 did not change the fluorescence intensity. Micelles of CTAC strongly catalyzed the reaction of thiols and 4-nitro-naphthalimides. 4-nitro-NBN and 4-nitro-NEHN are excellent probes for thiol quantification, and the products are stable in oxidant conditions. **Keywords:** 4-Nitro-naphthalimides, catalysis, micelles.
Supported by: FAPESP, CNPq, INCT-FCx, NAP-FCx

AA.08 - Topographic Analysis of Annexin A6-Proteoliposomes by Atomic Force Microscopy

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The liposomes are the most used membrane mimetic model. They have been developed to facilitate the study of the physicochemical properties of biological membranes and the interaction between lipids and proteins. Annexin A6 (AnxA6) is a protein found in three distinct regions of the matrix vesicle (MVs) membrane. These vesicles provide a suitable microenvironment for mediation of bone mineralization. The present study aimed to evaluate whether AnxA6 interacts with liposomes composed by lipids present in MVs´ membrane- (dipalmitoylphosphatidylcholine (DPPC), dipalmitoylphosphatidylserine (DPPS) and cholesterol (Chol)), using atomic force microscopy (AFM). For this, liposomes (10 mg. mL^{-1}) and proteoliposomes harboring AnxA6 (1:100 protein:lipid molar ratio) composed of pure DPPC and 5:4:1 (molar ratios) DPPC:Chol:DPPS were prepared. Liposomes and proteoliposomes were fixed using glutaraldehyde, dropped onto the surface of freshly cleaved mica and dried at room temperature. Measurements were performed a Shimadzu SPM-9600 Scanning Probe Microscope operating in tapping phase mode. Liposomes composed of DPPC and 5:4:1 DPPC:Chol:DPPS were identified as spherical particles with uniform size distribution and a smooth and homogeneous surface without any evidence phase segregation. Conversely, proteoliposomes harboring AnxA6 showed surface irregularities formed by protrusions that appear to agglomerate at various sites. The AFM topographic cross sections revealed that these protrusions were 21.37±4.83 nm wide and 1.15±0.41 nm height (N = 20) for DPPC proteoliposomes, whereas they were 26.97±7.62 nm wide and 2.01±0.89 nm tall (N=22) for DPPC:Chol:DPPS (5:4:1) proteoliposomes. This finding is consistent with the size of AnxA6 dimers or trimers assigned to the V-shaped AnxA6 with two flexible 6 nm-in-length loops. AFM images showed protrusions that suggest dimeric or trimeric AnxA6 domains on the surface of proteoliposomes, revealing that AnxA6 may adopt different conformations upon interaction with the lipid membrane. **Keywords:** Annexin A6, proteoliposome, atomic force microscopy. **Supported by:** CAPES

AA.09 - Matrix vesicle biomimetics carrying Annexin A5 and Alkaline Phosphatase bind to native collagen produced by human smooth muscle cell transdifferentiated in osteo/chondrocyte cells.

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Vascular smooth muscle cells (VSMCs) transdifferentiated ectopically trigger vascular calcifications, contributing to clinical cardiovascular disease in the aging population. AnxA5 and TNAP play a crucial role in (patho)physiological mineralization. The goal is study the affinity of proteoliposomes harboring AnxA5 and/or TNAP and different types of collagen matrix to simulate the MVs function during the ectopic calcification conditions. We performed affinity studies between DPPC and 9:1 DPPC:DPPS-proteoliposomes carrying AnxA5 and/or TNAP and different types of collagen matrix: type I, II, I+III and native collagenous extracellular matrix (ECM) produced from VSMCs with or without differentiation. AnxA5-proteoliposomes had the highest affinity for collagens, specially for type II. TNAP-proteoliposomes bound poorly and the simultaneous presence of TNAP in the AnxA5-proteoliposomes disturbed interactions between AnxA5 and collagen. DPPC proteoliposomes-AnxA5 affinities for ECM from transdifferentiating cells went up 2-fold compared to that from native VSMCs. The affinities of DPPC:DPPS-proteoliposomes were high for ECM from VSMCs with or without differentiation, underscoring a synergistic effect between AnxA5 and DPPS. Co-localization studies uncovered binding of proteoliposomes harboring AnxA5 or TNAP+AnxA5 to various regions of the ECM, not limited to type II collagen. AnxA5-proteoliposomes showed highest affinities for type II collagen, deposited during chondrocyte mineralization in joint cartilage. In all, TNAP in the lipid/protein microenvironment disturbs interactions between AnxA5 and collagen. These findings arise the hypothesis that TNAP would be cleaved from the MVs membrane just before ECM binding, that such will facilitate MVs anchoring to ECM via AnxA5 interaction. Proteoliposomes as MVs biomimetics are useful in the understanding of mechanisms that regulate the process and essential for the development of novel therapeutic strategies to prevent or inhibit ectopic mineralization. Acknowledgments: FAPESP, CNPq and CAPES. **Keywords:** proteoliposomes, collagen, matrix vesicles

AA.10 - A plug-and-play microfluidic device for efficient generation of monodisperse giant unilamellar vesicles

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Giant unilamellar vesicles, or GUVs, are cell-sized compartments widely used in biological and chemical applications. The monodispersity of GUVs is essential in terms of the quantitative analysis and the experimental reproducibility as biomimetic microreactors. Here we established a highly reproducible plug-and-play microfluidic-based method to generate the monodisperse GUVs without surface treatment[1] of the channel or precise flow-rate adjustment[2]. In this technique, the water-in-oil (W/O) droplets were generated in the microfluidic channel by hydrodynamic flow focusing and transferred across the W-O interface to form thin-shelled water-in-oil-in-water (W/O/W) droplets as a precursor of GUVs. The success rate of the W/O droplets transferring reached nearly 100% by stabilizing the co-flow of oil and water as well as adjusting the curvature of the interface. Owing to interfacial energy minimization, the thin oil layer of the W/O/W droplets was accumulated and detached to form GUVs. We performed a membrane protein insertion assay to confirm the unilamellarity of the GUVs and shrinkage of the GUVs caused by osmotic pressure. The present device can be operated right after bonding the monolithically replicated PDMS channel and plugging tubes. The stable state of the W/O/W droplet generation can be obtained within a short time (1–2 min) so that the amount of inner solution can be reduced down to ~20 µl. GUVs generated by the dewetting of the excess oil can be used as a unilamllar biological compartment as a cell mimic, and the present technology is especially useful to conduct rare or expensive reactions. We believe that this method will become one of the major platforms for artificial cell studies and quantitative biochemical studies which involve the lipid membrane. References [1] Deshpande, S. et. al., Nat. Commun. 2016, 7, 1–9. [2] Deng, N. N. Et al., J. Am. Chem. Soc. 2016, 138 (24), 7584–7591. **Keywords:** microfluidics, monodisperse GUVs, bioreactor. **Supported by:** JSPS grant

AA.11 - Effects of insecticide acephate on lipid monolayers and bilayers

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Due the large production of agricultural products in Brazil, the use of pesticides, such as herbicides, fungicides and insecticides, is highly widespread in the country. The irregular use of these chemicals (above the maximum limit or used in crops other than the allowed) is a concern, once they can be harmful to the environment and human health. Acephate is an insecticide of the organophosphorus family and it is the most irregularly used in Brazil. Once the main effect of this pesticide on insects and mammals is the inhibition of the enzyme acetylcholinesterase, it was chosen in order to assess its effects on lipid monolayers and bilayers as mimetic systems of the cell membrane. Three types of mimetic system were chosen: the Langmuir films (monolayers, analyzed by π-A isotherms), formed by DPPC (zwitterionic lipid), DODAB or DPTAP (cationic lipids); the giant unilamellar vesicles (GUVs - bilayers, analyzed by phase contrast microscopy), formed by hydration of DPPC films and hydration followed by vortex of DODAB or DPTAP films; and the large unilamellar vesicles (LUVs - bilayers, characterized by dynamic light scattering (DLS) and zeta potential), formed by the extrusion of the DPPC, DODAB and DPTAP multilamellar vesicles previously provided. The Langmuir films show that the acephate affects the cationic monolayers (DODAB and DPTAP) in concentration equal to 10^{-4} M, while the GUVs did not show any morphological effects in the presence of acephate at concentration of 0.8×10^{-4} M. On the other hand, the cationic LUVs in the presence of acephate at concentrations of 0, 0.1, 10, 25, 50, 75 and 100 μM show an increasing on their diameter and a diminishing of the superficial charge, mainly at the higher concentrations. Although the pesticide did not change the GUVs morphology, the results indicate that the acephate affects preferably the cationic monolayers and bilayers (LUVs).

Keywords: acephate, vesicles, lipids
Supported by: FAPESP (2018/01984-8), CNPq, CAPES

AA.12 - Comparative study of the native and mutant TNAP in *in vitro* biomineralization

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Osteoblasts are cells responsible for the bone biomineralization in a process mediated by the release of matrix vesicles (MVs). The MV´s membrane are enriched in Tissue Non-specific Alkaline Phosphatase (TNAP) when compared to the plasma membrane. TNAP is a phosphomonohydrolase enzyme able to produce inorganic phosphate (Pi) through PPi or ATP hydrolysis, thus initiating biomineralization. We also measured the ability of native and mutant TNAP either in solution or anchored to DMPC-liposomes, in propagating mineralization *in vitro*. In the present study, specific amino acids at positions Pro-244, Pro-307 or Ala-420 in native TNAP were replaced by cysteines for spin labeling and subsequent ESR analyses. Since TNAP is inserted in the membrane through a GPI anchor, we evaluated the influence of lipid microenvironments, which could cause conformational and functional changes in the properties of TNAP . The ability of the proteoliposomes to induce biomineralization *in vitro* was assessed by turbidimetry changes at 340nm/μg TNAP. Circular dichroism was used to confirm the structure of the mutants while kinetic studies were carried out to characterize phosphate production. We observed that *in vitro* mineral propagation for the TNAP-244 mutant and for the native TNAP was higher both in solution and into proteoliposomes. This finding agreed with the increase in the values of kcat/K0.5 (higher for TNAP in solution) previously obtained. FTIR results revealed changes in the intensity of the PO4-3/C=0 bands similar to apatite crystals for proteoliposomes containing TNAP-244 and TNAP-420. Unfortunately, ESR signals were of low intensity hindering any further analysis. However, with the aid of other techniques, we proved that the mutants with cysteines farther from the catalytic site and from the GPI anchor yielded higher mineralization propagation and higher catalytic efficiency for ATP hydrolysis. Finally, the lipid bilayer present in the proteolipossome were able to enhance *in vitro* mineral propagation.

Keywords: TNAP, Proteoliposomes, Matrix vesicles
Supported by: FAPESP (2016/21236-0, 2018/12092-0, 2019/08568-2), CNPq and CAPES.

AA.13 - Localization of Annexin A6 in Matrix Vesicles During Physiological Mineralization

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Annexin A6 (AnxA6, ~68 kDa) is the largest member of the annexin family of proteins present in matrix vesicles (MVs). MVs serve as nucleation sites for crystal deposition during physiological mineralization. Biochemical analyses revealed that AnxA6 is present in three distinct regions of the MV membrane. The first, corresponds to Ca^{2+}-bound AnxA6 interacting with the inner leaflet of MV membrane, the second, is AnxA6 localized on the surface of the outer leaflet of MV membranes, and the third, is AnxA6 inserted in the membrane's hydrophobic bilayer and co-localized with cholesterol. Here, we assess the localization of AnxA6 in the MV membrane using native MVs and MVs biomimetics. Using monolayers and proteoliposomes composed of either dipalmitoylphosphatidylcholine (DPPC) to mimic the outer leaflet of the MV bilayer or a 9:1 DPPC:dipalmitoylphosphatidylserine (DPPS) mixture to mimic the inner leaflet, we confirmed that AnxA6 interacts differently with MV membranes in agreement with the biochemical data. Thermodynamic analysis based on the measurement of the surface pressure exclusion, enthalpy and phase transition cooperativity (Δt1/2) showed that AnxA6 interacts with both the lipid models and that this interaction increases in the presence of cholesterol. The selective recruitment of AnxA6 by cholesterol molecules was observed in MVs as probed by the addition of methyl-β-cyclodextrin (MβCD). AnxA6-lipid interaction was Ca^{2+}-dependent as evidenced by the greater increase in surface pressure in negatively charged 9:1 DPPC:DPPS monolayers and a larger decrease in enthalpy in 9:1 DPPC:DPPS proteoliposomes caused by the addition of AnxA6 in presence of Ca^{2+} compared to zwitterionic bilayers composed of DPPC. We conclude that the different localizations and ways of interaction of AnxA6 with the lipid membrane suggest distinct functions in MV during biomineralization.

Keywords: Annexin A6, Proteoliposomes, Biomineralization

Supported by: FAPESP, CNPq and CAPES

AA.14 - Modulating membrane shape and mechanics by light

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Over the years, light has been widely used as an effective trigger in biotechnology to interrogate biological systems and provide a conditional control over the complex cellular processes. Of particular advantage are the reversibility, physiological compatibility and high spatiotemporal precision of photo-induced processes. Understanding the fundamental biophysics of light-triggered changes in bio-systems is crucial for cell viability and optimization of clinical applications of light-induced processes in optogenetics and photopharmacology. Here, we employ cell-sized giant unilamellar vesicles (GUVs) to investigate light-triggered changes on the material properties of membranes doped with azobenzene-phosphatidylcholine (azo-PC) as a photoswitch. In particular, we examined light-triggered vesicle area change, reversibility and kinetics of photoswitching and membrane bending rigidity. We show that light can be used to manipulate the shape and mechanics of these synthetic cells. Light-induced changes in vesicle area were quantified by employing vesicle electro-deformation. Our studies demonstrated membrane area increase due to cis photoisomerization of azo-PC which is consistent with results from molecular dynamics simulations. At high azo-PC fractions (>50 mol%), large and complex shape transformations were observed. Photoswitching was reversible but showed faster kinetics from cis to trans isomerization. Trans-to-cis isomerization of azo-PC rendered the membrane softer. With increasing fractions of trans azo-PC the GUV membrane stiffens, while increasing cis-azo-PC fraction softens the membrane. Our results demonstrate that membrane mechanics could be easily controlled for azo-PC containing membranes through light.

Keywords: biomembranes, biophysics, photoswitchable lipids

AA.15 - Self-organization of protocell accompanied by micro phase-segregation in a crowding solution

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Living cells on the Earth maintain their lives through the self-organization of their structure under the crowding conditions of biopolymers on the order of 30 - 40 weight%. However, the underlying physico-chemical mechanism why and how lives utilize the crowding condition still remains as a matter of unsolved problem. In this study, we aimed to unveil the fundamental problem how intracellular order, stability and functions are controlled by creating protocells under crowding environment. We report self-generation of a cell-like structure for water/water microdroplets in a crowding polymer solution. When the aqueous solution of poly(ethylene glycol) (PEG) and dextran (DEX) is mixed in the two-phase region near the binodal line, phase separation occurs and microdroplets of several 10 - 100 μm are formed. We found that long DNA (λ-DNA 49kbp) are entrapped spontaneously inside the droplets. In addition, we report the generation of a cell-sized droplet covered with phospholipid membrane, containing DNA molecules in a self-organized manner. We evaluated the stability of the droplets by experimenting with the time variation of the droplets. Compared to the control droplets, the droplets with DNA and lipid membranes were found to be more stable. Such experimental observations indicate that the droplets covered by lipid are rather stable over several hours, suppressing the fusion between the droplets. Based on these experimental observations on the spontaneous formation of stable cell-like structure, we propose a novel working hypothesis on the generation of primitive cell on the Earth.

Keywords: aqueous two phase separation, cell model, self-organization

AA.16 - Lipid hydroperoxide impacts in the lipid bilayer structure and decreases the bending modulus

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It is well known that oxidized lipids play an important role in diseases and cell death. Here, we studied how the presence of a lipid hydroperoxide derived from POPC, the lipid POPC-OOH, impacts in the lipid bilayer by coupling advanced fluorescence techniques and small-angle X-ray scattering (SAXS). SAXS data analysis pointed out the decrease of lipid bilayer bending modulus, which increased the swelling between different stacked bilayers. Furthermore, we used the fluorescent probes TMA-DPH and Laurdan to investigate the impact of the POPC-OOH in the apolar/polar interface. The TMA-DPH time resolved fluorescence analysis showed a decrease in the mean fluorescence lifetime in function of the hydroperoxide concentration, revealing a higher content of water molecules at the membrane interface. Also, there was an increase of the microviscosity in the TMA-DPH vicinity. Laurdan relaxation process in pure POPC-OOH membranes indicated a higher viscosity and hydration near the -OOH group. In conclusion, our results reveal that POPC-OOH alters the membrane order, increase hydration at the membrane interface, increase the microviscosity and decrease the bending rigidity.

Keywords: biophysics, oxidized lipid, x-ray scattering
Supported by: CNPq, CAPES, Ciências sem Fronteiras - CAPES, FAPESP, FCT

AA.17 - Can inhibitors of ATPase/Alkaline Phosphatase modulate the activity of Matrix Vesicles released by Chondrocytes?

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Na,K-ATPase(NKA) is physiologic essential for many cells and found in appreciable concentration in the matrix vesicles(MV) membrane[1]. We hypothesized that the NKA-membrane orientation may be used to understand the process of MVs biogenesis. Therefore, rightside/inside-out orientations could reveal whether the vesicles were protruded from the apical cell membrane or if first invaginated and later released and if NKA can be a backup mineralization pathway. Determine MV and NKA-proteoliposomes activity and ability to mineralize *in vitro* in the presence of ATPase inhibitors. MV released from growth-plate femurs chondrocytes of chicken embryos by collagenase were isolated[2]. Turbidity changes of synthetic cartilage lymph(SCL), 31P-NMR and colorimetric techniques along with inhibitors were used to estimate MV activity and mineralization. Precipitates analyzed by FTIR. DPPC and DPPC:DPPE(1:1w/w) proteoliposomes prepared by co-solubilization[3]. MV of 212nm diameter were used[2] for ^{31} P-NMR and colorimetric techniques estimated the MV activity in the presence of ouabain is ±95%, Levamisole and SBI-450 were more effective because they inhibit TNAP, which is the main phosphatase in MV. The proteoliposomes lipidic compositions are known to distribute different positions of NKA into the membranes. AFM images revealed the formation of protrusions related to the NKA insertion into the liposomes, associated with the height dimensions of the (αβ)-unit orientation[3]. The α-subunit domain, containing the active site of NKA, has a 4nm height, while the β-subunit domain is 8nm high. The height of the protrusions found in DPPC-NKA and DPPC:DPPE-NKA proteoliposomes were 2.1 and 0.5nm, respectively, revealing the exposure of ATP binding site outwards for DPPC:DPPE-NKA. The DPPC-NKA-proteoliposomes preserved activity was 61%, DPPC:DPPE-NKA 91%. Proteoliposomes with reconstituted NKA achieved mineral propagation by ATP hydrolysis and may be helpful to probe the mechanism of MVs mineral propagation. [1]Thouverey et al.(2011) J. Proteomics,74:1123. [2]Buchet et al.(2013) Methods Mol. Biol.1053:115. [3]Sebinelli et al.(2019) Soft matter,15:2737. Funding: FAPESP, CNPq and CAPES. **Keywords:** Na,K-ATPase, Matrix vesicles, Inhibitors

AA.18 - Obtaining, characterizing and reconstituting the salmonella enterica typhimurium PgtE protease in liposomes: a study of the mechanism of resistance to antimicrobial peptides

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Multi-resistant bacteria represent one of the most prominent public health problems. Bacteria use to produce proteases to inactivate the host's defense proteins, including antimicrobial peptides. PgtE is an aspartyl proteases present in the outer membrane of Salmonella spp. enterica tiphymurium that cleaves antimicrobial peptides rich in arginine. Obtaining of the recombinant protein PgtE in its soluble and active form to reincorporate it in liposomes to study the role of the lipid composition in PgtE insertion into lipid bilayer. The recombinant PgtE was obtained by cloning its gene from the DNA of the S. tiphy 5535 strain using the vector pET 28a (+) in *E. coli* BL21 (DE3). To obtain soluble and active form of the protein, the fractions of bacterial membrane were incubated with different detergents and later ultracentrifuged. The solubilization yield was evaluated by SDS-PAGE, protein dosage and the proteolytic activity in the supernatant. Our results indicate a good level of PgtE expression and a better recovery of the enzyme in its active form using the CHAPS detergent. The solubilized and active PgtE was reincorporated into DPPC or DPPC: DPPG 1: 1 liposomes using the co-solubilization method. The incorporation of the protein in the membrane was evaluated by differential scanning calorimetry (DSC), protein and lipid dosage in the liposomes and assays of proteolytic activity using the FRET method. The DSC tests showed a significant change in the profile of the membrane lipids phase transition thermogram including relevant changes in the ΔH and in the cooperativity of the lipids of proteoliposomes of DPPC: DPPG. In addition, the kinetic assays of PgtE in DPPC:DPPG membranes showed higher proteolytic activity. The presence of the negative charge on the membrane is an important factor in modulating PgtE insertion in the lipid bilayer. **Keywords:** antimicrobial resistance, proteases, proteoliposome

AA.19 - Effect of Annexin A5 in the interaction between DPPS-enriched membranes and Ca^{2+}

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The mineralization of bone is a process driven by matrix vesicles (MVs), which produce and release the first mineral particles into the collagenous extracellular matrix. Annexin A5 (AnxA5) is believed to play a crucial role in the mineral nucleation inside MVs influenced by both calcium and DPPS. We aimed to evaluate the stability of membranes composed of DPPC and DPPS in a medium containing Ca^{2+} ions, as well as the role of AnxA5 in this phenomenon and its implication for the biomineralization process. We assessed the effect of Ca^{2+} ions on the organization of membranes constituted by pure DPPC, 10%DPPS, 20%DPPS and pure DPPS (numbers depict molar percentage) formed both as bilayers (liposomes, characterized by differential scanning calometry (DSC) and dynamic light scattering (DLS) and monolayers (Langmuir monolayers, characterized by π-A isotherms and fluorescence microscopy). Our data revealed that calcium ions in the concentration range of 0.5-2.0 mM induced the formation of DPPS-enriched domains that make liposomes prone to vesicle fusion/aggregation, as observed by DSC and DLS measurements. However, in the presence of AnxA5, these domains are stabilized in such a way that the calcium fusogenic effect is retarded. In fact, fusion was observed only in concentrations higher than 1.0 mM Ca^{2+} while in the absence of protein it started at 0.5 mM Ca^{2+} . Microscopy images revealed the formation of small condensed lipid domains for pure DPPC, that became larger when DPPS was present and even larger when AnxA5 was present. We believe that this ternary behavior is central to the mineral nucleation inside the MVs that requires a significant amount of calcium and phosphate ions concentrated on a small region of the membrane, since the negatively charged DPPS-membrane associated to AnxA5 facilitates the nucleation, growth and release of mineral.

Keywords: DPPS, AnxA5, Calcium
Supported by: FAPESP, CNPq, CAPES

AA.22 - Clotrimazole fluidizes phospholipid membranes and localizes at the hydrophobic part near the polar part of the membrane

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Clotrimazole (1-[(2-chlorophenyl)-diphenylmethyl]-imidazole) is an azole antifungal drug belonging to the imidazole subclass that is being widely used in pharmacology and that it may incorporate in membranes. The objective to understand its interaction with model membranes. We have used DMPC and POPC as phospholipids and DSC, and 1H NOESY MAS-NMR and Molecular Dynamic Simulations as studying techniques. We have studied its interaction with DMPC phospholipid vesicles by using differential scanning calorimetry showing that clotrimazole decreases the temperature of the phase transition and at high concentrations it forms areas of high proportions of clotrimazole. 1H-NMR and 1H NOESY MAS-NMR was employed to investigate the location of clotrimazole in POPC phospholipid membranes. In the presence of clotrimazole all the resonances originating from POPC were shifted upfield but mainly those corresponding to C2 and C3 of the fatty acyl chains suggesting that the clotrimazole aromatic rings preferentially locate near these carbons. In the same way 2D-NOESY measurements showed that the highest cross-relaxation rates between protons of clotrimazole and POPC were with those bound to the C2 and C3 carbons of the fatty acyl chains. After molecular dynamics simulations it was seen that indicating that clotrimazole is located near the top of the hydrocarbon chains phase, the nitrogen atoms of the imidazole ring of clotrimazole being closest to the polar group of the carbonyl moiety. These results are in close agreement with the NMR ones and the conclusion is that clotrimazole is located near the water-lipid interface and located in the upper part of the hydrophobic bilayer. Clotrimazole fluidizes phospholipid membranes and localizes at the hydrophobic part near the polar part of the membrane.

Keywords: 1H NOESY MAS-NMR, clotrimazole, DSC

AA.23 - A complete membrane fusion system: determining fusion intermediates, kinetics and efficiency

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Membrane fusion is ubiquitous for cells to carry out seemingly unrelated processes such as fertilization and neurotransmission. Fusion occurs through specific intermediates; (i) docking, when the membranes bind, (ii) hemifusion, when lipids in the outer leaflet mix, and (iii) full-fusion (content mixing), when lipids on both leaflets as well as their aqueous contents mix. As a result, the end product is a larger compartment whose dimensions are the sum of the fusing membrane areas. Due to the high complexity, fusion is often studied using spectroscopic methods and reconstituted lipid vesicles, but these systems carry a number of limitations - the inability to detect intermediates, the increase in vesicle area, and the inherent complicated kinetics. Recently, we have developed a reconstituted system based on the fusion of charged large and giant unilamellar vesicles (LUVs and GUVs) that is able to circumvent these limitations (Lira et al., Bioph. J. 2019). It enables the detection of fusion intermediates, efficiency and kinetics in real-time using microfluidic devices. Here, we extended its capabilities by using a combination of independent and quantitative imaging and micromanipulation techniques, allowing us to quantify fusion efficiency, to estimate membrane's composition upon fusion in real-time and measure membrane mechanics on the level of a single vesicle. The quantification of area increase upon fusion is reported for the first time. The system is extremely efficient, and tens of thousands LUVs fused to a single GUV. Using a double optical tweezer setup coupled to a confocal microscope, we simultaneously detect fusion intermediates and measure the forces associated with the interaction of the fusing membranes, which is in the picoNewton force range. We anticipate that the insights here will help to unravel how regulatory factors mediate fusion in cells as well as it will support the development of highly efficient drug delivery systems.

Keywords: Membrane fusion, fusion intermediates, reconstituted systems
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AA.24 - The role of NPP1 in the biomineralization process: *In vitro* propagation of calcium phosphate minerals.

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The process of bone mineralization is mediated by matrix vesicles (MVs) that act as starting sites of hydroxyapatite formation. These vesicles are originated by the membrane budding of mineralization-competent cells with specific composition. Ecto-pyrophosphatase/phosphodiesterase (NPP's) consists of a large family of enzymes expressed in several mammalian tissue. Evaluate the behavior of NPP1 when incorporated into liposomes. Evaluate its influence on biophysical parameters in incorporated liposomes. Estimate whether NPP1 has the ability to propagate minerals *in vitro*. The expression of NPP1 was performed as described by Simão et al. 2010. Liposomes consisting of 1.5 mg/mL dipalmitoylphosphatidyl choline (PC) were prepared as described by Bolean et al. 2020. The incorporation of NPP1 into PC-liposomes was performed as described by Favarin, et al. 2019. For *in vitro* assays, NPP1:PC-proteoliposomes were incubated in SCL buffer, 74.0 mM Tris-HCl buffer (pH 7.4) and 2.0 mM of ATP, for 24h, at 37 ºC.The mineral formation was followed by changes in the turbidity at 340 nm. Infrared spectroscopy was used to investigate the chemical composition of the minerals precipitated. The mean diameter of the NPP1:PC-proteoliposomes was 115.8 with a polydispersivity index PI of 0.22, while 101.2 nm and PI 0.08 were obtained for the liposomes, indicating heterogeneity in the enzyme incorporation. Calorimetric analysis revealed a ΔHtrans of 4.3 and 5.8 kcal/mol for the NPP1:PC-proteoliposomes and PC-liposomes, respectively. Tc close to 39.8 ºC and t1/2 of 4.1 were obtained for the proteoliposomes. NPP1:DPPC-proteoliposomes induced mineral propagation only after incubation with either PS-CPLX or in the absence of nucleators, as indicated by increase in the turbidity close to 53 and 45%, respectively. We conclude that NPP1 is present in the studied liposomes, as well as affecting the thermodynamic properties of these liposomes. The *in vitro* mineralization data suggests that NPP1:DPPC-proteoliposome supports the propagation of phosphate minerals in a biomineralization model.

Keywords: Biomineralization, Liposomes, Proteoliposomes
Supported by: CNPq

AA.25 - Interaction of metallic nanoparticles with DOPC vesicles by dynamic light scattering
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Nanomaterials of all sorts have been widely explored in scientific studies, and new properties and applications are constantly being discovered. In biotechnology, including biomedical applications, the nanomaterials stand out to integrate a new generation of cell probes or carriers for drug delivery, especially because of their small size and tunable surface properties. Thus, is important to evaluate the interaction of nanoparticles with the membranes component to understand the interaction mechanism. Thus, in this work, we evaluated the interaction of silver nanoparticles (AgNp), silica-coated silver nanoparticles (AgNp@SiO2), and gold nanoparticles (AuNp) with the DOPC (1,2-dioleoyl-sn-glycero-3-phosphocholine), a phospholipid present in the cell membrane. Thus DOPC large unilamellar vesicles (LUVs) with ~130 nm (biomimetic system) were exposed to 200 µL of AgNp, AgNp@SiO2, or AuNp colloid for 180 minutes and evaluated by dynamic light scattering (DLS). The variation of DOPC LUVs sizes was performed in function of intensity, volume, and number percentage. A significant decrease in the sizes, as well as the number of vesicles in the system, was observed only for the AgNp up to 90 minutes. A small variation was observed to AgNp@SiO2 up to 90 minutes, which can be scribed to AgNp residues present in the AgNp@SiO2 colloid, once the AgNp was applied as a precursor in the nanoparticle synthesis. No changes were observed for DOPC LUVs in presence of AuNp. The results suggesting a high surface reactivity for AgNp onto DOPC lipid, which promotes a break in DOPC bilayer. The latter can be related to the action mechanism as an antimicrobial agent. On the other hand, the SiO2 coat decreases the surface reactivity, and as well as AuNp can be classified as biocompatible nanoparticles. The DLS measurements showed to be an important tool for analysis of the nanoparticle interaction at the biomimetic system
Keywords: pesticides, surface properties, vesicles
Supported by: FAPESP

AA.26 - Characterization of lipid membranes fluidity in Bacillus subtilis adaptive response to cold shock.
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Homoviscous adaptation in Bacillus cells upon a decrease in the ambient growth temperature occurs by an increment in the proportion of low-melting-point fatty acids in the membrane lipids. The short- term adaptation involves desaturating the acyl chains of their membrane phospholipids. The temperature dependence of unsaturated fatty acids (UFAs) biosynthesis relies on the transcriptional control of the des gene, which codes for the sole desaturase of this microorganism, Δ5-Des, and is upregulated upon a cold shock. The increased expression of des in response to low temperatures is strictly controlled by a canonical two-component system constituted by the histidine kinase DesK and its cognate response regulator DesR. This regulatory pathway has been dissected at the genetic, biochemical and structural levels. However, the membrane biophysical changes regulating this pathway remain poorly studied. Therefore, the main objective of this work is to characterize and determine parameters associated with lipid membrane fluidity changes in Bs, as well as with model membranes with a similar composition, upon temperature variations. In order to obtain membranes with different lipid composition we resorted to a set of Bs strains. A wild type strain exhibits 2 % UFAs at 37ºC and 8 %UFAs at 25ºC in the acyl chains of membrane phospholipids. Overexpression of the des gene (Des+ strain) leads to membranes containing up to 12% and 20% UFAs at 37ºC and 25ºC, respectively. In contrast, a mutant strain lacking the des gene (Des-) is unable to produce UFAs. Lipid membranes of these strains were extracted and purified. We have determined quantitative parameters associated with membrane fluidity by fluorescence spectroscopy and microscopy techniques. These studies provide the first step towards the biophysical characterization of B. subtilis plasma membrane adaptation upon a temperature decrease. The information obtained here could be extended to other bacteria and signaling pathways. **Keywords:** Bacillus subtilis, lipid membranes fluidity, Fluorescence Spectroscopy
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AA.27 - The Protective Effect of Artepillin C in Healthy Model Membrane Structure Against Reactive Oxygen Species

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Artepillin C is the majority compound found in Brazilian green propolis, a well-known product used for disease and antioxidant prophylaxis. Independent of its protonation state, artepillin C interacts with model membranes, either by disrupting the lipid organization when in neutral state (acidic pH) or only by interacting preferentially in the surface when in deprotonated state (physiological pH), without affecting structurally the membrane. The latter enforces the advantage of using Artepillin C against lipid peroxidation caused by free radicals, which is a first step before degenerative diseases. The protective effect of artepillin C against lipid peroxidation was evaluated by the analysis of the surface pressure (SP) stability of Langmuir monolayers, after inducing reactive oxygen species (ROS) generation by photoactivation of erythrosin in the presence of model membranes. Lipid films were formed of dioleoylphophatidylcholine (DOPC). The pH of the system was controlled by using a Hepes buffer solution at 10 mM + 150 mM NaCl. Langmuir monolayers were formed after spreading 0.5 mg/mL of DOPC onto the subphase containing erythrosin and/or artepillin C (10 μM), and the SP stability analyses were performed when the lipid film reaches 30 mN/m, keeping the trough area constant over data acquirement. Erythrosin was photoactivated by using a LED source at 530 nm. Without photoactivation, the SP of the lipid film has decreased near 10% for all system, independent of the presence or the absence of erythrosin and artepillin C. When erythrosin was photoactivated, the ROS generation lead the lipids to a lysis after peroxidation, increasing the area per lipid in the film and, consequently, leading the SP for even lower values. The antioxidant potential of artepillin C and its interaction with Langmuir monolayer preclude the lipid peroxidation caused by the ROS.

Keywords: Artepillin C, Model Membranes, Lipid Peroxidation
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AA.29 - Triglyceride lenses at the air-water interface as a model system for studying the initiating stage in the biogenesis of lipid droplets

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Lipid Droplets (LD) are intracellular structures consisting of an apolar lipid core, composed mainly of triglycerides (TG) and steryl esters, coated by a lipid-protein mixed monolayer. The mechanisms underlying LD biogenesis at the endoplasmic reticulum membrane are a matter of many current investigations. Although models explaining the budding-off of protuberances of phase-segregated TG inside bilayers have been proposed recently, the assumption of such initial blisters needs further empirical support. To evaluate the dispersability and wettability of TG-phase (lenses) excluded from a PC monolayer. To describe the oil-water interfacial tension at the lens in relation to the compression of the laterally surroundng PC monolayer. Langmuir films of PC/TG where compressed up to lateral packing where TG molecules are excluded from the monolayer into Collapse Structures (CS). Surface Spectral Fluorescence Microscopy (SSFM) was used characterize the solvatochromism of Nile Red (NR) both in monolayers and inside CS. Brewster Angle Microscopy (BAM) was used to characterize the topography of films and thickness and lateral size of individual CS. Upon TG exclusion from Langmuir TG and PC/TG films, CS exhibited highly reproducible lateral size (∼1 micron lateral radius) not varying with lateral packing changes, and being highly stable at surface pressures beyond collapse. NR solvatochromism both in monolayers and inside CS indicated that CS corresponded to a phase of liquid TG. These lenses were dramatically flattened when PC was present (6-12 nm compared to 30-50 nm for lenses on PC/TG and TG films, respectively). The oil-water interfacial tension acting at each individual microscopic lens varied with compression states of the laterally surrounding monolayer at the air-water interface. Lenses formed on air-water Langmuir films can serve to assess variables of relevance to the initial step of LD biogenesis -such as the degree of dispersion of excluded-TG phase and shape, spatial distribution and oil-water interfacial tension of TG lenses-.

Keywords: Lipid Droplets Biogenesis, Langmuir monolayers, Neutral Lipids

AA.30 - Stability of membranes containing different types of anionic lipids

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Membrane stability is fundamental to sustaining life. When subjected to strong stimuli, such as electric pulses, pores open in the membrane. Usually these pores reseal after that and membrane integrity is restored, as observed in neutral PC (phosphatidylcholine) giant unilamellar vesicles (GUVs). However, GUVs containing charged lipids display a different response to strong DC electric pulses: some micron-sized pores open indefinitely leading to vesicle burst within less than a second after the pulse while GUVs that apparently restore their integrity after macropore closure exhibit a long-lasting high permeability revealing the persistence of sub-microscopic pores minutes after the end of the pulse. These phenomena are supposed to correlate with the fraction of the anionic lipid used. Here, the stability of GUVs composed of PC and increasing fractions of other physiologically relevant anionic lipids, such as cardiolipin (CL), phosphatidylinositol (PI) and phosphatidylinositol 4,5-bisphosphate (PIP2) is investigated with phase contrast optical microscopy. First, the occurrence frequency of disturbing events is quantified in a large population of GUVs. Then, the dynamics of vesicle contrast loss due to long-lasting permeability is assessed on individual GUVs. Finally, the edge tension, which reflects the energy penalty per unit length to arrange lipids in pore rims, is measured from the dynamics of macropore closure. A significant membrane destabilization occurs already at 10 mol% CL, whereas for PI and PIP2-containing membranes substantial vesicle perturbation was induced only at 50 mol% PI or PIP2. The edge tension for membranes composed of 50% of anionic lipid were significatively reduced when compared to pure PC membranes. Membranes containing higher fractions of charged lipids are more unstable as a result of a reduced pore edge tension. The results obtained are important to understanding the response of cells to electroporation, a widely used protocol that renders biomembranes transiently permeable for several medical applications. **Keywords:** electroporation, GUVs, anionic lipids
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AA.31 - Construction and characterization of microreactors of Chlorocatechol 1,2- dioxygenase using low complexity domains as molecular adhesives

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Low Complexity Domains (LCDs) can be used as molecular adhesives on proteins and are capable of undergoing Liquid-Liquid Phase Separation (LLPS. LLPS has been emerged as an interesting strategy to produce microreactors with usefulness in vaccines development and tissue engineering. The 1,2- Choloratechol Dioxygenase (CCD) is an enzyme with an increasing potential in bioremediation. Therefore, the construction of the CCD-LCD2 chimera could lead to the development of microreactors capable of spatially and temporally controlling reactions for application in environmental decontamination. The initial step was based on the construction of the DNA coding for both chimera proteins – GFP-LCD2 and CCD-LCD2 – with subcloning in a vector containing the LCDs codes. The protocol for expression in *E. coli* and purification was established and the behavior for LLPS was determined by DIC and Fluorescence Microscopies. The stability of the proteins was determined by spectrophotometer, fluorimeter and DLS. Finally, the structural stability was determined by Circular Dichroism. As a control, GFP-LCD2 gene code was successfully constructed, and the recombinant protein was produced by a heterologous expression. CCD-LCD2 gene coding is still in construction. The success in gene cloning was verified by electrophoresis in agarose gel for both chimera proteins. The expression and purification of GFP-LCD2 resulted in a protein with 43 kDa, with great stability of their LLPS in temperatures below 37ºC and in the pH range of 5,0 to 7,0. The secondary and tertiary structures were conserved with a slight increase in content of other structures such as intrinsically disordered regions. The gene construction, expression and purification steps of GFP-LCD2 were well established, and the protein thermal stability and structural characterization have been determined. Therefore, we expect, in the future, to optimize the same protocols for CCD-LCD2.
Keywords: Biorremediation, 1,2-CCD, 1,2-CCD
Supported by: FAPESP, CNPq and CAPES

AA.32 - PHOSPHO1 interactions with membrane models

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Bone biomineralization is a process mediated by osteoblasts through the release of matrix vesicles (MVs). The most accepted theory describes the MVs´ biogenesis by budding from cell membranes, secreted at specific sites in the bone extracellular matrix. The presence of Ca2+ and inorganic phosphate (Pi) inside the MVs provides a suitable environment to nucleate the first apatite crystals. The internal reservoir is composed by enzymes such as PHOSPHO1, a phophatase that hydrolyzes phosphocholine and phosphoethanolamine, and generates Pi through phospholipids degradation(1). In this study, we aimed to investigate the interactions of PHOSPHO1 with lipids enriched in the membrane of MVs. We used DPPC and DPPS (8:2 molar ratio), in Tris/HCl (100 mM containing NaCl, 2 mM MgCl2) buffer, pH 7.4, and Langmuir monolayers as a mimetic membrane model. 0.76 µg.mL-1 PHOSPHO1 was added to the subphase. Chloroformic 1 mmol.L-1 DPPC and DPPS solutions were dripped onto the air-liquid interface, and the monolayer was compressed. The DPPS monolayers were expanded in the presence of PHOSPHO1 (minimum molecular area 63 Å2), and the compressibility was reduced (Cs- 1 592.1 mN.m-1) when compared to pure DPPC (56.9 Å2 and 167.6 mN.m-1), evidencing a better lipid packing. The presence of the enzyme reduced the minimum area occupied per lipid and the compressibility in DPPC monolayers. Thus the composition of the monolayers and the lipid organization is crucial for the interaction of PHOSPHO1 with MVs´membrane model, shedlighting in the mechanisms of bionegis driven by this enzyme.

Keywords: PHOSPHO1, membrane, interactions
Supported by: FAPESP: (2021/02768-0; 2019/08568-2) and CNPq (304021/2017-2).

BA - Membrane Permeation: Channels and Transporters

BA.01 - (Na^+,K^+)-ATPase activity in different tissues.

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The (Na^+, K^+)-ATPase, found in the plasma membranes of all animal cells, underpins many homeostatic processes and is directly responsible for the asymmetrical, electrogenic counter-transport of Na^+ and K^+ that results in strong ionic gradients across their membranes. The (Na^+, K^+)-ATPase also hydrolyzes other phosphate-donating substrates (K+-phosphatase activity), such as p-nitrophenylphosphate, O-methylfluorescein phosphate and acetyl phosphate. The activities of (Na^+, K^+)-ATPase from three different rat tissues homogenates (kidney, brain and heart) were characterized using the synthetic substrate p-nitrophenylphosphate (PNPP). The tissues were homogenized and centrifuged at 10,000 × g for 35 min at 4 °C, the supernatant was rapidly frozen in liquid nitrogen and stored at –20 °C. PNPP hydrolysis (PNPPase activity) by the gill microsomal fraction was assayed at 37 °C, monitoring the release of the p-nitrophenolate ion. The difference in measured PNPPase activity in the absence and presence of ouabain was considered to represent the K^+-phosphatase activity. K^+-phosphatase activity in homogenate from kidney is 5- and 12-fold higher than in homogenate from brain and heart, respectively. Differences are also observed in sensitivity to ouabain. The results suggest that different isoforms of (Na^+, K^+)-ATPase are expressed in these tissues and the (Na^+, K^+)-ATPase can be regulated to the different cellular needs.
Keywords: peptides, proteins, scorpion
Supported by: CNPq, CAPES and UFMS

BA.02 - Mechanosensitivity and electric field modulates the water flow through plant and animal aquaporins

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Aquaporins (AQPs) are part of a large but conserved family of transmembrane tetrameric proteins that include water channels as well as water-solute and/or gas channels. Each subunit has its own permeable pathway. Experiments in oocytes show that the water-transport rate of plant and animal aquaporins decreases by increasing the osmotic gradient and that this effect correlates with membrane tension increments. By other side, molecular dynamic (MD) simulations predict how the water molecules move through the permeable pathway as well as how they might respond to electric fields. Using the heterologous xenopus oocytes system, we studied the mechanosensitivity of homo and heterotetramers of the plant FaPIP2;1. In addition, to test the effects of electric fields on the water transport rate we performed molecular dynamic simulations on homotetramers of FaPIP2;1 and AQP4. Functional parameters were obtained from the kinetics of cell volume changes with different osmotic gradients. Simulations were performed with NAMD v.2.7 and the CHARMM27 force field, using a homology model of FaPIP2;1 developed with the crystal of SoPIP2;1 (PDB 2B5F) and the structural data of human AQP4 (PDB 3GD8). Our experimental results show that FaPIP2;1 behaves as a mechanosensitive aquaporin. In analogy with the study of ion channels, the transport capacity of AQPs can be evidenced in a plot of water flux versus osmotic gradient (Jw-Δosm). For mechanosensitive AQPs the Jw-Δosm plots show deviations from linearity with high gradients. On the other hand, molecular dynamic simulations reveal that FaPIP2;1 has higher water load capacity than other aquaporins, such as SoPIP2; 1 and AQP4. In addition, MD simulations predict that the water transport rate can change with the applied electric field in both AQP4 and FaPIP2;1. Our results suggest that changes of membrane tension or electric field perturbates the water flow through aquaporin channels.
Keywords: mechanosensitivity, gating, electric field
Supported by: CONICYT, Millennium Scientific Initiative of the Chilean Ministry of Economy, Development, and Tourism (Chile); CONICET, ANPCYT, UBA (Argentina).

BA.03 - Transitions During the Voltage Sensor Activation in the Voltage-gated Proton Channel (Hv1) Changed According to the Absolute pH

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The voltage-gated proton channel (Hv1) is a dimeric membrane protein able to dissipate acute acid loads in cells. This is achieved because the aperture of the channel is regulated by both the voltage and ΔpH across the membrane. Structurally, its function relays in the voltage sensor domain, as deletion of the N- and C-terminal domain produced monomers that maintains the biophysical properties of the dimer. We wanted to study the coupling between the voltage and pH sensing in the monomeric Hv1. We measured Hv1 currents to study the pH dependence of the monomeric channel in excised membrane patches of Xenopus laevis oocytes. As in the dimeric channel, the monomeric channel G-V curves were shifted according to the ΔpH established across the membrane, but this was not the case for the kinetics of activation, which were different for the same ΔpH. Then, we measured the effect of pH in gating currents of the monomeric Hv1 using a non-conducting mutant channel. In this case, the Q-V curves were changed according to the ΔpH established across the membrane, but like the ionic currents, the kinetics of decay of the ON-gating currents were not similar at the same ΔpH. Using different voltage protocols, we seek to find the transitions effected at different pHs during the movement of the voltage sensor. Finally, we fit our data using a Markov chain Monte Carlo method to try to quantify the modulation by pH. Our results showed that for the case of the monomeric channel, ΔpH determines the initial and final states during activation of the channel, but the kinetic mechanism to reach the states does not simply depend on the ΔpH, but it changed according to the absolute internal and external pH. **Keywords:** voltage-gated proton channel, pH-dependence, gating currents
Supported by: Supported by CONICYT-PFCHA/Doctorado Nacional/2017-21170395 to E.C., Fondecyt 1180464 to C.G., and 1161672 to A.N. Millennium Scientific Initiative of the Chilean Ministry of Economy, Development, and Tourism (P029-022-F).

BA.04 - Automatized and optimized analysis of video-registered volume time courses of aquaporin-expressing Xenopus oocytes

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Biophysical characterization of aquaporins expressed in oocytes is based on monitoring cell volume changes. This model allows the recording of sequential images or videos with low-cost videomicroscopy equipment. However, one bottleneck in the experimental workflow is image processing. Timelapses recorded at high temporal resolution may contain thousands of images from where oocyte volume information must be extracted. The first step is oocyte segmentation, a user-dependent process that requires training and experimental criteria and is prone to bias included by the operator. Afterwards, oocyte area in each image is computed and converted to a relative volume curve (V_t/V_0), assuming spherical shape of oocytes. Then, the osmotic permeability coefficient (P_f) is calculated using the slope of the V_t/V_0 curve. To achieve an accurate P_f estimation, detection of the initial frame is mandatory, and not trivial to determine. This initial frame corresponds to an in-focus and stable oocyte image. Moreover, this frame must be temporarily located as near as possible to the instant the oocyte is subjected to the osmotic gradient. The challenge of this work is to present a new Phyton-based tool which allows us to process images, extract required information, and calculate the initial frame, V_t/V_0 curves and P_f of each oocyte. We developed this new script to produce an automatic, faster, and operator-independent end-to-end solution to perform P_f analysis. To develop this tool we used various image and data processing techniques such as shape recognition algorithms and in-depth classification of image focus using cluster analysis. Comparison of results with the traditional approach show equal P_f values but with better determination of the initial frame and significantly lower time of analysis. With this tool it is possible to greatly increase the number of oocytes per experiment, gaining statistical power and substantially reducing the efforts spent in manual analysis. **Keywords:** Cell volume, Aquaporins, Image processing. **Supported by:** UBA (UBACyT 2018-2020 number 20020170200049BA and UBACyT 2020-2021 number 20020190200141BA awarded to M.O) and ANPCyT (PICT 2017-0368 awarded to M.O. and PICT2017-2338 to G.A.)

BA.05 - Hydrolysis of nucleotide and non-nucleotide substrates by the Spf1p P5A-ATPase.

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P-ATPases are a large and ubiquitous family of transporters that use the energy of ATP hydrolysis to keep the concentrations of different compounds on both sides of biological membranes out of thermodynamic equilibrium. The P5-ATPases have atracted much interest after the reports of mutations in the human P5-ATPase ATP13A2 that cause a juvenile form of Parkinson's disease. Spf1p from *Saccharomyces cerevisiae* is the best characterized P5A-ATPase. Recent studies showed that Spf1p has a peptidyl translocase activity and directly interacts with the transmembrane segment of certain proteins, allowing their translocation when they are incorrectly inserted into the ER membrane and place P5-ATPAses within the cellular proteostasis control network. The present work aimed to explore the hydrolytic activity of Spf1p against different phosphorylated compounds using a purified preparation of the recombinant protein in C12E10 micelles. The reaction media contained Tris-HCl 50mM (pH de 7,2 a 28°C), MgCl2 2mM free, Na3N5mM, mM EGTA 0,5mM, the indicated amount of ATP o NTP and 1 µg of Spf1p supplemented with 40 µg of PC andC1 2E10. Pi was estimated with Baginski method after 20 minutes. We found that in addition to ATP, Spf1p was capable of hydrolyzing other nucleotidic substrates as ITP, GTP and, to a lesser extent, UTP. Interstingly, Spf1p did also hydrolyze non-nucleotide substrates, as pNPP with a maximum velocity approximately 4 times higher than that of ATP. As with ATP, the activity increased with the concentration of pNPP along a double hyperbolic curve with two components of low and high affinity. Based in these results, it might be possible that under conditions of low cellular energy Spf1p function is preserved by using alternative substrates. Knowledge of the functioning of P5A-ATPases can contribute to the design of strategies to avoid the loss of cellular proteostasis that underlies different types of human diseases.

Keywords: NTPs, P5-ATPASE, SPF1

BA.06 - Mechanistic insight into pH gating in PIP aquaporins: role of specific loopD amino acids

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Aquaporins are membrane channels that transport water and other solutes. Their transport activity is regulated by different stimuli such as pH, binding of cations, phosphorylation/dephosphorylation or interaction with other proteins. All aquaporins share a general fold and tetrameric quaternary structure. Each protomer present two conserved regions that regulate the specificity of transport: the Asn-Pro-Ala (NPA) motives and the aromatic/arginine (Ar/R) selectivity filter. The plant PIP subfamily of aquaporins distinguish from other members of the family due to a longer intracellular loopD which is involved in its gating mechanism. The open/closed conformational transition in PIP channels is triggered by intracellular acidification, and different pH0,5 are found for homotetramers and heterotertamers form by PIP1 and PIP2 paralogues. Our goal is to elucidate the role of specific LoopD' s amino acids in the modulation of PIP gating mechanism by intracellular pH. Our *in vitro* and *in silico* experiments, for wild type and mutant PIP show that: i- a conserved Leu residue in the cytoplasmic constriction is the structural element that determines pore blockage and, ii- a Pro residue present in PIP2 but not in PIP1 channels is involved in differential pH0.5 of homo and heterotetramers dose-response curves. So, two loopD residues, Leu 206 and Pro 194, works in combination with the pH sensor His 202 to control effective pore closing.

Keywords: aquaporin, gating, water transport
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BA.07 - EXPLORING THE CX26 HEMICHANNEL using MD simulations

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Gap junctions provide a communication pathway between adjacent cells. They are formed by two connexons that correspond to different cells. In this work, we investigated the dynamic behavior of a Cx26 connexon in a POPC lipid bi-layer using Molecular Dynamics simulations. Specific amino acid interactions with calcium and their stability were found. Few of these sites such as, GLU42, GLU47, GLY45 and ASP50, were already suggested in the literature. Besides, we identified novel calcium biding sites: ASP2, ASP117, ASP159, GLU114, GLU119, GLU120 and VAL226. Molecular Dynamics simulations. Furthermore, we focus our attention on the membrane protein interactions and the ion flux though the connexon pore. We analyzed extensive atomistic simulations with and without calcium ions. We found that lipid-protein interactions were mainly mediated by hydrogen bonds. Specific amino acids were identified forming hydrogen bonds with the POPC lipids (ARG98, ARG127, ARG165, ARG216, LYS22, LYS221, LYS223, LYS224, SER19, SER131, SER162, SER219, SER222, THR18 and TYR97, TYR155, TYR212, and TYR217). In the presence of calcium ions, we found subtle differences on the HB lifetimes. Finally, these MD simulations are able to identify and explain differential chlorine flux through the pore depending on the amount of the calcium ions and its distribution within the pore

Keywords: CONNEXIN, Molecular Dynamics, POPC
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BA.08 - Hydroperoxidized lipid membranes properties favor ion permeability

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The self-assembled lipid bilayer is an effective semipermeable membrane. Indeed, water molecules cross the membrane with a permeability coefficient around Pm≈10 mm/s, whereas ionic species such as salt display permeabilities many orders of magnitude smaller (Pm≈10-11 mm/s for Na+). In the biological context, such small permeabilities have led to the neglect of passive membrane permeation in favor of assisted transportation. Yet, the exact pathways that allow ions to translocate across the membrane are still a matter of controversy [1]. In particular, it is likely that common biological phenomena such as phase behavior or lipid oxidation [2-4] play not only a direct role in passive membrane translocation but also in protein assisted transport. In the pursuit of a deeper understanding of the effect of lipid oxidation in ionic transportation [5], we study the electropermeability and the phase transition of lipid bilayers containing different amount of their hydroperoxidized form. We measure the ionic current induced by an electric potential across model membranes of 1-palmitoyl-2-oleoyl-glycero-3-phosphocholine (POPC) with different fractions of hydroperoxidized POPC. Even non-oxidized membranes from a single lipid species such as POPC display a complex behaviour that has fascinated several generations of scientists [6-7]. At low voltage, the intrinsic permeability results in a small but measurable current across the membrane. As the strength of the electrical field is increased, current spikes can be observed, generally associated to water channels piercing the membrane allowing ions to migrate across the membrane. In parallel, we study the main phase transition temperature between gel and fluid phase and its evolution for two different single lipid model membranes containing increasing amount of their hydroperoxide forms, giving us more information on hydroperoxydized membrane properties. We show with this work how lipid hydroperoxidation fundamentally changes the membrane properties and conductive behaviour. We then tentatively discuss the possible consequences for the insertion and functioning of membrane ionic channels that may be hampered by the structural modification of oxidized membranes.

Keywords: membranes , Hydroperoxidized , lipid

BA.09 - A solution NMR study of a prokaryotic Na+/Ca2+ exchanger incorporated in detergent micelles

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Na+/Ca2+ exchangers (NCXs) are essential for the maintenance of Ca2+ homeostasis in different cell types and are therefore considered important pharmacological targets. However, high-resolution structures for eukaryotic NCXs have not been obtained to date. NCX-Mj, an orthologue from the thermophilic archaea M. jannashii, has emerged as a structural model of the NCX transmembrane domain. While NCX-Mj kinetic properties have been extensively studied, only its outward-facing state structure has been solved and, hence, a complete model of the ion translocation mechanism is missing. This project aims to study the NCX-Mj intrinsic dynamics in DDM micelles by solution NMR spectroscopy. A synthetic gene coding for NCX-Mj was cloned in pET24 to express a protein in fusion with a 9xHis tag at the C-terminal end. *E. coli* C43 harbouring the pET24a-NCX-Mj plasmids were cultured in minimal media with 15NH4Cl as nitrogen source. Harvested cells were sonicated and membrane proteins were solubilised with 2% sarkosyl. After centrifugation, NCX-Mj was purified from the supernatant by batch Ni2+-affinity chromatography, at which point sarkosyl was exchanged for 0.3% DDM. NCX-Mj was further separated by size-exclusion chromatography, yielding 2 mg NCX-Mj per culture litre. SEC-MALS analyses revealed monodispersity of the protein detergent complex (PDC) fractions, as well as different elution times for empty micelles and PDCs, albeit with surprisingly longer retention times for the latter. These analyses suggested that monodispersity could be compromised by changes in the protein:detergent ratio. The observation of well-dispersed peaks along the 1H dimension in preliminary 1H-15N TROSY spectra is consistent with a folded protein. Analyses of NCX-Mj mutants that stabilise a given state (outward- or inward-facing) are underway. By comparing 1H-15N TROSY spectra of wild-type and mutated NCX-Mj we expect to identify sets of peaks for each state.
Keywords: Na+/Ca2+ exchanger, Solution NMR spectroscopy, Structural biology
Supported by: FAPESP

BA.10 - BaCopA, a Cu(I) ATPase from the Antarctic bacterium Bizionia argentinensis

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Copper ions are cofactor for several enzymes and participate in some cellular redox reactions. Intracellular excess of copper ions generates reactive radicals that cause damage to DNA, proteins and lipids. For this reason, intracellular levels must be regulated to avoid toxic concentrations. A subfamily of P-ATPases (denoted as PIB-1) are present in prokaryotic and eukaryotic organisms, and constitute one of the main transporters responsible for the elimination of excess copper ions from the cytosol. In this work we characterize a putative PIB-type ATPase belonging to Bizionia argentinensis(BaCopA), a gram-negative bacterium isolated from the superficial seawater of Potter Cove, Antarctica. BaCopA was cloned and expressed in *Saccharomyces cerevisiae* as a GFP-fusion His-tagged protein for its subsequent purification and detection. Activity assays indicate that purified BaCopA is able to catalyze ATP hydrolysis at 5°C. ATPase activity of BaCopA increases when Cu (I) and ATP are added to the reaction medium.However, an inhibitory effect of ATPase activity occurs with the addition of vanadate, a specific inhibitor of P-ATPase-type enzymes.A structural model was built by homology modeling using the resolved structure of L. pneumophilaCopA as template (PDB: 4BBJ). The structural alignment shows a high degree of similarity, with the typical topological pattern of PIB-1 ATPases. Comparison with its mesophilic and hyperthermophilic counterparts led to the identification of key residues conserved in functional domains and differences in non-covalent interactions and surface charges. The detailed analysis of this interaction network suggests greater structural flexibility in BaCopA and, therefore, a better adaptation to low temperatures.
Keywords: ion transport ATPases, psychrophilic enzymes, bioinformatics

BA.11 - The VRAC blocker DCPIB opens Ca^{2+}-activated BK channels and increases intracellular Ca^{2+} in melanoma and pancreatic duct carcinoma cell lines

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The Volume Regulated Anion Channel (VRAC) is known to be involved in cancer cell behavior and response to therapies. Since ion channels play an increasingly recognized role in cancer we investigated the effect of DCPIB, a presumably specific VRAC blocker, in pancreatic duct adenocarcinoma (PDAC) and melanoma. We sought to define the mechanisms of DCPIB in two PDAC lines (Panc-1, MiaPaCa-2), as well as on a primary (IGR39) and a metastatic (IGR37) melanoma line. We performed whole-cell patch clamp electrophysiology, gene expression analysis and calcium measurements. DCPIB induced a dramatic increase of currents in Panc1, MiaPaca2 and IGR39, but not in IGR37 cells. Currents were sensitive to tetraethylammonium and thus not mediated by K2P channels, known to be activated by DCPIB. Rather, currents were mostly mediated by the Ca^{2+}-dependent BK channel. DCPIB activation of BK as verified in transfected HEK293 cells. Further experiments showed that in IGR39, and to a smaller degree also in Panc-1 cells, DCPIB induces a rapid Ca^{2+} influx. This, in turn, indirectly potentiates not only BK but, in IGR39 cells, additionally activates other Ca^{2+}-dependent channels. However, Ca^{2+} influx is not required for BK activation by DCPIB. The direct activation of BK by DCPIB involves the extracellular part of the protein, as no effect was detectable when DCPIB was delivered inside the cell via the patch pipette. We conclude that the BK channel is a new target of DCPIB, and that the compound can acutely increase intracellular Ca^{2+}, elongating the list of DCPIB side-effects that need to be taken into consideration for future development of DCPIB-based activators/inhibitors of ion channels and other membrane proteins.
Keywords: BK channel, PDAC cancer, Melanoma
Supported by: AIRC, MIUR

BA.12 - Steady state kinetic analysis of Legionella pneumophila Cu+ transport ATPase. The activation by Cu+ and ATP

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P-type ATPases are a family of membrane proteins which couple ATP hydrolysis to the transport of substrates across biological membranes. Within them, Cu+-ATPases are the most widespread and conserved heavy metal ion transporting ATPases (PIB-ATPases). Its reaction cycle is assumed to be described by the so-called Albers-Post model postulated for the most studied P-ATPases such as the Na+,K+-ATPase or the Ca2+-ATPases. However, as some structural and functional particularities arise for Cu+-ATPases, several authors posit some doubts about their reaction cycle mechanism. The aim of our work is to perform a functional characterization of Legionella pneumophila Cu^{+}-ATPase (LpCopA) by measuring steady state ATPase activity. Cu+-ATPase activity of the enzyme presents a maximum at ∼37ºC and pH 6.6-6.8. Phospholipids enhance LpCopA Cu+-ATPase activity in a non-essential mode where optimal activity is achieved at an asolectin mole fraction of 0.15 and an amphiphile-protein ratio of ~30000. As described for other P-ATPases, Mg2+ acts as an essential activator. When evaluating the role of ATP and Cu+ in the reaction cycle of LpCopA we observed that ATPase activity increases as Cu+ concentration increases with a functional dependence that can be described by a sum of two hyperboles. On the other hand, the increment on ATP concentration in the reaction media produces an increment of ATPase activity that can be described by a hyperbola plus a constant value. Based on that, and the [Cu+] and [ATP] dependencies of the best fitting parameters of the functions pointed above, we propose a minimal reaction scheme for LpCopA catalytic mechanism that contemplates two enzyme conformations with different affinities for ATP, enzyme phosphorylation and binding of at least two Cu+ ions with different affinities. This model is compatible with the structural information available and the main characteristics of the reaction cycle models for the most characterized P-Type ATPases.
Keywords: Cu(I) transport ATPase, Kinetic mechanism, reaction cycle
Supported by: UBA, CONICET and FONCyT

BA.13 - Unveiling the mechanism of irreversible inhibition of aquaporin-10 by organogold compounds by biophysical methods and metadynamics

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Aquaporins (AQPs) are membrane protein channels that facilitate the diffusion of water and small solutes across cell membranes. For their involvement in a variety of pathologies, AQPs have emerged as potential drug targets, unveiling the use of selective AQP modulators as promising strategies for treatment of AQP-related diseases. Organometallic gold(III) complexes have gained interest with Auphen being discovered as a potent human AQP3 inhibitor. The mechanism behind this interaction was also reported, showing a reversible binding between the gold metal and AQP3 Cys40 residue. Here, we investigated the inhibitory effect of new organogold compounds in human AQP10 (hAQP10), an aquaglyceroporin expressed in the adipose tissue with relevance in body energy homeostasis. Using aqy-null yeast cells transformed with a plasmid encoding hAQP10, we tested the compounds' effect on glycerol permeability using stopped-flow fluorescence. Knowing that these compounds can react with cysteine residues and lead to the formation of stable and irreversible C-S bonds, we evaluated the reversibility of organogold compounds bond to hAQP10 cysteine residues, in the presence of β-mercaptoethanol. Moreover, we investigated their binding mechanism through molecular modelling and metadynamics atomistic simulations. Permeability assays revealed Au(III) CCON complex as one of the most potent of the cyclometalated Au(III) C^N compound series to inhibit hAQP10-mediated glycerol transport. These compounds revealed to irreversibly inhibit hAQP10-mediated glycerol permeability, probably due to the establishment of the stable covalent bond C-S. Computational results showed a local arylation of hAQP10 Cys209 residue by Au(III) CCON complex, resulting in alteration of the glycerol conductance pathway with overall shrinkage of the pore while water flux was barely affected. Thereby, even if the arylation occurs at a distance from the channel selectivity filters, the whole pore responds to this local modification. Altogether, we found Au(III) CCON complex as a potent inhibitor of hAQP10 glycerol permeability and identified a new mechanism of hAQP10 irreversible modulation by establishment of a covalent and stable C-S bond. **Keywords:** aquaporin, inhibitors, organogold compounds. **Supported by:** The authors acknowledge FCT - Fundação para a Ciência e Tecnologia, grant PTDC/BTM-SAL/28977/2017, fellowship 2020.04974.BD to C. Pimpão and projects UIDB/04138/2020 and UIDP/04138/2020 to iMed.ULisboa

BA.14 - Inactivation properties of ORIC, VRAC-like current of filamentous fungus *P. blakesleeanus*: the role of ATP and the first glimpse of the single channel behavior

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Outwardly rectifying, inactivating current (ORIC), recorded in cytoplasmatic droplets of *P.blakesleeanus* is characterized by a mild outward rectification, selectivity for anions, and a characteristic inactivation on depolarizing potentials. In absence of ATP, ORIC has a fast rundown with increasing speed of inactivation, while both processes are delayed by ATPpip (for at least 8min) and the non-hydrolysable AM-PCP (minimum of 5min). Extracellularly applied, ATP has a blocking effect. Flavonoid querzetin leads to a gradual loss of current despite the presence of intracellular ATP. Our research group has shown azide to be the potent respiration inhibitor of *P. blakesleeanus*, leaving the cells viable but significantly depleted from ATP. We are presenting data recorded in whole cell configuration, showing that: 1.ORIC can be activated despite the depletion of ATP in the cytoplasmatic droplet. However, its inactivation profile is significantly different comparing to normal conditions with or without ATP in the pipette in the first minute of recording, with reduced τ on lower depolarizing potentials. The fraction of current blocked by querzetin with AM-PCP in pipette solution is greater comparing to experiments with ATPpip , even with the 5-fold increase of AM-PCPpip concentration. The time required for current to recover from depolarizing steps is shorter at more hyperpolarized potentials. We have also started to look for the first insights into ORIC single channel activity in out-out configuration, and recorded, upon stimulation of ORIC in whole cell, and patch excision, the step-like pattern of inactivation that strongly resembles one of ORIC. Based on current data, we can conclude that ATP has a significant role in the inactivation process, with a more potent effect comparing to AM-PCP, whether by the means of greater affinity for the binding site or an additional mechanism involving kinase activity. **Keywords:** biophysics, ion, channels. **Supported by:** The Ministry of Education, Science and Technological Development of the Republic of Serbia

BA.15 - Cisplatin induced cardiotoxicity: macroscopic and cellular aspects.

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Cancer and cardiovascular diseases are the main death-causes in Uruguay and in developed countries. These pathologies have similar age of presentation, mainly in patients 60 years old and above, so it is frequent in clinical practice to be in need of administrating chemotherapy agents to patients with cardiovascular comorbidities. Cisplatin (CPT) is frequently used alone or in combination with other agents to treat: lung, testicular, ovarian and gastrointestinal cancers. The aim of this work was to characterize the possible detrimental effects in cardiac function by the acute exposition to CPT using isolated heart and cardiomyocytes from guinea pigs (*C. Porcellus*). Methods: All the procedures regarding animal experimentation were performed following protocols submitted to and approved by the "Comisión Honoraria de Experimentación Animal" (Exp #070153-000118-17). Isolated hearts were placed in a Langendorff system and perfused with Tyrode 1.8 mM Ca^{2+} alone (control) or with CPT (0-100μM). Strain was recorded through a gauge force transducer at the base of the papillary muscle. Electrical responses were measured with Ag-AgCl electrodes in the papillary muscle. Cardiomyocytes were isolated by enzymatic methods. Data were obtained by patch clamp sometimes joined with confocal microscopy with Rhodamine and Fluo dyes for Ca^{2+} imaging. Statistic tests between treated and not treated were non- parametric t-tests (Mann Whitney-Wilcoxon). The best fit of Hill´s equation to dose-response curves was done using nonlinear regression methods. Results are shown as mean +/- s.e.m (n=3). Results: In isolated hearts, CPT showed a biphasic effect over both, tension development and cardiac heartbeat. CPT increased the tension development with concentrations lower than 3.3 μM, whereas it decreased tension development with concentrations higher than 10 μM. In isolated cardiomyocytes, calcium currents were reduced accordingly by cisplatin. Confocal microscopy showed an increment of fluorescence by the calcium binding dyes, implying an increase of cytoplasmatic calcium with 3.3 μM CPT. At higher doses than 10 μM, the fluorescence diminished. A positive chronotropic effect was shown with concentrations lower than 3.3 μM, whereas a negative chronotropic effect was seen with concentrations of 49.5 μM. Conclusions: Our results indicate that CPT, may affect cardiac automatism and contraction, presumably by blocking L-type (Cav1.2) calcium channels and interference with molecules involved in maintaining the homeostasis of intracellular Ca^{2+}.
Keywords: Cancer, cardiovascular diseases, Cisplatin

BA.16 - Going messy: Assessing SIRAH's ability to simulate Intrinsically Disordered Proteins

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Simulate intrinsically disordered proteins (IDPs) is very challenging since these proteins have labile folding or conformational preferences easily changing upon environmental conditions or in the presence of molecular partners(1). Consequently, a lot of atomistic and coarse-grained (CG) simulations end up needing to develop and reparametrize their current force fields to work with it. This is because the majority of fully atomistic force fields have been created to reproduce folded or globular structures. Consequently, they may tend to over-stabilize initial conformers or dynamics(2). On the other hand, the SIRAH force field uses three beads to represent the protein's backbone (corresponding to the nitrogen, Cα carbon, and carbonyl oxygen, respectively). This mapping permits a direct evaluation between the conformational space explored at the CG and fully atomistic levels(3). However, in analogy with many atomistic force fields, SIRAH was also developed to primarily reproduce secondary structure elements. Therefore, conformational biases could not be ruled out. Studying the dynamical behavior of IDPs with the CG SIRAH force field suggests that the current version attains a fair description of IDPs' conformational flexibility.
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Keywords: Coarse-Grained simulations, IDPs, SIRAH

BB - Membrane-Active Peptides

BB.01 - Experimental and simulation study: Interaction of CARC peptides from HlyA with lipid membranes

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Escherichia coli alpha hemolysin (HlyA) is a pore-forming protein which belongs to the 'Repeat in toxins' (RTX) family. Although HlyA does not need cholesterol as a receptor to be active, the presence of this lipid in target cells enhances its activity. Several CRAC (Cholesterol Recognition/interaction Aminoacid Consensus sequence) and CARC (similar to CRAC but with the opposite orientation) were found in HlyA sequence. Only one CARC is present in the membrane insertion domain of the toxin. The aim of this work was to study the role of the CARC domain present in the N-terminal portion of the toxin in the interaction with membranes. Two peptides derived from HlyA were synthesized: PEPY: corresponds to a CARC sequence in the transmembrane domain of HlyA and PEPA: similar to PEPY but with residue Y^{347} substituted by A. Peptides were synthesized by the solid phase peptide synthesis method (Fmoc strategy) and purified by HPLC; peptide molecular mass and structure were determined by mass spectrometry and circular dichroism. Langmuir monolayer assays and Surface Plasmon Resonance (SPR) were performed to study insertion and association of the peptides into different POPC:Cho lipid mixtures (1:0, 4:1; 2:1). In addition, Molecular Dynamics (MD) simulations of the peptides with bilayers of those same compositions were carried out by using the united-atoms force field GROMOS. MD simulations and SPR assays show that PEPY has more affinity for POPC:Cho (4:1) membranes and also that PEPA presents less affinity for lipid bilayers in general. On Langmuir monolayer experiments, the same results were observed regarding insertion into monolayers. These results indicate that the CARC peptide derived from the N-terminal region of HlyA has a strong affinity for POPC:Cho (4:1) membranes, and that peptide-lipid interaction strongly depends on the presence of residue Y^{347}.
Keywords: alpha hemolysin, biophysics, cholesterol. **Supported by:** CONICET, UNLP.

BB.02 - PROJECT - *In vitro* evaluation of the antitumor potential of the Pep 5 peptide and its effects on Na,K-ATPase activity and analysis of the lipid composition of the plasma membrane

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Cancer is one of the most studied diseases today, as it has a high mortality rate and affects people of all ages. The development of this disease is due to the disordered growth of cells that invade tissues and organs and tend to be very aggressive and uncontrollable. The Na,K-ATPase (NKA) pump is an integral enzyme of the plasma membrane that depends on ATP for the active transport of the Na+ and K+ ions and is responsible for maintaining a low intracellular Na+ / K+ ratio. The enzyme has three subunits: The alfa subunit, responsible for catalytic activity and ionic transport; The beta subunit, responsible for the normal activity of NKA and contains several glycosylation sites; And the gama (or FXYD2) subunit that has a modulating function and influences the kinetic properties of the enzyme. This pump plays a fundamental role in the survival of cells, and is closely related to the amount of phospholipids and cholesterol in the membrane, which are necessary for its correct operation. Generally, the NKA pump has its expression increased in tumor cell lines compared to non-tumor cells, which makes this enzyme an excellent target for anticancer therapy. Bacteriocins are peptides or proteins secreted by bacteria that have antimicrobial and anticancer properties. Therefore, the present study aims to evaluate the interaction of the bacteriocin-like peptide Pep 5, secreted by Staphylococcus epidermidis 5, with the NKA pump and the plasma membrane in tumor cells. Cytotoxicity, cell migration, enzymatic activity and expression of its subunits will be performed, in addition to determining the dosage of phospholipids in the membrane of lung carcinoma cells, in order to propose new treatments against cancer, more effective and with lower side effects. **Keywords:** Cancer, NKA, Bacteriocins
Supported by: Universidade Federal de São João del-Rei – UFSJ

BB.03 - CaDef2.1G27-K44, an antimicrobial peptide bioinspired on defensin from *C. annuum* fruits: anti-Candida and antimycobacterial activities, mechanisms of action on yeasts and cytotoxicity on mammalian cells

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The growing resistance of microorganisms to multiple drugs associated with the high host toxicity of drugs in the current scenario is a major concern in Public Health. Therefore, new therapeutic strategies are necessary to cope with these problems. Antimicrobial peptides, natural or synthetic, appear as promising molecules for antimicrobial therapy because of their both broad antimicrobial activity and mechanism of action. To determine the anti-*Candida* and antimycobacterial activities, mechanism of action on yeasts, cytotoxicity activity on mammalian cells in the presence of *Ca*Def2.1$_{G27-K44}$ peptide (sequence under patent review). The *Ca*Def2.1$_{G27-K44}$ peptide was designed meeting the following criteria: positive net charge; low molecular weight (<3000 Da); Boman index ≤ 2.5; total hydrophobic ratio ≥ 40%. The mechanism of action of was studied by grow, plasma membrane permeabilization, ROS induction, mitochondrial functionality, and caspase activity assays. The cytotoxicity on Murine macrophages RAW 264.7 cells, human monocytes cell line THP-1 and hemolytic activity were also determined. *Ca*Def2.1$_{G27-K44}$ inhibited the growth of *C. albicans*, *C. buinensis*, *C. parapsilosis* and *C. tropicalis* with MIC100 values ranging from 12.5 to 25µM. For *C. albicans*, *C. buinensis* and *C. tropicalis* the MIC100 value corresponds to the lethal dose that kill 100% of the treated cell. *Ca*Def2.1$_{G27-K44}$ showed inhibitory activity against laboratory *Mycobacterium tuberculosis* strain H37Rv and highly virulent strain of *M. tuberculosis* with MIC50 of 33.2 and 55.4µM, respectively. Additionally, we demonstrate that *Ca*Def2.1$_{G27-K44}$ is active against yeasts even at different salt concentrations, induced morphological alterations such as, smaller cell size and cytoplasm condensation, presented cell membrane permeabilization and increased ROS levels, causes loss of mitochondrial functionality and activation of caspases. *Ca*Def2.1$_{G27-K44}$ has low cytotoxicity against mammalian cells. *Ca*Def2.1$_{G27-K44}$ has great potential as an antimicrobial agent with low toxicity to host cells.

Keywords: Antimicrobial peptide, Candida sp., Mycobacterium tuberculosis

Supported by: FAPERJ, UENF, CNPq, CAPES

BB.04 - Design, synthesis, purification and characterization of the wild type and four analogues of the SARS-CoV-2 fusion peptide (FP2) to evaluate the specific interaction with calcium ions

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The SARS-CoV-2 fusion process occurs through interaction with membranes of the so-called fusion peptide, a relatively hydrophobic and highly conserved amino acid fragment of the Spike protein within the *Coronaviridae* family, which is essential for the virus's fusogenic activity. It has been hypothesized that coordination of calcium ions at peptide sites is essential for the peptide binding to the target membranes. Thus, this study evaluates the importance of calcium ions in the modulation of the fusion peptide conformation. For this, solid phase peptide synthesis of the wild type fusion peptide (FP2) was performed, designed with a polyglycyl-lysine tail at C-terminus to increase peptides' solubility. In addition, three peptide analogues were synthesized containing strategic substitutions at positions 4 (Glu), 5 and 15 (Asp) by Gln and Asn, respectively, to determine which residues modulate calcium binding. Also, another peptide analogue containing a Asn replacement at position 9 by Asp was synthesized, aiming to hypothetically increase fusogenicity, by potentially creating a second calcium binding site. Further, purification, characterization of the synthesized peptides by HPLC and Mass Spectrometry were successfully evaluated and Circular Dichroism was performed to analyze secondary structure formation with and without calcium ions in solution and in lysophosphatidylglycerol micelles. Interestingly, the results evidenced that the secondary structures of FP2, Asn5 and Ans15 analogues are modulated by calcium binding, indicating that both Asp5 and Asp15 residues of the FP2 may not represent the calcium coordination sites. In contrast, the secondary structures of both Asp9 and Gln4 analogues are no longer affected by calcium binding, even in the micellar environment. This result suggests that the FP2 Glu4 residue is a putative calcium binding site. In conclusion, our results showed that a Ca2+-dependent secondary structure formation of FP2 and its analogues, identifying the putative Ca2+-coordination site, which most likely affects the peptide fusogenicity.
Keywords: Biophysics, Peptides, SARS-CoV-2. **Supported by:** FAPESP

BB.05 - Effect of membrane potential on entry of lactoferricin B-derived antimicrobial peptide into single bacterial cells and lipid vesicles

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Recently, we reported that antimicrobial peptide (AMP)-induced damage of membrane, increased with increasing negative membrane potential (∆φ) (JBC. 2019). AMP LfcinB(4-9) (sequence-RRWQWR) derived from lactoferricinB, can enter *E. coli* cells without damaging cell membranes. Thus, LfcinB(4-9) is a cell-penetrating peptide (CPP)-type AMP. Effect of ∆φ on the action of CPP-type AMPs is not well known. In this study, we investigated the effect of ∆φ, on interactions of Rh-LfcinB(4-9), with single *E. coli* cells, spheroplasts and giant unilamellar vesicles (GUVs) to reveal the mechanism of its antimicrobial activity (J. Bacteriol, 2021). First, we investigated interaction of Rh-LfcinB(4-9) with single *E. coli* cells and spheroplasts containing calcein by adding peptide to their vicinity and observed their fluorescence intensity change using CLSM. Next, we examined effect of ∆φ on interaction of Rh-LfcinB(4-9) with *E. coli* lipid-GUVs containing AF647 and small GUVs using single GUV method. At low peptide concentrations, Rh-LfcinB(4-9) entered cytosol of *E. coli* and spheroplasts without damaging membranes and the protonophore carbonyl cyanide m-chloro-phenylhydrazone (CCCP) suppressed its entry. Studies of time-kill method indicate that these low concentrations of peptides exhibit antimicrobial activity, but CCCP inhibits this activity. We also found that, Rh-LfcinB(4-9) entered the GUV lumen without pore formation in the presence of ∆φ and the rate of peptide entry into GUV lumen increased with increasing ∆φ. All the results show that ∆φ increases the rate of Rh-LfcinB(4-9) entry into the cytosol of *E. coli* cells and spheroplasts, and the lumen of GUVs without pore formation. To the best of our knowledge, this is the first report demonstrating the role of ∆φ in the antimicrobial activity of CPP-type AMPs and in their entry into the cytoplasm, which is a key process to determine their antimicrobial activity. These results indicate that antimicrobial activity of Rh-LfcinB(4-9) and hence LfcinB(4-9) increases with increasing negative ∆φ.
Keywords: antimicrobial activity, membrane potential, entry. **Supported by:** JSPS

BB.06 - Translocation of the Nonlabeled Antimicrobial Peptide PGLa across Lipid Bilayers and its Entry into Vesicle Lumens without Pore Formation

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Fluorescent probe-labeled peptides are used to investigate the interaction of antimicrobial peptides (AMPs) with cells and lipid vesicles to elucidate the mechanism of their activity. However, it is reported that labeling can significantly change the interactions of AMPs with plasma and lipid membranes. To eliminate the effect of fluorescent probe labeling, we investigated the entry of nonlabeled AMP PGLa into the lumen of single giant unilamellar vesicles (GUVs) using the new method devloped in our lab (Biochemistry, 2020). First, we investigated using confocal laser scanning microscopy the interaction of nonlabeled PGLa with single DOPG/DOPC (4/6)-GUVs containing fluorescent probe AF647 and DOPG/DOPC (8/2)-large unilamellar vesicles with self-quenched calcein in their lumen. Second, we simultaneously measured the PGLa-induced increase in fluorescence intensity of the GUV lumen due to calcein ($I_{calcein}$) and the PGLa-induced fractional area change of the GUV membrane (δ), as well as the lumen intensity due to AF647. In the interaction of nonlabeled PGLa with single GUVs, ($I_{calcein}$) increased with time without leakage of AF647 after a lag time from starting the interaction. This result clearly shows that PGLa can enter into the GUV lumen without damaging the GUV membrane, i.e., without pore formation. With increasing PGLa concentration, the fraction of PGLa entry increased. The results of simultaneous measurement show that PGLa entry occurs during the second increase in (δ), indicating that PGLa enters the lumen only during the translocation of PGLa from the outer to the inner leaflet of GUV membrane. Membrane tension due to an external force elevated the rate of PGLa entry. This is the first report to demonstrate experimentally that nonlabeled AMPs enter the vesicle lumens without pore formation (BBA-Biomembrane, 2021). Based on these results, we discuss the mechanism of nonlabeled PGLa entry into lipid vesicle. **Keywords:** PGLa, entry, giant unilamellar vesicle. **Supported by:** JSPS

BB.07 - Semi-quantitative RT-PCR analysis of an antimicrobial peptide expression in hemolymph and hepatopancreas of shrimp Macrobrachium rosenbergii (De Man, 1879) challenged with Aeromonas hydrophila

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Macrobrachium rosenbergii, known as freshwater giant shrimp, stands out for being one of the most economically important crustaceans with a high market value, being cultivated on a large scale in the world. The species was introduced in Brazil in 1970 and is currently the most favored for cultivation in the country. In the production environment, shrimp are exposed to several microorganisms that can burden the rearing systems, causing the crop to be threatened. Thus, crustaceans need an efficient and active immune system, responding through quick reactions to stimuli caused by the environment. Small chains of amino acids called antimicrobial peptides (AMPs) are secreted and actively react to fight infections. AMPs are considered essential molecules of the innate immune system, being present in several species. This work aimed to evaluate the production of a specific type of AMP expressed *in M. rosenbergii*, named Pellino, in response to septic injury caused by Aeromonas hydrophila after 12 hours of injection. The transcriptional profile of genes involved in the production of Pellino was analyzed by semi-quantitative RT-PCR analysis, with the β-Actin gene as expression normalizer. Different primers were used for the cDNA reaction. Results indicated that it was possible to express Pellino AMP under the conditions tested. It was observed Pellino gene expression in hemolymph of animals challenged with *A. hydrophila* and no expression was observed in the control sample, indicating a possible immune defense activation. **Keywords:** freshwater shrimp, hepatopancreas, immune system. **Supported by:** Fundação Araucária and CAPES

BB.08 - Introducing the multispectral phasors: a tool for the analysis of fluorescence excitation-emission matrix and spectral signal unmixing

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The use of phasors to analyze fluorescence data was first introduced for time-resolved studies in order to reduce the mathematical complexity of the models used. Recently, this approach was extrapolated to steady-state experiments, in what is called spectral phasors (SP), which are obtained from the Fourier transform of the fluorescence emission spectrum. In this work, we revise key mathematical aspects that lead us to re-interpret SP as the characteristic function of a probability distribution. This allows us to introduce a new tool, called multispectral phasor (MSP), that seize not only the information from the emission spectrum, but from the full excitation-emission matrix (EEM). Additionally, here we also developed an open-source Java software in order to facilitate the data processing. Using simulated spectra, we prove that there is a tight relationship between the MSP polar coordinates and the characteristics of the EEM, similar to what is known for SP. Due to our mathematical re-interpretation, we also show that MSP can be a powerful tool to tackle spectral signal unmixing problems in a more general case than SP. In addition, we proposed two main biophysical applications of MSP: protein conformational studies and Föster Resonance Energy Transfer (FRET). As a prove of concept, we experimentally measure the EEM changes upon denaturation of the human serum albumin (HSA) and during a HSA-ANS FRET. In this sense, we were able to use MSP to interpret the results in a simpler and finer way and in complete agreement with what is known in the literature. Understanding a protein's EEM as a molecular conformation fingerprint will open a door for the use of MSP as a tool to analyze and comprehend protein conformational changes. **Keywords:** Spectral phasor, fluorescence excitation-emission matrix, signal unmixing. **Supported by:** CONICET (PIP2013-2015),FonCyT (PICT2012-1377, PICT2015-2575). L.B.P.S. holds a PhD fellowship from CONICET and E.E.A is a Career Member of CONICET. We are also grateful to all the members of the biophysical area at CIQUIBIC for helpful discussions

BB.09 - Biophysical and structural studies of an antimicrobial membrane-modifying peptide of Piscidin family

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Antimicrobial peptides are potential templates for the design of novel antibacterial drugs due to their low propensity for resistance development and their ability to kill multi-drug-resistant bacteria. The ecPis-4s peptide isolated from the fish species *Epinephelus coioides* has been described as a potent antimicrobial agent against several bacterial and fungal strains1. It is composed of 22 residues (FFRHIKSFWKGAKAIFRGARQG-NH2) and carries a natural C-terminal carboxamide. The mode of interaction of ecPis-4s with membrane mimetic media is studied employing a set of biophysical techniques. The mode of interaction of ecPis-4s with membrane mimetic media is studied employing a set of biophysical techniques. Firstly, the ecPis-4s was chemically synthetized by SPPS using the Fmoc strategy, purified by HPLC, and characterized by mass spectrometry. Circular dichroism (CD) spectroscopy shows a high content of peptide helical structure when it is associated with POPC:POPG vesicles, and a low helix structuration in the absence of membranes. Solution NMR experiments have been performed in the presence of negatively charged SDS micelles to determine the three-dimensional structure of the peptide. The structure confirmed the helical conformation throughout the linear chain of ecPis-4s. Proton-decoupled 15N and 2H solid-state NMR of macroscopically oriented lipid bilayers have been used to determine the peptide topology in membranes made of POPC:POPG or *E. coli* lipid extract. The chemical shift around 70 ppm indicates that the peptide adopts a surface orientation with a tilt angle close to 90°. Carboxyfluorescein (CF) release experiments demonstrate a lytic activity of this peptide in the presence negatively charged vesicles made of POPC:POPG. In conclusion, the ecPis-4s peptide presents a high affinity to anionic membranes where it adopts an amphipathic helical conformation that aligns parallel to the phospholipid bilayer surface. This mode of interaction suggests a membrane-lytic activity once the local concentration or peptide reaches a threshold.
Keywords: antimicrobial peptide, peptide-membrane interaction, peptide topology

BB.10 - Whole cell 2H Solid-state NMR of Antimicrobial Peptides Interacting with Cell Envelopes: Role of Lipopolysaccharide

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Antimicrobial Peptides (AMPs) have been studied for more than two decades because they promise to help overcome the problem of resistance conventional antibiotics. However, AMPs have not been as successful as hoped, perhaps because we lack a detailed understanding of their mechanisms of action. To understand these mechanisms, numerous biophysical techniques, including solid-state 2H NMR, have been used to study membrane disruption both in model lipid system and in intact bacteria. In the real biological context of AMPs, studies have suggested that, in addition to interaction with lipids, it is essential to consider the non-lipid component of Gram- negative bacteria. Which has the lipopolysaccharide layer (LPS), one of the outer membranes of cell envelope component that protects bacteria from AMPs. This study investigated how LPS affects AMP-induced membrane disruption. MSI-78 is an AMP that has been shown to disrupt lipid membranes of target bacteria. We disrupt the LPS layer of Escherichia coli cells (*E. coli*) via chelation of the stabilizing divalent cations. However, the 2H NMR spectra of *E. coli* demonstrated that the 2.5 mM and 9.0 mM EDTA concentration used does not affect the lipid acyl chain order. MSI-78 is an AMP that has been shown to disrupt lipid membranes of target bacteria. Interestingly, we found that the 2H NMR spectra of *E. coli* with a 9.0mM concentration of EDTA in the presence of MSI-78 shows a slight increase of the lipid acyl chain disorder compared to MSI-78 alone. Thus, our results suggest that disruption of the LPS layer very slightly sensitizes bacteria to membrane-disruption by MSI-78.
Keywords: NMR, Peptides, Lipopolysaccharide
Supported by: Natural Sciences and Engineering Research Council of Canada

BB.11 - Studying flavivirus capsid proteins interactions with viral RNA

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Dengue virus (DENV) and Zika virus (ZIKV) are mosquito-borne flaviviruses, sharing structural features. The nucleocapsid core of the mature virion is formed by the 11 kb viral (+) single- stranded RNA condensed with multiple copies of the capsid (C) protein [1,2]. This is an essential protein, conserved among flaviviruses, which is involved in key steps of the viral life cycle, namely encapsidation and viral assembly [3]. One key step, essential for viral replication, requires DENV C specific binding to intracellular lipid droplets (LD), an interaction that was fully characterized. In addition to the interaction with LDs, it was also demonstrated by us that DENV C interacts specifically with host very low-density lipoproteins (VLDL) and viral RNA [3]. Those findings led to the development of pep14-23, a patented peptide based on the region comprising amino acids 14 to 23 of DENV C, which is able to inhibit DENV C binding to LDs and VLDL [3,4]. Then, in order to characterize DENV and ZIKV C binding to the viral RNA, through biophysical approaches, we started examining locations within the viral RNA to which the protein has higher affinity, with specific RNA sequences being identified [5]. Preliminary circular dichroism data show that some analogous DNA sequences used as proxies of selected RNA sequences do indeed interact with DENV C, causing changes in the protein secondary structure. Other biophysical approaches, such as dynamic light scattering, fluorescence and nuclear magnetic resonance spectroscopies, are being applied to better characterize this phenomenon, DENV C regions to which the viral RNA is prone to bind. These data will allow to select, test and develop inhibitors against this essential interaction of DENV C with viral RNA. This methodology used for DENV might be applied to other related flaviviruses (such as ZIKV), as well as other human viral pathogens.
Keywords: Dengue virus, Zika virus, flaviviruses
Supported by: Fundação para a Ciência e a Tecnologia—Ministério da Ciência, Tecnologia e Ensino Superior

BB.12 - The possible anticancer role of bioactive peptides from European amphibian skin secretions

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Bioactive peptides with potential therapeutic value have been isolated from amphibians for decades. They can exhibit many physiological activities such as antimicrobial, analgesic, antioxidant and anticancer (Xu and Lai 2015). Four peptides were isolated from the skin of Pelophylax perezi, an amphibian captured in Azores islands (Portugal), and later also produced by chemical synthesis. One in particular, PpT-2, was found to belong to the tryptophyllin family (TPHs) (Chen et al. 2004), a heterogeneous small peptide group characterized by the presence of a tryptophan together with one or two proline residues. TPHs bioactivity remains uncertain, but their neuromodulator and antioxidant role were already studied. Indeed, some TPHs showed a sedative effect on birds, together with relaxation events in rat arterial and urinary bladder smooth muscle, as bradykinin antagonists (Wang et al. 2009). Since bradykinin is implicated in cancer progression, their antiproliferative effect in human prostate cancer cells was also tested and the proliferation inhibition was proven to be effective (Wang et al. 2013, 2014). Therefore, in this work, we evaluated and further investigated the anticancer potential of PpT-2 and of three new histidine-rich peptides from Pelophylax perezi skin secretion, as they displayed a moderate antiproliferative effect against prostate cancer cells.

Keywords: Bioactive peptides, Tryptophyllins, Anticancer

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CA - Applications in Biomedical and Materials Science

CA.01 - Luminescence of complex cis-[Ru(phen)2(3,4Apy)2]2+ in model membranes

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The study of the interaction between the transition metal complex cis-$[Ru(phen)_2(3,4Apy)_2]^{2+}$ (RuApy) and cell model membranes was motivated by previous studies from our laboratory that demonstrate that RuApy has spectroscopic properties (in physiological environment pH 7,4: λ_{abs} = 480nm, ε_{480nm} = 9500 mol^{-1} Lcm^{-1} , λ_{em} = 655 nm, τ_{em} = 120 ns) that allow its use as a luminescent probe for the β-amyloid peptide(Aβ). These properties are important as the toxicity of Aβ can be influenced by those in neuronal cell membranes. In this context, this work investigates the interaction between RuApy and cell model membranes such as large unilamellar vesicles (LUVs) and giant unilamellar vesicles (GUVs) prepared with the negatively charged lipid DOPG. The luminescence auto suppression studies of RuApy in aqueous solution pH 7.4 (phosphate buffer) indicated a limit for the steady state luminescence studies at 60 μM. The complex was a sensitive probe in the presence of the DOPG(LUVs) vesicle, and this can be observed by the continuous suppression of RuApy emission at 655 nm. Studies carried out to determine the sensitivity of the RuApy complex against the LUV vesicle of DOPG indicated that 10 μM of complex is sensitive to a concentration of 5μM of vesicle. The results obtained so far indicate an electrostatic interaction between RuApy and DOPG(LUVs).

Keywords: fluorescence, model membranes, ruthenium complex
Supported by: CNPq, FAPESP and Capes

CA.02 - Improvement of the Methodological Strategies to Product Functionalizes Antibodies using Small Angle Neutron Scattering (SANS)

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Antibodies are used by jawed vertebrates for defense against invading pathogens. Usage of those versatile tools in a plethora of settings in clinics and biomedical sciences hinges on functionalization strategies that retain native antibody reactivity. To this date, antibody functionalization is performed by trial and error. We aim to reduce costs by providing general principles to allow the full spectrum of antibody functionalization by correlating functionalized antibody reactivity to cognate antigen by small angle neutron scattering, SANS, measurements and mathematical modeling of antibody and antibody-antigen super-complexes, obtained by titration experiments. For this research we have used for as antibody pure goat anti rabbit immunoglobulin, and for the antigen, pure Horseradish Peroxidase Preliminary results show that the systems (antibody and antibody-antigen complexes) do not change in the range of a temperature related to storage temperature (25º C), body temperature (37º C) and 40º C. These results will give us the pair distribution function of these systems and the results will be viewed in light of published precedence to highlight areas where future effort is needed to refine such versatile tools and improve their production. However, between the antibody and the complexes structure, different conformations were observed. The antibody has a globular structure with a radius of gyration around 33 Å, and the complexes display an elongated cylindrical shape with radius of gyration around 63 Å. This study shows how the scattering techniques (SANS) can provide useful information about the conformation of the antibody and antibody-antigen formation and help to shed light in the understanding the physical, chemical, and structural changes on the organization of these important antibody functionalization for the immunological system.

Keywords: Antibody-Antigen, Biophysics, SANS
Supported by: CNPq

CA.03 - Evaluation of Photoinduced Membrane Damage by Substituted Magnesium Porphyrazines

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Photodynamic Therapy (PDT) is an innovative and efficient treatment modality for a wide array of diseases, including infectious and many types of cancer. PDT is usually based on a photosensitizer (PS), its excitation by light and the subsequent energy or electron transfer to molecular oxygen ($^3O_2$), generating reactive oxygen species (ROSs), specially singlet oxygen ($^1O_2$). However, new experimental evidences suggest that contact-dependent reactions between the PS and biomolecules in the cell membrane – mainly lipids – are essential to cause membrane leakage, therefore unbalancing the chemical gradients that keeps the cell functioning and generating cell death. In order to advance in the development of this treatment, research on PSs that act by the mechanism of contact-dependent reactions with the cell membranes is essential. We have prepared a series of magnesium porphyrazines octa-substituted with fluor and trifluoromethyl groups (FMgPz and CF_3MgPz, respectively) and have investigated their properties in interaction with membrane models, more specifically SUVs and GUVs (small and giant unilamellar vesicles, respectively), which are made of unsaturated lipids. The membrane damage was investigated in the presence of the porphyrazines after red light irradiation (λ = 630 nm), which is the wavelength of maximum absorption by the porphyrazines (ε=~6x10 ^{4} L.mol $^{-1}$ .cm $^{-1}$). Both FMgPz and CF_3MgPz have equal $^1O_2$ quantum yields (Φ_Δ = 0,34). Our results showed a greater photoinduced membrane leakage in the presence of CF_3MgPz when compared to FMgPz. We attributed the higher efficiency of CF_3MgPz to the contact-dependent reactions between the CF_3MgPz and the vesicles, which also causes PS photobleaching. Interestingly, the photobleaching rate of CF_3MgPz was also far greater than that of FMgPz, indicating that the greater membrane damage is parallel with the higher photobleaching rate. Therefore, in order to develop more efficient PS, we need to consider strategies to have the bleached photosensitizer replenished during PDT. **Keywords:** Redox Biochemistry, Photochemistry, Membrane. **Supported by:** FAPESP

CA.04 - Effects of *Amburana cearensis* dichloromethane extract in cerebral ischemia models focusing on glial cells

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Glutamatergic excitotoxicity is a pathophysiological mechanism present in chronic neurodegenerative diseases (Alzheimer's Disease) and acute (Brain Ischemia), and it especially affects the hippocampus, as it has a high density of glutamatergic neurons. Under these conditions, neurons and oligodendrocytes can be severely affected. However, astrocytes can attenuate or prevent cell death through this mechanism by re-uptake excess glutamate. It is known that secondary metabolites of *Amburana cearensis* may be related to neuroprotection mechanisms against excitotoxic damage. The aim of this work was to investigate the neuroprotective effects associated with treatment with *A. cearensis* Dichloromethane Extract (EDAC) in models of cerebral ischemia. Hippocampal slices from wild-type Wistar rats (P6-8) or transgenic SOX10-EGFP and GFAP-EGFP reporter mice (P10-12) were used to identify oligodendrocytes and astrocytes, respectively. These slices were submitted in two models treated with EDAC: 1) oxygen and glucose supply (OGN) or deprivation (OGD); and 2) in organotypic culture (OHSC) submitted to glutamate excitotoxicity. Protein expression, cell morphology, cell viability and genetic transcription tests were performed. Under OGD conditions, our results showed that EDAC prevented the reduction of cellular processes of SOX10 expression, without an increase in the expression of astrocytic proteins between the OGN and OGD control groups. However, EDAC increased GFAP expression under OGD conditions. In OHSC, we observed that excess glutamate induced an increase in cell death and that this was inhibited by treatment with EDAC. However, under these conditions, EDAC does not protect neurons. On the other hand, GFAP, GLT1, GLAST and GS were overexpressed in cultures treated with EDAC under glutamatergic excitotoxicity. We also observed, by RT-qPCR, a slight increase in transcription in GDNF, GLT1, GS, NGF and OLIG2 in EDAC-treated hippocampal slices. Our results demonstrate that EDAC has a potential pharmacological effect in brain ischemia models. **Keywords:** Amburana cearensis, Neuroprotection, Glia. **Supported by:** FAPESB, CAPES and CNPq

CA.05 - PROJECT: Multiplatform Metabolomic Prospection of Biomarkers and Biochemical Aspects of Sickle Cell Disease and Osteonecrosis Secondary to Sickle Cell Disease.

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Sickle cell disease is the most frequent hemoglobinopathy in Brazil and is a worldwide public health problem, with a great impact on the morbidity and mortality of the affected population. In patients with this pathology, the predominant clinical manifestation of the joint is osteonecrosis, which commonly progresses to a terminal disease. Metabolite profiling approaches in complex systems have become a powerful tool to investigate metabolic processes, identify potential biomarkers and unravel metabolic reprogramming in various diseases. Thus, metabolomic studies can reveal new ways to study this pathology as well as a better understanding of the sickling process of red blood cells, dysregulated pathways, unrecognized biomarkers, and new therapeutic possibilities. In this context, this work aims at identifying biomarkers related to osteonecrosis secondary to sickle cell disease, using nuclear magnetic resonance and high-performance liquid chromatography in a multiplatform metabolomics approach. Blood plasma from control individuals and from sickle cell disease patients with and without osteonecrosis will be used. Based on the clinical stage of osteonecrosis, graded with the modified Ficat and Arlet method, the samples will be classified as early, intermediate and advanced stage of osteonecrosis. After processing the data, the identification of metabolites will be carried out using the Chenomx NMR Suite 8.4 software and the search in a database (Metlin, HMDB). The metabolic pathways will be associated through the KEGG metabolic pathway bank. The statistical treatments of the data will be performed in MetaboAnalyst 3.0 software. Preliminary results identified differences between the metabolic profile of the study groups in this work, as well as a map of metabolic alterations associated with sickle cell disease. The present study may provide support for the validation of biomarkers for osteonecrosis secondary to sickle cell disease.
Keywords: metabolomics, osteonecrosis, sickle cell disease

CA.06 - Interleukin-10 gene promoter region polymorphism is associated with worse sudden cardiac death risk and echocardiographic parameters in chronic Chagas disease

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Functional genetic polymorphisms involved in the immune response may modulate the clinical variability in Chagas disease (CD). Several studies have proposed a protective role for IL-10 in CD pathophysiology. One study found an association between the G allele in rs1800896 single nucleotide polymorphism (SNP) and the cardiac form of CD, while others were not able to find the same results. This study aimed to evaluate the association between rs1800896 SNP and clinical and echocardiographic manifestations in CD patients. We conducted a cross-sectional study with 185 chronic CD patients, of which 95 were male and 90 females, with a mean age of 47,9 ± 11,6 years. They were genotyped by the polymerase chain reaction-restriction fragment length polymorphism method. The clinical forms, risk of sudden death (Rassi score), the score of cardioembolic ischemic stroke scores, and echocardiographic parameters were obtained by accessing the patients' medical records. P-value < 0.05 was considered significant. We observed that 72 patients presented the AA genotype, 96 AG and 17 GG. The expected and observed genotype frequencies obeyed the Hardy-Weinberg equilibrium. The statistical analyses conducted showed no significant associations between the allelic and genotypic frequencies and the clinical forms of chronic CD (cardiac, digestive, or cardiodigestive) nor the score of cardioembolic ischemic stroke. On the other hand, the G allele was associated with higher sudden cardiac death risk (Rassi score) (p=0,025), systolic (p=0,004) and diastolic (p=0,035) left ventricular diameters above echocardiographic reference values and lower left ventricular ejection function (p=0,026). This study found an association between the G allele of rs1800896 and worse sudden death risk and echocardiographic parameters compatible with CD dilated cardiomyopathy pathophysiology. Therefore, rs1800896 polymorphism seems to be a promising biomarker for CD, although other studies may be necessary to reinforce those findings.
Keywords: Chagas Disease, Interleukin-10, Single Nucleotide Polymorphism. **Supported by:** CNPq and CAPES

CA.07 - Molecular cloning, expression and purification of Fbxl17 fragments for antibodies produce

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Fbxl17 is one of the 69 human F-box proteins, which interacts with SKP, Cullin and RBX to form the SCF-type E3 ubiquitin ligases. In addition to the F-box domain of interaction with SKP1, Fbxl17 contains the LRR domain (Leucine Rich Repeat) which interacts with its substrates. Analysis of 1992 patient samples from the METABRIC (Molecular Taxonomy of Breast Cancer International Consortium), showed that FBXL17 was mutated in 135 samples in the region that encodes its LRR domain. Truncating Fbxl17 LRRs impaired its association with other SCF components and decreased its ubiquitination activity. We are investigating the consequences of FBXL17 truncations in cellular level, therefore, we are interested in producing antibodies for use in western blotting and immunofluorescence. Clonning, expression and purification of the N-terminus, F-box and C-terminus fragments of Fbxl17 from bacterial expression vectors. To obtain anti-Fbxl17 antibodies from these fragments that interact with Fbxl17 in mammalian cells by western blotting. Synthetic FBXL17 codon usage to bacteria was used as template to cloning Fbxl17 fragments into pET28a vector. Induction of heterologous expression was performed with IPTG 0,2 µM at 37 ºC for 4 hours. Insoluble Fbxl17 N-terminus were purified by affinity chromatography and submitted to SDS-PAGE. The Coomassie stained band of Fbxl17 N-terminus was excised and used to immunization of rabbits. The specificity of total immunoglobulins obtained were tested in western blotting. Cloning of the Fbx117 fragments into pET28a was confirmed by sequencing. The N-terminus fragment was expressed in insoluble fraction and purified. The anti-Fbxl17 N-terminus was specific and selective for the purified fragment, however did not probe Fbxl17 from cell extracts. The anti-Fbxl17 N-terminus obtained was unable to identify Fbxl17 in cell extracts, but it will be used in protein-protein interaction assays when this fragment was used as a bait.
Keywords: Fbxl17, breast cancer, E3 ubiquitin ligase
Supported by: FAPESP

CA.08 - Development of an electrochemical device for the fast and label free detection of the biomarker ADAM-33 related to the triple negative breast cancer

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The classification of breast cancer is defined by clinicopathologic parameters and immunohistochemical markers. Recently, the lack of expression of ADAM-33 was related to the triple negative breast cancer, basal-like markers and correlated with shorter overall survival and metastasis-free survival. Usually, the detection of ADAM-33 is performed by immuhistochemistry, which is laborious and time consuming. In this context, electrochemical immunosensors are a good alternative for protein detection as they may provide fast, straightforward, and label-free determinations. We developed an electroanalytical immunosensing platform consisting of a gold electrode modified with self-assembled monolayers of cystamine with anti-ADAM-33 IgG monoclonal antibodies covalently attached. Cyclic voltammetry and electrochemical impedance spectroscopy (EIS) experiments were employed to characterize the immunosensor. The ADAM-33 detection was based on the variation of the charge transfer resistance (∆Rct), obtained through EIS using [Fe(CN)6]3-/4- as probe, after incubating the immunosensor in the presence of the biomarker. ∆Rct increased linearly with ADAM-33 concentration in the range of 0.5-1.5 µg mL-1, with a detection limit of 0.31 µg mL-1. The immunosensor was able to successfully detect ADAM-33 in cell extracts from human breast cancer lines, without suffering significant influence from other coexisting biomolecules. The results suggest that the developed device is a promising, feasible, and sensitive tool to detect ADAM-33 in breast cancer isolated material. Thus, it could consist in an alternative to support fast breast cancer diagnosis and prognosis.
Keywords: breast cancer, ADAM-33, electrochemical immunosensor
Supported by: UEPG, Propesp, C-LABMU, Fundação Araucária, CNPq and CAPEs

CA.09 - Direct visualization of virus removal process in hollow fiber membrane using an optical microscope

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Virus removal filters developed for the decontamination of small viruses from biotherapeutic products are widely used in basic research and critical step for drug production due to their long-established quality and robust performance. A variety of imaging techniques have been employed to elucidate the mechanism(s) by which viruses are effectively captured by filter membranes, but they are limited to 'static' imaging. Here, we propose a novel method for detailed monitoring of 'dynamic process' of virus capture; specifically, direct examination of biomolecules during filtration under an ultra-stable optical microscope. Samples were fluorescently labeled and infused into a single hollow fiber membrane comprising cuprammonium regenerated-cellulose. While proteins were able to pass through the membrane, virus-like particles (VLP) accumulated stably in a defined region of the membrane. After injecting the small amount of sample into the fiber membrane, the real-time process of trapping VLP in the membrane was quantified beyond the diffraction limit. The method presented here serves as a preliminary basis for determining optimum filtration conditions, and provides new insights into the structure of novel fiber membranes. For details, please refer the following publication. https://www.nature.com/articles/s41598-020-78637-z
Keywords: molecular imaging, dynamics of virus filtration, VLP visualization

CA.10 - Imidazolium-based ionic liquids as additives to preserve green fluorescent protein activity at room-temperature and under stress

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Advances in biotechnology have allowed the development of fluorescent proteins (FP) for several industrial applications. However, there are still difficulties in their use at large scales and in novel fields due to the low stability of FP, which limit their application, distribution, and storage. The discovery of additives capable of preserving the activity of FP at room temperature and under stress conditions is needed to help expand and facilitate their commercial use. Hence, we aimed to evaluate the use of ionic liquids (ILs) as additives capable of preserving the activity of the Enhanced Green Fluorescent Protein (EGFP), an important biomarker and biosensor, at different storage times and under unfavorable conditions. We evaluated the effect of 1-alkyl-3-methylimidazolium chloride-based ILs ([Cnmim]Cl) aqueous solutions on EGFP fluorescence at short (48 h) and long-term studies (3 months), and then their ability to protect the EGFP in the presence of denaturing agents. All [Cnmim]Cl ILs (at 0.100 M) were able to preserve EGFP fluorescence for longer than the phosphate-saline buffer (PBS) and NaCl solutions, increasing from 1 to 3 months. ILs solutions with shorter to medium cation alkyl chain length were the most effective for maintaining EGFP fluorescence, as well as protectors of EGFP activity in the presence of the surfactant SDS, an acid of guanidine hydrochloride, and for H2O2. [Cnmim]Cl solutions can be added to aqueous solutions to preserve EGFP fluorescence activity at room temperature for long-storage times and to reduce the negative impact of denaturing agents on EGFP. Therefore, there is a massive potential for the application of ILs as additives to preserve FP in the long-term without refrigeration and under unfavorable conditions, which is fundamental to expanding their industrial and commercial uses.
Keywords: protein stability, ionic liquids, preservatives
Supported by: FAPESP (projects 2018/50009-8, 2014/19793-3, 2014/16424-7, 2018/25511-1, 2016/07529-5, 2018/06576-5, 2020/14144-8, 2018/01858-2, 2018/20833-0, CAPES (001), CNPq, ATN, FCT (projects UIDB/EQU/00102/2020 and UIDP/EQU/00102/2020).

CB - Biotechnology and Biomaterials (agricultural, human and animal)

CB.01 - A new approach for purification of the catalytic site of the Angiotensin Conversion Enzyme, N domain, mediated by the ELP-Inten system

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Angiotensin-converting enzyme I, ACE, is a key part of the renin-angiotensin system whose main function is to regulate blood pressure and balance of salts in the body. ACE1 has two isoforms, somatic, sACE, and testicular, tACE. sACE possesses two domains, N- C-, with catalytic sites which exhibit 60% sequence identity. These domains differ in terms of chloride-ion activation profiles, rates of peptide hydrolysis and sensitivities to various inhibitors. N-domain has specific action in the hydrolyze of Alzheimer's diseases beta amyloid bodies and angiotensin 1-7, which active the MAS receptor and triggering anti-thrombotic and anti-inflammatory actions. The objective this work was to obtain catalytic site Ala361 to Gli468 of the N-domain region, csACEN, isolation without chromatographic and denaturant chemical process. For that, a new methodology was used in the expression of the csACEN peptide, in which the peptide was linked to the elastin-like polypeptide, ELP, and Intein, and expressed at 37C. The characterization of catalytic site was made by SDS-PAGE and dot blotting. The culture temperature at 37C significantly increased the expression of the ELP/Intein/csACEN fusion protein. This culture was lysed at a low temperature allowing the fusion protein to become soluble. The precipitation of ELP at high concentrations of ammonium sulfate were obtained in 0.57 M and 0.8 M. Intein autocleavage occurs at acidic pH and it is important to pay attention to: pI 6.65 for csACEN and pI 6.87 for ELPcsACEN, which are very low. The best autocleavage efficiency was with MES and TriHCl buffers, pH 6.3 and 6.8, respectively, in which pure csACEn peptide was obtained. The strategy used to obtain the Ala361 to Gli468 catalytic site in soluble and pure form was obtained with success and the protocol for obtaining similar peptides was established. **Keywords:** N Catalytic site of ACE1, elastin-like polypeptide/Intein, high temperature of cultivation

CB.02 - Entrepreneurial university: the search for transfer of technology from a schistosomiasis diagnostic kit.

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Schistosomiasis is a neglected tropical disease caused by the helminth infection from species of the genus Schistosoma. Globally, it is estimated that the disease affects over 250 million people in 78 countries of the world and is responsible for some 280,000 deaths each year. In the Americas, the only pathogenic human schistosome species is *Schistosoma mansoni* (*S. mansoni*). Available methods for the diagnosis of schistosomiasis comprise microscopic, molecular and serological approaches, with the latter detecting antigens or antibodies associated with *S. mansoni*. Throughout the last decades, efforts are aimed at developing news strategy for diagnostic to be used especially for low intensity infections in lowly endemic areas. With a view to innovation and technology transfer from the university to the private company, the objective of this work is to evaluate the optimization parameters of an ELISA diagnostic kit using rationally designed chimeric proteins. Thus, one of the steps evaluated was coating buffer using PBS pH7.2, Tris-HCl pH8.5 and coating buffer provided by the company were used. The conditions established for the ELISA were: 96-well polystyrene microplates, concentration of protein 140ng/well, dilution of serum 1:20, the conjugate was an anti-IgG antibody peroxidase-conjugated dilution of 1:5.000 (v/v), and measure the absorbance at 405 nm. The ratio of positive to negative was used to determine which of them is the best to discriminates the samples. The Tris-HCl pH 8.5 coating buffer showed the best discrimination between positive and negative samples when used SM1 protein; however, the company's coating buffer was better to discriminate the same samples groups when used SM2 protein. In this way, SM2 protein is more advantageous than SM1 protein and it had better results with the company's coating buffer standardized. In conclusion, the protein SM2 is a potential tool to be investigated for schistosomiasis diagnosis on an industrial scale. **Keywords:** S. mansoni, innovation, diagnosis. **Supported by:** UFSJ, CNPq, and Capes

CB.03 - The Presence of Solvents can Induce Cubic-to-Inverted Micelar and the Cubic-to-Hexagonal-to-Inverted Micellar phase transitions on phytantriol-based cubosomes

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Cubosomes are nanoparticles composed of a specific combination of amphiphilic lipids, like phytantriol (PHY), and a nonionic polymer. They have a high hydrophobic volume, as compared to regular liposomes and are good candidates for drug delivery applications. Due to their unique structure, these nanoparticles possess the ability to incorporate highly hydrophobic drugs. A challenge for the encapsulation of hydrophobic molecules is the use of organic solvents in the sample preparation process. In this study, we investigated the structural influence of different solvents (acetone, ethanol, chloroform, octane, DMSO, and methanol) on the inner structure of cubosomes. Small Angle X-ray Scattering (SAXS) technique was used to get the structural information and the phase behavior of cubosomes. The presence of high amount of acetone and ethanol (around 16% v/v) are not able to change the cubic symmetry (Pn3m), despite the increase in the unit cell parameter, observed in the case of ethanol. In both cases, a cubic-to-inverted micellar phase transition was observed. Chloroform and Octane, on the other hand, have different effects over the cubosomes as compared to acetone and ethanol, both of them induced a cubic-to-reversed hexagonal phase transition at ~0.25 and 2.5 v/v% for chloroform and octane respectively. those are attributed to the insertion of the solvent in the hydrophobic region of the cubosomes, increasing its volume and inducing such a transition. Before this phase transition, no change in the unit cell parameter was observed at smaller solvent concentrations. Moreover, a second phase transition from reversed hexagonal-to-inverted micellar was observed 1.0 and 17 v/v% for chloroform and octane, respectively. Our data also suggest that after 24 hours of solvent/cubosome incubation, some structural features of cubosomes changes. We believe that this study could shed light on researchers working on drug delivery systems using cubosomes.

Keywords: Cubosome, nanoparticles, solvents, SAXS, phase diagram, lyotropic liquid crystal, LLC,
Supported by: FAPESP, CNPq, CAPES

CB.04 - Rubella diagnostic assay using rp1, a novel rationally designed antigen

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Rubella is a disease of epidemiological importance, especially when pregnant women are infected, causing congenital rubella syndrome. Thus, the specific diagnosis is essential since the clinical manifestations are similar to other diseases. In this context, the development of local antigens with high sensitivity and specificity, at low cost, can be a promising alternative compared to imported antigens. This study aimed to the development of an in-house IgG Rubella-specific ELISA using a novel antigen. The new antigen was designed and predicted by bioinformatic tools. After synthesis, two in-house ELISA assays were performed: The first one, for titration of the peptide and the other one to evaluate the reactivity of the peptide against different human sera. Two potential epitopes were predicted as the potential antigenic target was selected, originating the RP1. The results of the in-house ELISA showed an optimal concentration of 200 ng of peptide/well. This new peptide was able to discriminate between positive and negative human samples. Our results demonstrate the potential of the peptide for the detection of rubella-specific IgG antibodies, being use full for the development of national kits in the future.

Keywords: rubella virus, german measles, diagnosis rubella
Supported by: CNPq

CB - 05 - Photopolymerizable liposomes: Biophysical Caracterization

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The design of nano-photopolymerizable liposomes relies on two advantages: lipid self-assembly properties and photoactivable bonds in diacetylene lipids moiety along their acyl chain. Closely packed and adequately ordered, diacetylene lipids undergo UV irradiation polymerization via 1,4-addition to form alternating ene-iyne polymer chains upon irradiation with 254 nm light. Lipopolymers can aggregate into various vesicular and non-vesicular assemblies depending on the lipid concentration, structure, and processing conditions. Thus, this study aimed to evaluate the potential use of the binary mixtures of polymerizable diacetylene lipid 1,2-bis(10,12-tricosadiynoyl)-sn-glycero-3-phosphocholine (DC8,9PC) with DMPC, DOTAP, DSPE PEG 2000 and DSPE PEG 2000 amine and ternary mixtures of DC8,9PC:DOTAP: DESPE PEG 2000 and DC8, 9PC.DMPC. DSPE PEG200 amine. An overview is given over UV diacetylene nano-sized liposomes, their characterization structurally and functionally. The lipopolymers were studied by UV-Vis, and FTIR spectroscopy, Bright Field, Confocal microscopy, DLS, and its interaction with red blood cells. The degree of polymerization over 35 UV irradiation cycles and polymer length formed (between 5-11 monomers units) was calculated. We performed a detailed deconvolution analysis of ATR-FTIR peaks of the DC8,9PC over the range 2300-1900 cm-1 shows, an expected significant change: reducing the triple carbon bond, accompanied by the increase of a double carbon-carbon bond (C=C), which is initially absent in monomers. Region 1750-1650cm-1study the hydrogen bonding of the headgroup. The deconvolution revealed at least three main peaks in the monomer state and broad background peaks that appear upon UV exposure. The region 1500-1450cm-1 corresponds to CH2 in-plane bending and CH2 symmetric stretching. They show an anisotropic packing of alkyl chains. According to its biophysics, the NPs are spherical with amorphous-like areas, and micro-crystal domains were observed with variable-sized liposomes. The liposomes had variable toxicity reaction for red blood cells according to lipids involved. **Keywords:** Polymerizable Liposomes, biophysics, drug delivery

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CB.06 - Evaluation of the immunomodulatory effect of the rhodium chloride/polycyclodextrin complex

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The Rhodium chloride ($RhCl_3$) shows several biological activities, such as anti-inflammatory, and its complexation with polycyclodextrin ($RhCl_3$/pCD) could enhance its biological activities.The aim of this study was to synthesize and characterize the $RhCl_3$/pCD and to evaluate its immunomodulatory effect in macrophage response and in carrageenan-induced paw edema model. Materials and methods: Physical-chemistry characterizations of RhCl3/pCD were performed by FTIR, 1 H-NMR and ITC. RAW264.7 was cultured in 96-well plates at 2×10 5 cells·mL -1 in supplemented RPMI-1640. Cells were maintained for 3h in the presence or absence of RhCl3/pCD. NF-κB expression was measured in RAW264.7 cells stimulated with LPS (1 μg·mL -1) and IFN-γ (0.9 ng·mL -1). The paw edema model was induced by carrageenan (2.5%) injection (20 μL) into the left footpad, and 20 μL of PBS into the right footpad, of all groups. The left and the right paws were measured at 1, 2, 3 and 4h after the injection of carrageenan and the difference were calculated. 30 minutes before the paw edema induction, the $RhCl_3$/pCD (5 mg/Kg) were administered intraperitoneally (100 μL). FTIR and 1 H-NMR confirmed the formation of the complex in the solid state and in solution. Moreover, ITC suggest a 1:3 $RhCl_3$/pCD stoichiometry, per monomer of pCD. The $RhCl_3$/pCD was more potent to reduce the NF-κB expression in RAW 264.7 and J774 A.1, than $RhCl_3$, at all tested concentrations. Moreover, both RhCl3 and $RhCl_3$/pCD were able to reduce paw edema. Both $RhCl_3$ and $RhCl_3$/pCD were able to reduce the paw edema. However, the $RhCl_3$/pCD showed better ability to reduce the NF-κB expression than $RhCl_3$, suggesting a better imunomudulator effect. Further studies are necessary to determine the molecular mechanisms of the immunomodulation. **Keywords**: $RhCl_3$, Cyclodextrin, immunomodulation

Supported by: FAPEMIG, CNPq, CAPES and UFJF

CB.07 - Project - Efficacy and safety of magnetic-fluorescent nanoparticles for tracking mesenchymal stem cells

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Stem cells are characterized by their capacity for self-renewal and differentiation into several specialized cell types. Interest in these cells has increased in recent years due to their plasticity and regenerative potential. For the implementation of advanced therapies, such as therapies with mesenchymal stem cells, pre-clinical and clinical studies that demonstrate the absence of adverse effects and the presence of desired therapeutic effects are essential. Stem cell tracking allows obtaining information on the lifespan and better route of cell administration, enabling a greater understanding of the effects of these new therapies. The superparamagnetic iron oxide nanoparticles have been explored with the aim of tracking stem cells, as they have applicability in clinical studies in the construction of magnetic resonance images, allowing the comparison of functional data with tracking data simultaneously, in addition to being biocompatible. This project proposes evaluate the efficacy and safety of magnetic-fluorescent nanoparticles to label mesenchymal stem cells for posterior tracking. The nanoparticles used in this project will be the Molday ION Rhodamine-B (MIRB), efficacy and safety evaluations will be carried out in mesenchymal stem cells extracted from human adipose tissue. To assess viability, the MTT colorimetric test will be performed; proliferation and cell death will be evaluated by immunocytochemistry using antibodies to anti-ki67 and anti-caspase 3, respectively; and the cells will be induced to differentiate into adipocytes, osteocytes and chondrocytes. With this project we hope to determine if MIRB is efficient and safe for tracking mesenchymal stem cells for long-term use in preclinical models.
Keywords: mesenchymal stem cells, cell tracking, superparamagnetic iron oxide nanoparticl
Supported by: FAPERJ and CNPq

CB.08 - PROJECT: *In silico* molecular characterization of the prenyltransferase gene family and its contribution to the genetic improvement of Ricinus communis

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Ricinus communis L. is an oilseed species with great potential for pharmaceutical and other industrial applications. Thus, it is important to research mechanisms to guarantee its production despite the abiotic stress conditions that most crops are subjected to, since the largest planting in Brazil is in the semi-arid area of the northeast region. The prenyltransferase (PT) gene family is responsible for the formation of enzymes that catalyze the formation and structural modification of isoprenoids that give rise to an extensive group of natural products present in plants and involved in primary and secondary metabolisms and responses to environmental stresses. These products can have cis and trans stereochemistry and in other species, such as A. thaliana, they have highly conserved domains. In this project, bioinformatics will be applied to characterize this important gene family, whereas *in silico* gene expression patterns of PT genes will be analyzed by microarray. R. communis PT genes will be submitted to several bioinformatics tools such as phylogenetic analysis, identification of conserved domains, prediction of cell sublocation, physical-chemical characteristics as well as analysis of the promoter upstream regions. The presence of conserved domains PF00432, PF01239, PF01255, PF01040 and PF00348 referring to prenyltransferases identified based in PFAM domain database will assist the characterization of the genes. The results of this project aim to promote the understanding of the genes of the PT family and establish a basis for the study of this family in R. communis, as well as the possibility of developing strains more resistant to abiotic stresses.
Keywords: bioinformatics, prenyltransferases, R. communis

CB.09 - Development of Sars-CoV2 Spike protein binding peptides for covid diagnosis

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COVID-19 is an infectious disease caused by the new coronavirus called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The virus is highly contagious and can be transmitted through direct and indirect contact with infected people through respiratory droplets when sneezing, coughing, or even speaking. Real-time polymerase chain reaction (RT-PCR) testing is the standard reference for confirming Sars-CoV2 infection, and with the rapid increase in the number of infected people, most countries are finding a shortage of diagnostic kits. This project intends to develop a new molecule capable of binding to the virus that causes COVID-19 to develop new diagnostic kits. Through protein engineering techniques and prior knowledge about the interaction between the virus and its receptors in the human host cell, the project generated a chimeric peptide with binding capabilities to SARS-CoV-2. Computational analyses were performed to create a peptide with the binding domain of the human ACE-2 coupled to a rigid peptide that improves stability. The synthetic gene for this peptide was acquired, followed by protein expression and purification. ELISA assays using the Sars-CoV2 Spike protein as an antigen were successful. Further studies are necessary to validate new diagnostics tests using the protein developed in this study.
Keywords: diagnosis, Immunoassays, SARS-CoV-2
Supported by: CAPES

CB.11 - Identification of beta-casein A1 and A2 in cow kinds of milk using a chicken IgY based ELISA

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Beta-caseins A2 and A1 are the most abundantly proteins found in bovine milk. Each animal, depending on its genetics, can produce proteins A1 and A2 simultaneously or independently protein A1 or A2. The difference between protein A2 (ancestral origin), is a mutation suffered in the codon responsible for position 67, with a substitution of a proline for a histidine. A bioactive peptide known as beta-casomorphine-7 (BCM7), which is derived mainly from partial proteolysis of version A1, has been linked, by several clinical studies, as the trigger of several serious disorders of the human gastrointestinal system. The objective of the project was to develop an immunodiagnostic method based on antibodies capable of recognizing, in a discriminatory way, beta-caseins A1 and A2 in milk samples from producing cows. With this detection using our method, due to the low cost of the methodology when compared to genetic sequencing methods, we will be able to identify A2A2 milk producing matrices of the order of thousands of animals in a relatively short time, expand the milk market A2 and ascertain the quality of batches produced in the dairy industry. In the immunodiagnostic test type ELISA, IgYs were used as primary antibodies from international production and another from national production. For better test reliability, the 25 animal samples were previously genotyped. The sensitivity and specificity were evaluated, obtaining sensitivity and specificity of 100% when using the commercial IgY anti-A1 antibody and 95.2% sensitivity and 100% specificity when using the national anti-A1 antibody. Thus, the technique developed with both primary antibodies could differentiate the A2A2 milk from A1A1 and A1A2 types.
Keywords: Dairy industry, ELISA, Milk A2,

CB.12 - Project: Use of anti-pgl 1 serology as an active search tool for sources of infection by *Mycobacterium leprae* in hyperendemic municipalities – Mossoró/RN

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Leprosy is an infectious and contagious disease, caused by the bacillus *Mycobacterium leprae*, which can cause physical disabilities, becoming a worldwide public health problem. In 2019, Brazil ranked second in the detection of new cases, with Rio Grande do Norte being the state with the lowest detection coefficient, however, from 2001 to 2013, about 20% of its university students had very high or hyperendemic coefficients. Among them is the municipality of Mossoró, in the western region. Therefore, the present study intends to investigate the usefulness of anti PGL-1 serology through the rapid test to detect cases of multibacillary leprosy in residents of the hyperendemic area of the city of Mossoró / RN, contributing to blocking the transmission of M. leprae to the community, through an innovative strategy. The collection of 500 people belonging to the male sex has been carried out since June 2021 at the Laboratory of the Clinical Center Vingt-un Rosado, in Mossoró / RN. The greetings are identified by the laboratory team, after identification, they are transported in a thermos box to the Laboratory of Biochemistry and Molecular Biology - BIOMOL of the State University of Rio Grande do Norte - (UERN). Serology for anti-PGL-1 analysis is performed by depositing 5 µl of serum into the lateral flow test receptacle (ML Flow) together with carrier solution. Results are interpreted after 10 minutes, and the test positivity is scaled up according to the color intensity of the test line. According to the serological analyzes of 101 that were carried out from June to July 2021, with approximately 17 analyzed weekly, a total of 8 desired tested positive and 7 were considered doubtful, even with the preliminary results, this is a project that aims to screen patients and colonize through clinical diagnoses.

Keywords: Leprosy, Epidemiology, Serology.

CB.13 - Toxicity evaluation of *Bauhinia monandra* extract containing phytochemicals and leaf lectin (BmoLL) on embryos of *Biomphalaria glabrata*

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Biomphalaria glabrata mollusks are the main vector of schistosomiasis in Brazil and the search for natural molluscicides interrupting the parasite evolutionary cycle is necessary. Plants of the Bauhinia genus are widely found in endemic continents for the disease and leaves of Bauhinia monandra contain a BmoLL lectin with biocidal action. To analyze the extract (E) from leaves of B. monandra and the embryotoxic effect on B. glabrata. E was obtained with powder from B. monandra leaves in 10 mM citrate-phosphate buffer, pH 6.5, containing 0.15 M NaCl. Secondary metabolites were investigated by thin-layer chromatography (TLC); quantitative protein, hemagglutinating activity (HA) and specific HA (SHA) were evaluated. B. glabrata embryos at the blastula, gastrula, trocophore, veliger and hippo stages (300 of each) were exposed to E for 24 h at different concentrations (12.5-600 µg/mL). Subsequently, the embryos were washed and transferred to plates with filtered and dechlorinated water, analyzed with a magnifying glass for their viability (normal) and unviable forms (malformations and death). TLC detected cinnamic derivatives, flavonoids and saponins; protein concentration (21.2 mg/mL) and SHA (96.6) confirmed the lectin presence. In the concentration of 100 µg/mL, E provided 65.3% and 88.7% of unviable blastula and gastrula, respectively, while at 200 µg/mL 100% of unviable embryos were observed for both stages. In trocophore 400 and 600 µg/mL of E made 90.3% and 100% of non-viable embryos. At the same E concentrations, the velliger and hippo stages showed lower unviability at 400 µg/mL of 59.3% and 51.6%, respectively, and 100% of malformations and/or deaths at 600 µg/mL. E showed teratogenic/toxic effects in all evolutionary stages of B. glabrata, proving to be an efficient molluscicide.

Keywords: Lectin, Embryotoxicity, Schistosomiasis

CB.14 - Bactericide activities of purified tripsin inhibidor from seeds of Enterolobium timbouva

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Among the biggest problems facing the world, microorganism resistance to antibiotics could, by 2050, kill more than 10 million people. Within the biomolecules studied for new antibiotics, protease inhibitors from plant organs have shown high antimicrobial and antibiotic potential. In this study we aimed to verify the bactericidal, antibiofilm and synergistic activities of a trypsin inhibitor from the seeds of *Enterolobium Timbouva* (EtTI). Using five Gram-positive and five Gram-negative bacterial species, the minimum inhibitory concentration was defined as the lowest concentration of EtTI capable of inhibiting visible microbial growth by the Müller Hinton broth microdilution technique. The minimum bactericidal concentration was the lowest concentration of EtTI that did not allow bacterial growth on solid medium. The effects of EtTI on bacterial biofilm inhibition and on mature biofilms were done in 96-well microplates and after the incubation period, seeded onto BHI agar plates for counting. Mechanism of action: SYTOX Green was used as indicator of membrane damage. To evaluate the synergistic activity, the test, also called "chessboard", was based on the CLSI protocol. EtTI had a MIC/MBC for *S. haemolyticus*, *S. saprophyticus* and *S. epidermidis* and only MIC for *E. coli*. EtTI showed the best result for the *S. saprophyticus* strain, with MIC and MBC of 4.5 µM. This strain was the chosen for antibiofilm treatments with MIC and 10 X MIC that caused reduction 61,5% in biofilm formation and 50% in mature biofilm. EtTI had a linear damage kinetics pattern, with 48% death after 2 hours. In addition, EtTI was shown to be synergistic with the standard drug vancomycin (ΣFIC: 0,28µg/mL), reducing the MIC value for EtTI by 32 times and the MIC of vancomycin by 4 times. In conclusion, the biological activity of EtTI shows the potential as antimicrobial and, as further steps are taken, possible drug.
Keywords: Biofilm, Synergism , SYTOX Green; **Supported by:** FUNDECT

CB.15 - Metal-Organic Frameworks Formed by Nucleotides, Transition Metals and Quantum Dots

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Nucleotides are excellent metal ligands able to stablish coordination interactions with metal ions, specially with transition metals. The co-assembly of nucleotides and metal ions through coordination interactions at specific sites give raise to nanoscopic scaffolds called metal-organic frameworks (MOFs). These materials display a range of promising applications in biomedicine including drug loading, molecular delivery and bioimaging. In the current work, we perform the synthesis and structural characterization of MOFs based on coordination polymers formed between nucleotides, metal ions and quantum dots. OBJECTIVE: our purpose is to develop a synthesis protocol to produce complexes involving purine-based nucleotides, metal ions and ZnO quantum dots. We aim to provide detailed characterization on the structure of these nanostructured materials and test their *in vitro* behavior toward tumor cell lines. The synthesis protocol consists in the solubilization of guanosine or adenosine monophosphates, which have versatile binding sites, in the presence of metal ions such as Ca, Mg, Mn, Fe, Co, Cu, Ni and Ag. ZnO quantum dots (QDs) are prepared through a hydrolisis reaction of zinc acetate in the presence of sodium hydroxide and methanol. This procedure leads to the formation of QDs that emit greener light upon UV irradiation. The QDs are co-solubilized with nucleotides and metal ions leading to the spontaneous formation of nanoparticles over a course of hours or days. To perform structural and morphological characterization of the complexes, we have used fluorescence microscopy, atomic force microscopy (AFM), fluorimetric assays and circular dichroism (CD). The characterization techniques allowed to select complexes that are most viable for the research. Interestingly, metal ions that participate in biological roles (e.g., Ca and Mg) have led to better results in comparison to those that do not have a biological function. The formation of complexes has been observed for atoms with neighbors regarding their valence layers and having relatively similar atomic masses. Importantly, the incorporation of ZnO has been observed and complexes have been found to form well ordered, crystal-like, nanoscopic assemblies. A viable protocol to produce ordered nanoparticles with QDs embedded in the crystal structure has been developed. From the results obtained so far, the nanostructured materials are promising for tests with cell membranes.
Keywords: coordination polymers, quantum dots, metal-organic frameworks; **Supported by:** CNPq

CB.16 - PROJECT Characterization of the antifungal activity of metabolites from native Brazilian plants against the strains of the genus Cryptococcus and Candida

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The frequency of invasive fungal infections caused by opportunistic pathogens has increased worldwide. Cryptococcosis and candidiasis are systemic fungal infections of great medical relevance worldwide. Therapeutic options for the treatment of these infections are restricted, both because of the increased resistance of *Cryptococcus* and *Candida* strains and because of the drugs used and the high toxicity of antifungal agents for the human body, all of which have made drug treatment difficult. Thus, the search for prototypes for the development of new antifungal agents is an important public health issue and requires investment in research so that new drug candidates can be discovered. Thus, this work aims to characterize the antifungal activity of native plants in Brazil against strains of the genus *Cryptococcus* and *Candida*. The extracts and fractions and/or subfractions with the best anticryptococcal and anticandida activity will be subjected to chemical characterization (high performance liquid chromatography and nuclear magnetic resonance) and cytotoxicity analysis. Regarding the antifungal activity, the Minimum Inhibitory Concentration (MIC) and the fungicidal/fungistatic concentration against *Cryptococcus* and *Candida* strains will be determined. After this initial screening, tests will be carried out to understand the effect of extracts on the oxidative stress of fungal cells (dosing of antioxidant enzymes, reactive oxygen and nitrogen species, and analysis of mitochondrial and lysosomal stability by flow cytometry) in the cell membrane (ergosterol dosage), cell wall (sorbitol test), capsule (morphometry and zeta potential) and cell volume (morphometry). Thus, it is expected that this project can contribute to the management of candidiasis and cryptococcosis, since native plants are an excellent option for these diseases that are often neglected.

Keywords: Cryptococcus, Candida, treatment
Supported by: FAPEMIG

CB.17 - Detection of SARS-Cov-2 Protein Using an ACE2-Like Protein

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COVID19 is a respiratory disease caused by the SARS-CoV-2 virus and is transmitted by aerosols. The penetration of SARS-CoV-2 into respiratory tract cells is mediated by the Spike protein using the ACE-2 (angiotensin-converting enzyme 2) receptor as a gateway. The structure of ACE-2 responsible for the interaction with the viral Spike protein is constituted by an alpha-helix. Based on this, by protein engineering technique, an ACE-2 peptidomimetic denominated K2ECA2 was developed to bind SARS-CoV-2 spike protein. The aim of this study is to develop new technologies for SARS-CoV-2 detection. The protein was successfully expressed in *E. coli* and purified to homogeneity. The protein gene was cloned into pET32a and transformed into *E. coli* BL21 PLysS bacteria for expression. ELISA assays were performed by sensitizing a 96-well plate with 75 ng ECD (Spike Extracellular Domain) protein and detected with 5 µg/mL biotinylated K2ECA2 protein. A Dot Blot was performed by immobilizing 100 ng of ECD on a nitrocellulose membrane and detected with increasing amounts of K2ECA2 protein. Protein was expressed during 6 hours of induction. In both ELISA and Dot Blot, the recombinant protein was able to detect the immobilized ECD protein. The K2ECA2 protein was able to detect the ECD portion of the Spike protein of SARS-CoV-2. More complex trials using samples obtained from infected patients will be performed.

Keywords: COVID19, Diagnosis , Immunoassays
Supported by: CAPES

CB.18 - Construction of a recombinant chimeric protein using epitopes of *T. evansi* ISGs75

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Trypanosoma evansi, the causative agent of Surra, main strategy of evading the host's immune systemis antigenic variation, where VSGs undergo remodeling instead of ISGs that do not present this variation. Previous bioinformatics analyzes demonstrate a variety of unique epitopes in *T. evansi* ISGs 75. This fact, combined with the potential use of chimeric proteins, can enhance their use for differential diagnosis. In which, these tests using recombinant antigens generally have greater specificities when compared to raw antigens. The aim of this work was the expression of a chimeric protein consisting of sequential peptides epitopes of the *T. evansi* ISGs75. The synthesis of peT31 with the peptide chimera was made by the company FastBio. The expression was made according to the peT31 manual. To determine the best expression time, the total protein profile was analyzed before the addition of IPTG, T0 (time zero) and after (0.1 mM IPTG) until T6 (six hours). Pellets were sonicated (0°C) and centrifuged to obtain total proteins. The analysis and visualization of the protein profile (*E. coli*, pLysS) and determination of the best expression time was performed by SDS-PAGE. And confirmation of the expression was made by marking the His-Tag by Dotblotting. In the analysis of the electrophoretic profile of the cell lysate, bands of proteins with different molecular masses were found. The recombinant protein is located at the 21KDa position, between 20 and 29 kDa markers, and the best expression time was 6 hours. For confirmation, the soluble and insoluble T6 fractions were subjected to Dotbloting and anti-His-Tag antibodies were detected. Successful construction and expression of the recombinant chimera was achieved. It was possible to confirm the presence of the recombinant protein and from that, new studies will be developed in order to use the chimera as a target for diagnosis or vaccine production.
Keywords: Glycoprotein, Recombinant protein, , Surra.

CB.19 - PROJECT: Biochemical and physiological effects of plant growth promoting bacteria (PGPB) as nutrients solubilizer in the soil during the growth and vegetative development of Zea mays

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Corn (Zea mays L.), belongs to Poaceae family, originates from a tropical American region. The adaptive qualities are a result of the work done by Native Americans. However, the domestication of primitive corn became extremely dependent on man. Submitted to suitable field conditions, the seed develops from the absorption of water, starting its plant development. The corn crop presents particularities in the absorption of chemical compounds during its cultural cycle. Fertilization using chemical compounds as a means of nutritional gain for the soil is one of the pillars of modern agriculture. However, the exaggerated use of mineral fertilizers in agriculture has become a problem. Plant growth promoting bacteria (PGPB) are microorganisms known in the literature due to their great potential for agricultural production. Plant macronutrients such as phosphorus are often found in large amounts in Brazilian soils, but the vast majority are not available for absorption due to mobility, insolubility or their structural shape. In this context, the PGPB present a sustainable alternative for soil management, acting as biofertilizers, which can help in cultural growth and development, providing micro and macronutrients for plant absorption. The aim of this master's degree project is to evaluate the physiological and biochemical changes of corn seedlings germinated in different PGPB preparations. Physiological parameters such as germinability, emergence speed, fresh mass, radicle and shoot growth and biochemical parameters such as Sugar Composition, Production of Non-enzymatic Antioxidant Compounds, Activity Determination of Catalase and Superoxide Desmutase Antioxidant Enzymes, Identification of Isoform of SOD in addition to evaluation of the micronutrient and macronutrient content of the soil before planting and after seedling establishment. With the results obtained, it is expected to indicate a PGPB lineage capable of improving corn production.
Keywords: Corn, Antioxidant Activity, Plant Biochemistry

CB.20 - Histopathological evaluation of ZEUS® toxicity on the gills of *Oreochromis niloticus*

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Corn is one of the most cultivated crop worldwide, but is commonly affected by stink bugs. To avoid crop losses due to pests, insecticides have been used. However, insects acquire resistance, requiring the use of new compounds. One of them is the insecticide ZEUS® (Ihara), a combination of dinotefuran and lambda cyhalothrin, produced in order to reduce their environmental impacts. Therefore, our objective was to analyse the histopathological effects of sublethal concentrations of ZEUS® on the gills of Nile tilapia (O. niloticus). Juveniles tilapia were purchased from a commercial fish farm in Santa Catarina state, Brazil, acclimatized for 81 days, and then exposed for 96 hours to 0.01 mg.L-1 (T1), 0.05 mg.L-1 (T2) and 0.1 mg.L-1 (T3) ZEUS® concentrations, in addition to the control group (CT). After the exposure period, fish were sacrificed and their gills were fixed in buffered formaldehyde, followed by dehydration, diaphonization and inclusion in paraffin. The hematoxylin and eosin stained slides were analysed under light microscope at 1,000 x. The histopathological alterations in the gills were hyperplasia, lamellar fusion, cell necrosis, parasitosis, and aneurysm. For all ZEUS® concentrations, the most frequent damage was the aneurysm, which can lead to the disruption of pillar cells and structural disarrangement of the branchial lamellae, possibly affecting the gas exchange and osmotic regulation. In general, fish of CT, T1 and T2 groups were classified as containing soft damages, while the T3 group presented moderate damage. Although damage was apparently different among treatments, there was no statistical differences between exposed and control group. Therefore, we can suggest that 0.1 mg.L-1 of ZEUS® can cause concerning histopathological changes on O. niloticus gills.
Keywords: Histology, Insecticide, Toxicity

CB.21 - Selection of binding peptides against the Spike protein of SARS-CoV2 using Phage display

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The worldwide outbreak caused by the pathogen called SARS-CoV-2 originated from Wuhan in China has spread to 6 continents and claimed to date over 4 million human lives. Therefore, the development of fast diagnostic strategies, therapy, and vaccines is urgent. On the other hand, the Spike protein of SARS-CoV-2 and its proteins derivatives such as the ECD (ExtraCellular Domain) or even the tiny receptor-binding domains (RBD) has become an essential target to many researchers since these proteins are needed to recognize the ACE2 human cell receptors and subsequent invasion. Therefore, specific ligands identified against RBD or ECD may be required tools for developing new diagnostic or even drug candidates. The phage display technology allows the display of peptides or proteins on the surface of bacteriophages, a technique used to identify specific ligands to virtually any biological target moderately fast. Our goal was to test a synthetic Kunitz phage-displayed library to discover specific ligands to the ECD SARS-CoV-2. After five rounds of library selection against ECD protein immobilized on ELISA microwells, phages binders were collected and re propagated in *E. coli* XL1 Blue bacteria. Input and output Phage populations were quantified on each selection round to assess the evolution of sub-libraries. In the last round, some colonies were selected, amplified individually, and tested as monoclonal phage in ELISA. We obtained two clones that showed strong binding to the ECD protein compared to the control. Thus, our synthetic Kunitz phage display technique was able to identify specific ligands against SARS CoV-2 ECD protein. The phage display technique proved to be efficient for detecting ligands and possible inhibitors
Keywords: Peptide library, ELISA, Kunkel mutagenes
Supported by: UDESC

CB.22 - Ultrastructural and viability analysis of biofilm formed by oral streptococci under the action of N-acetylcysteine

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Biofilm is the name given to the aggregated microbial life form, possible through the adhesion of these microorganisms to a matrix or extracellular polymeric substance (EPS), which provides stability, protection and resistance to disinfecting agents. N-acetylcysteine (NAC) has been proposed as an alternative for the control of bacterial biofilm in several human infections, as it disturbs the stability of the biofilm matrix and exposes microorganisms to antimicrobials. The aim of this study is to evaluate the ultrastructure of oral biofilms, under the action of NAC, formed by oral streptococci from the groups: *S. gordonii*, *S. mitis*, *S. mutans*, *S. oralis*, *S. salivarius*, *S. sanguinis*, and the structure of the multispecies biofilm, under scanning electron microscopy (SEM) and confocal microscopy (MC), in hydroxyapatite (HA) discs. The bacterial inoculum was standardized by means of spectrophotometry due to optical density (630 nm). Final bacterial concentrations of $1.5x10^8$ CFU/mL were obtained, which corresponds to tube No. 0.5 on the MacFarland scale. Biofilm susceptibility to NAC at different concentrations (0.78 to 25 mg/mL) was evaluated by colorimetric assay with crystal violet. NAC at a concentration of 12.5 mg/mL was shown to inhibit biofilm formation for all species, while, concentrations of 0.78 to 25 mg/mL inhibited biofilm formation by *S.oralis*. On the next step, HA discs will be soaked in saliva and then distributed in 24-well microplates along with bacterial inocula to obtain mono and multispecies biofilms. Biofilm degradation activity will be evaluated under different concentrations of NAC: 6,25 and 12,5 mg/mL. The ultrastructure of biofilms after treatment will be analyzed under SEM and CM, using SYTO 9 (excitation wavelength of 488 nm and emission wavelength of 500-550 nm) to analyze the biofilm in x–y planes. The parameters of the coefficient of thickness and roughness of biofilms will be measured using the MATLAB software. **Keywords:** oral biofilm, orals streptococos, scanning electron andconfocal microscopy. **Supported by:** Fundação de Âmparo à Pesquisa do Amazonas

CB.23 - PROJECT: Ecotoxicological effects of microplastic leachates in *Ruditapes decussatus*

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Plastics are the main category of waste reported in the oceans; they are ubiquitous in all ecosystems, especially marine environments. These compounds became notably important because they are made from raw materials like natural gas or refined crude oil; most are not biodegradable. Plastics can be produced in different size scales, from macro-, meso-, micro- and nanoplastics. When in the marine environment they can suffer from the action of waves, photodegradation, and weathering, leading to their fragmentation into smaller particles. These processes can also leach of their structural chemical components, which in turn might cause negative effects on marine biota, especially on filter-feeding organisms. Therefore, this project aims to evaluate the response of biochemical biomarkers in different tissues of the clam *Ruditapes decussatus* exposed to microplastic leachates under laboratory conditions. Clams will be exposed for 30 days to two environmentally relevant concentrations of plastic leachates (1 mg/L and 100 mg/L), made from plastics collected at different beaches of the Algarve coast, south Portugal. The biochemical biomarkers include those involved in the antioxidant defence system: superoxide dismutase (SOD), catalase (CAT), glutathione peroxidases (GPx), glutathione reductase (GR) and glucose-6-phosphate dehydrogenase (G6PDH), xenobiotic biotransformation system: glutathione S-transferase (GST), oxidative damage: levels of lipid peroxidation (LPO) and protein carbonylation (PC), neurotoxicity: acetylcholinesterase (AChE), genotoxicity (comet assay) and filtration rate (FR). Chemical analyses will be performed to identify the chemical compounds present in the microplastics leachate. Finally, bioaccumulation analysis of contaminants will also be evaluated in the clam tissues. Overall, we expect to provide valuable information on the toxicity of microplastics leachate in order to guide future decisions on the use and destination of plastics worldwide. **Keywords:** biomarkers, environmental pollution, chemical analysis. **Supported by:** RESPONSE - JPI OCEANS

CB.24 - *Bothrops jararacussu* ontogenetic venom variability: from birth to adulthood

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Variability in activity and composition of venoms is a fact previously described and occurs at various levels, such as ontogenetic, geographic, sexual, diet, inter and intra-specific. Ontogenetic changes are observed in snake species from different genus. *Bothrops jararacussu* snake belongs to Viperidae family. These individuals show sexual dimorphism in both size and color. Their diet undergoes ontogenetic change, young individuals feeding on ectothermic animals and adults predominantly preying on small mammals. Individuals of this species have large amounts of myotoxic PLA_2 in their venom composition. This present study aims to perform an ontogenetic analysis of venom from *B. jararacussu*, following the development of newborn individuals in Instituto Butantan until adulthood. Venom samples were obtained from *B. jararacussu* snakes born from the same litter at the Laboratory of Herpetology of Instituto Butantan. Venom samples were subjected to SDS-PAGE, followed by HPLC and L-amino acid oxidase enzymatic activity. A protein band within the range of 15-10 kDa appears on male individuals around 9 months of age, while in females this band appears around 15 months. HPLC profile shows a peak eluted around 45-minute that also increases over time, while the peaks eluted at 80-90 region decrease in same proportion. This peak/band, possibly a PLA_2 with myotoxic activity, increases in intensity with each subsequent extraction. LAAO activity was higher in female individuals up to 15 months of age, when the difference with male individuals started to became less noticeable. We observed that transition of protein profile from young to adult occurs around 15 months in female and 9 months in male individuals. We observed that this change happens gradually. A trend of increased LAAO activity in these individuals was observed. The greatest advantage of our work was following the growth of venom from same individuals, from birth to adulthood. **Keywords:** variability, ontogeny, biotechnology. **Supported by:** CAPES, CNPq, FAPESP (2018/25786-0; 2017/16908-2; 2018/20651-0)

CB.25 - Structural and mechanical characterization of self-assembled guanosine-hydrogels loaded with methylene blue

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Hydrogels are attractive biomaterials finding many applications, including the biomedical field. Guanosine is a low molecular weight gelator, being a building block to produce supramolecular hydrogels. Its self-assembling in aqueous medium can produce flexible and long fibers (G-quadruplex), which can entangle into a 3D network. Being a natural compound, the guanosine-derived hydrogel exhibits biocompatibility and biodegradability, which promote its bio-application. The aim was characterizing the guanosine-based hydrogels loaded with methylene blue (MB) comparing to the drug-free formulation. Stable hydrogels were formed by mixing: Guanosine (G)-neutral and Guanosine 5'-monophosphate (GMP)-negatively charged (G:GMP molar ratio = 1:6; 1:2; 1:1). AFM, rheology and SAXS were applied to study the structural and mechanical features of the G-hydrogels. AFM revealed the G-wires in the sample containing MB like those in the absence. The Tgel-sol obtained by rheology was 62.3°C, 58.2°C, and 47.2°C for MB-free and 62.2°C, 57.4°C and 45.5°C for MB-loaded to 1:1, 1:2, and 1:6 samples, respectively. The MB-loaded hydrogels were less viscous compared to the MB-free, mainly for the 1:6 sample, due to the higher GMP proportion, enhancing the electrostatic interaction with MB (positively charged). The volume-fraction distribution of the different chemical groups from SAXS showed the MB position overlaps with the ribose and phosphate groups, with slight differences related to the G:GMP and MB concentration. It was shown that the Gibb's free energy for MB binding to GMP is lower (-30 kJ/mol) than to G, (-20 kJ/mol) demonstrating a preference of MB to bind to GMP. Other interesting properties of guanosine-hydrogels such as a pH-dependent swelling behavior and drug release, besides the self-healing property were demonstrated. Concluding, the encapsulation of a drug model did not significantly perturb the structure of guanosine-based hydrogel. The results qualify the G:GMP hydrogel as an excellent biomaterial that can entrap and deliver bioactive molecules. **Keywords:** biomaterial, drug encapsulation, supramolecular hydrogel
Supported by: FAPESP - grant number: 2018/07194-9, CNPq and CAPES

CB.26 - PROJECT: Comparative study on yield, chemical composition and antifungal activity of essential oils from Croton blanchetianus Baill from different populations in serra do lima, Patu-RN

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Croton blanchetianus Baill (Quince), belonging to the Euphorbiaceae family, is a widespread species in the Caatinga biome of northeastern Brazil. Several studies report the importance of this species' essential oil for folk medicine and pharmaceuticals. Therefore, the objective of this study will be to analyze the yield, chemical composition and antifungal activity of essential oils from different populations of C. blanchtianus from Serra do Lima in Patu, Rio Grande do Norte. For the study, branches and leaves will be collected over a year, both in the dry season and in the rainy season, at 6, 12 and 17 h. The collected plant material will be packed in nylon bags and taken to the Plant Physiology and Biochemistry Laboratory (LFBP) for the extraction of essential oils, which after being extracted will be dehydrated and sent for analysis of chemical compounds by means of gas chromatography. The oils will also be evaluated for their antifungal activity against Fusarium oxysporium, an important pathogen that causes disease in bananas. For this step, tests will be carried out on spore germination and mycelial antifungal activity, using a 96-well plate, each well containing 100 µL of different concentrations of essential oils at different concentrations and 100 µL of potato-dextrose broth containing spores . To inhibit the mycelia, a thin layer of PDA medium will be deposited in a Petri dish, where they will be deposited on a solid surface, the plates will be incubated at 35°C for 72h and the diameters of the inhibition zones will be observed every 24h. It is expected to identify in which period of the year (dry or rainy) there is greater production of essential oils, and if there will be any change in their chemical composition. It is expected to prove antifungal activity of the oil against the deposited fungus.
Keywords: Antifungal activity, C. blanchetianus, Essential oil

CB.27 - PROJECT - Analysis of the effect of exposure to pesticities in pregnants and newborns and its relationship with changes in oxidative metabolism and DNA repair

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In today's world human beings are exposed to pesticides throughout their entire lives, including during pregnancy due to the passage of these products via the placenta. Exposure to these compounds has been associated with changes in oxidative metabolism and DNA, with consequences on the development and health of the newborn. Despite the evidence pointing to a relationship between fetal exposure to pesticides and the development of diseases, knowledge about the genotoxic effects of exposure to pesticides on the fetus is negligible, with no conclusive studies. Therefore, the objective of this work is to evaluate the association between exposure to pesticides, occurrence of DNA damage and changes in DNA repair pathways in mothers and newborns. The present study will be carried out from maternal peripheral blood and umbilical cord blood samples from newborns treated at a city hospital in Palotina/PR. The samples will be submitted to tests to detect the presence of organophosphates, carbamates, organochlorines and glyphosate, as well as to verify possible damage to the genetic material through micronucleus tests, comet assay and expression tests of genes encoding repair proteins. In addition, a possible state of oxidative stress will be investigated by checking the activity of the enzyme Glutathione Reductase, the products of lipid peroxidation and the levels of 8-oxodg. It is expected with this to verify the existence of transplacental passage of pesticides, and consequent exposure of the fetus still in the gestational period. Through this, we will be able to understand how exposure to these products can damage the genetic material and interfere with maternal-fetal health.
Keywords: DNA damage, Pesticides, Pregnancy

CB.28 - Project: Development on the Genome Surveillance Methodology of SARS-CoV-2 Independent of Genetic Sequencing

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COVID-19 is a disease caused by the Sars-CoV-2 virus that belongs to the Coronaviridae family and the Betacoronavirus genus, which comprises a group of large RNA positive viruses simple tape. This disease, which currently affects more than 205 million confirmed cases of COVID-19 in the world, and more than 4 million deaths, due to the high transmissibility and the seriousness of the cases. The COVID-19 pandemic has generated a worrying epidemiological situation that highlights the importance of epidemiological surveillance in ensuring effective actions for rapid diagnosis, tracking of the disease for early control, in order to minimize transmission and prevent future outbreaks. Among the laboratory techniques used, the test considered standard for diagnosis is the RT-PCR, and for genomic analysis and alterations it is the genetic sequencing method. However, one of the biggest concerns is the emergence of viral variants, with genetic mutations that confer greater transmissibility to the virus, which emphasizes the need for studies of methodologies that help genomic surveillance more quickly. This study is an applied research that will be carried out at the Laboratory of Infectious Agents and Vectors (LAIVE) of the Federal University of Western Bahia - UFOB, which aims to develop an alternative method of genomic surveillance, in a less expensive and simpler way. An analysis of the genomic sequences of SARS-CoV-2 will be performed to find conserved regions that will be targeted for amplification and subsequent enzymatic restriction. Through the analysis of the restriction fragmanides polymorphism (RFLP) it is expected to find a specific pattern that identifies the different viral variants. With this research, it is expected that an alternative, simpler and cheaper genomic surveillance methodology capable of differentiating the SARS-CoV-2 variants will be developed. And with that, knowledge is generated that will help in preventive measures to control the disease.

Keywords: COVID-19, Coronavírus, Mutation
Supported by: FAPESB

CB.29 - Biochemical characterization of plant growth-promoting fungus Penicillium chrysogenum 34-P to enhance the salinity tolerance of non-halophytic crops

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Soil salinity is one of the major causes of abiotic stress in plants, decreasing food productivity in many regions of the world. Plant growth-promoting fungi can help plants to tolerate stressful conditions and confer an improved growth on saline soils. In the present study, we evaluated the potential of Penicillium chrysogenum 34-P to promote plant growth under salinity stress. The tolerance of isolate to saline conditions was investigate in Potato-Dextrose-Agar (PDA) with different concentrations of NaCl (0-20% w/v). The fungal was grown in PD broth (0 and 5% NaCl) for 7 days at 30ºC (110 rpm) to measure dry weight. The strain has been investigated for indole-3-acetic acid (IAA), ammonia and siderophores production, and phosphate solubilization in presence of NaCl at 5% (w/v). Amylase, CMCase, pectinase, protease and xylanase production was investigated under saline conditions (5% w/v) in TLE medium for 168 hours. P. chrysogenum 34-P showed tolerance to different concentrations of NaCl (3, 5, 10 and 15% w/v). The fungus growth in PD broth with NaCl showed significant decrease (27.9%) of dry weight when compared with the control, from 257 to 201 mg, respectively. The presence of NaCl reduced in 71% the synthesis of IAA (17.67 µg mL-1). On the other hand, phosphate solubilization was increased by an average of 24% in relation to control (92.54 mg L-1). The isolate exhibited ammonia and siderophores production under NaCl presence. The enzymatic activity of amylase (11.3 U mL-1 in 72 h) and xylanase (5.82 U mL-1 in 120 h) were decreased in presence of salt, however, activities of CMCase (0.84 U mL-1 in 48 h), pectinase (0.81 U mL-1 in 120 h) and protease (10.02 U mL-1 in 120 h) were increased. Our results indicate that the P. chrysogenum 34-P displays plant growth-promoting abilities showing potential for applications in saline environments.

Keywords: salt stress, plant growth-promoting fungi, enzymes. **Supported by:** CNPq and CAPES

CB.30 - PROJECT: Study of the interaction and modulating effect of lectin from seeds of *Dioclea violacea* and the antibiotic neomicin applied to skin wounds using carboximethylcellulose and alginate biological membrane.

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Skin lesions can increase the risk of local and systemic infections by pathogens. To speed up the healing process, bacterial contamination levels need to be reduced and dressings associated with traditional antibiotics have been an option in this treatment as they release the drugs in a controlled manner, reducing the risk of toxic effects in patients. Lately, studies of the action of lectins have converged in this purpose of fighting infections, especially when associated with aminoglycoside antibiotics, since lectins have DRC (Carbohydrate Recognition Domain). This work will aim to analyze the modulating effect of Dioclea violacea (DVL) seed lectin on the antibiotic activity of Neomycin in multiresistant bacterial strains present in mouse skin lesions. The DVL lectin will be extracted and purified from the seeds of D. violacea which, by means of a hemagglutinating assay, will characterize its interaction with neomycin. Inoculums of multiresistant bacterial strains will be added to wounds induced in Swiss mice that will be divided into 06 groups, 01 uninfected group, with aseptic wounds treated with saline (50 µL); and 05 infected groups treated with: saline (50 µL); the formulation (membrane); formulation containing the D. violacea lectin; formulation containing the neomycin and formulation containing the lectin and neomycin, respectively. In each group with its defined treatment, clinical analysis of the lesions, count of viable bacteria and histopathological analysis will be performed. Results will be expressed as the mean ± standard error of the mean. Inhibition percentages will be calculated as the mean of the inhibitions obtained for each individual experiment. The statistical evaluation of the results will be performed using analysis of variance (ANOVA), followed by the Boferroni and Kruskal-Wallis test, for parametric and non-parametric data, respectively. The significance level equal to 0.05 will be adopted. **Keywords:** lectins, membrane, neomycin

CB.31 - Ontogenetic and sexual comparison of *Bothrops erythromelas* (jararaca-da-seca) snake venom

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The genus *Bothrops* is responsible for approximately 85% of the more than 27,000 snakebite envenomations reported yearly in Brazil. In the northeast region, *Bothrops erythromelas* is one of the most medically important species. The snakes from this species shift their diet from predominantly amphibians and lizards to mainly mammals as they develop from juveniles to adults. As ontogenetic changes in snake venoms were observed in other species, we aimed to analyze the shift in the venom of *B. erythromelas* snakes from newborns to adults. Thirteen *B. erythromelas* snakes born and maintained in captivity in the Laboratory of Herpetology at Butantan Institute had their venoms milked and pooled every six months after they were born, separated in males and females (at 24 months-old the venoms were stored individually). Additionally, thirteen adult snake venoms were milked and stored individually. All venoms were submitted to protein quantification by Bradford method, phospholipase A_2 (PLA_2; 4-nitro-3-(octanoyloxy)benzoic acid substrate), L-amino acid oxidase (L-methionine substrate), and caseinolytic (azocasein substrate) activities; the latter was also performed with previous incubation with antibothropic serum to measure this activity's inhibition. Venoms from newborns presented less protein quantification, PLA_2, and caseinolytic activities, which increased as the snakes aged. Male venoms had slightly higher PLA_2 and proteolytic activities in general. L-amino acid activity, however, was not detected in any of the pools and was low in 6 individuals. Individual variation was observed among the venoms in all assays, including inhibition of the caseinolytic by the antivenom, which inhibited male venom slightly better (average of 10.9% and 9.9% inhibition of male and female venoms, respectively). In conclusion, the *B. erythromelas* venoms studied in this work showed that ontogeny might influence them more than gender, individual variation is also remarkable in this species, and antivenom is not able to efficiently neutralize proteolytic activity over casein.
Keywords: Bothrops erythromelas, Ontogeny, Individual variation
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CB.32 - Bactericidal and antioxidant potential of microalgae *Skeletonema costatum* and *Diacronema lutheri*, by the influence of temperature and light incidence variation.

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Microalgae have been a growing object of scientific interest due to their relevance in several areas. With their photosynthesizing characteristics and amplitude of occurrence in the planet, they are an efficient alternative producer of bioactive compounds with application in several areas and with high biological and biotechnological value. Modifications in abiotic conditions can promote changes in the production of metabolites and, consequently, in antibacterial and antioxidant properties of microalgae. The project proposes to evaluate the increase in the production of metabolites with antibacterial and antioxidant properties of the microalgae *Skeletonema costatum* and *Diacronema lutheri*, through responses induced by temperature variation and light incidence, in their production of algal biomass. For temperature conditions, the project will use the regular cultivation one (22ºC) and 30ºC. For light incidence, microalgae will be subject to 70 and 100 µmol of photons m^{-2} s^{-1}. Extracts will be prepared by maceration in 99.5% ethanol. To evaluate the antibacterial activity, the method of disk-diffusion in agar and microdilution in broth will be performed for six strains of bacteria. The antioxidant activity (AAO) will be evaluated by the radical scavenging activity method using 2.2-diphenyl-1-picrilhidrazil radical (DPPH). With the results obtained from the previous steps, the profile of the microalgae metabolites with the best potential in the activities tested will be analyzed using the Ultra-Efficiency Liquid Chromatography (CLUE) method coupled to a Mass Spectrometer (MS). As an expected result, the project aims to obtain the profile of metabolites to understand how abiotic changes in the cultivation of these species can alter the production of their bioactive compounds. In addition, to identify the antibiotic and antioxidant potential of the studied microalgae, contributing to the isolation or synthesis of new pharmacological targets. **Keywords:** Bioactivity, Cultivation condition, Microalgae
Supported by: CAPES, FINEP

CB.33 - *In vitro* antitumor effects of sodium selenite in chronic myeloid leukemia cell lineages.

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Selenium compounds have been studied as an antitumor agent due their oxidant role and the negative interaction with glycolysis pathway, leading cancer cells to death by starvation. In this scenario, the objective of this work is to evaluate an antitumor activity of sodium selenite (Na2SeO3) in chronic myeloid leukemia cell lineages K562 and LUCENA. LUCENA cells is a resistant lineage obtained through treatment with low doses of vincristine, an antitumor agent used in the clinic treatment. Cell viability was measured by MTT assay and the mitochondrial membrane potential (MMP) was performed by JC-1 probe. The cells were previously treated with 10 µM, 100 µM or 1000 µM of Na2SeO3 for 48 hours, where a proliferation of 75% of control K562 cells was observed, while a significant reduction was observed for the concentrations of 100 µM and 1000 µM of Na2SeO3. For the LUCENA lineage, the proliferation rate was 63% for the control, whereas a 66% reduction in cell number occurred for the 1000 µM concentration of Na2SeO3. On these conditions, the cell viability was significant reduced only in 100 µM or 1000 µM of Na2SeO3 for both cell types. To MMP, the treatment was performed for 24 hours, and the result obtained was a significant reduction for all concentration for K562 cells, while for the LUCENA lineage only at concentrations of 100 µM and 1000 µM of Na2SeO3 was performed a significant reduction. The preliminary results of this work show that K562 and LUCENA cells are sensitive to Na2SeO3 supplementation and that the treatment affects mitochondrial physiology.
Keywords: metabolism, cancer, selenium
Supported by: FAPERJ

CB.34 - The use of metformin as adjuvant to restore allantoin-reduced cisplatin cytotoxicity *in vitro*

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The tumor lysis syndrome (TLS) is a metabolic disorder frequently associated to hyperuricemia. As a tool for the treatment of TLS, rasburicase has been employed, producing allantoin. In recent studies by our group, was observed that high levels of allantoin promote the reduction of the cytotoxic effect of cisplatin both in H460 and K562 cells lines. Therefore, in view of the maintenance of the concomitant use of cisplatin and rasburicase, search for adjuvant agents, like metformin, that can maintain their efficience it is of great interest. Recently, our group demonstrated the capacity of metformin in restores cisplatin cytotoxicity in the presence of allantoin in K562 cell line. Through this observation, this study sought to understood how metformin promoted the recuperation of cisplatin cytotoxicity. To realize this study, the K562 cell line was maintained in RPMI 1640 media supplemented with 10% of fetal bovine serum and 0.5% of penicilin-streptomicin at 37ºC and 5% of CO2. For the assays, the cells were incubated in RPMI 1640 low glucose media (0.5mM) for 2h and treated with metformin (0.5 to 3mM), cisplatin (15 to 33µM), allantoin (100 and 200µg/ml), rotenone (0.125 to 2µM) and their combination for 48h. Cell viability, cell cycle, morphology analysis and NMR assays were performed. Our results showed: (1) the synergism between metformin and cisplatin, (2) the cytotoxicity recuperation of cisplatin by metformin at morphology level, (3) the alterations of cisplatin effect in cell cycle after combination with allantoin, (4) the absence of metformin-cisplatin interaction, (5) the interaction between metformin and allantoin, (6) the increase of cisplatin cytotoxicity after its combination with rotenone and (7) the similarity between metformin and rotenone effects in restores cisplatin cytotoxicity in the presence of allantoin. This study demonstrated that metformin restores allantoin-reduced cisplatin cytotoxicity independently and dependently of the mitochondria.

Keywords: Metformin, Cisplatin, Allantoin
Supported by: FAPERJ

CB.35 - PROJECT: FTIR for diagnosis and prognosis of COVID-19

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The Sars-Cov-2 virus belongs to the Coronaviridae Family, is the causative agent of the Coronavirus Disease of 2019. The gold standard for COVID-19 diagnosis is Reverse Transcription Polymerase Chain Reaction (RT-PCR) and uses the respiratory tract specimen for the analysis. Among the main COVID-19 biomarkers included acute phase proteins like C Reactive Protein (CRP), IL-6 cytokine and Lactate Dehydrogenase (LDH). However, the collection of respiratory tract specimen brings a series of limitations that range from patient discomfort and high risk of transmission to health operators. Vibrational spectroscopic techniques are applied for the human disease's diagnosis and are already well knowledge in the literature for biological fluids analysis such as urine, blood and saliva. The advantages of its use are reproducibility, small amount of sample and specificity in the biochemical arrangement recognizing of the different biomolecule's classes. Therefore, the aim of this project is evaluating the use of Fourier Transform Infrared absorption spectroscopy (FTIR), with multivariate analysis, as a new diagnosis tool, besides detect and quantify salivary biomarkers associated with clinical forms of COVID-19 for determining patient's prognosis. This is an epidemiological study, prospective cohort, to be carried out at the Laboratory of Infectious Agents and Vectors -LAIVE of the Federal University of Western Bahia- UFOB. The sample will consist of 200 patients attended in the LAIVE services. The method to be used involves the following steps: (i) saliva and nasopharyngeal swab collection; (ii) samples processing; (iii) PCR analysis; (iv) implementation and validation of the FTIR diagnostic method; (v) implementation and validation of the quantification of salivary biomarkers by FTIR. Thus, a new, faster and less expensive diagnostic and prognostic method of COVID-19 it is expected, and that will be able to prioritize the analysis of non-invasive biological samples, ensuring patient comfort, safety of the health operator and good results. **Keywords:** Sars-CoV-2, Spectroscopy, Saliva

CB.36 - PROJECT: Pharmacological activity of carvacrol cigarette in a smoke-induced acute lung injury

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Acute lung injury is a condition characterized by tissue damage and increase of oxidative stress and inflammation, increasing inflammatory marker such as inflammatory cells and cytokines. Cigarette smoking is one of the risk factors for reactive oxygen species (ROS) generation, which induces oxidative stress and lung inflammation. Carvacrol (CAR) is a promissor terpene that have been used against pulmonary inflammation and antioxidative activity. This study aims to evaluate of anti-inflammatory and antioxidant activity of carvacrol in Cigarette smoke-induced acute lung injury. Mice C57BL/6, male, (25-28g) will be divided into two groups: control and cigarette smoke (CS). The CS group will be exposed to 12 cigarettes per day for 5 days. The control group will be exposed to sham smoking (without cigarrete smoke). The CS group will be treated with CAR (1, 3 or 10 mg/mL) or vehicle by inhalation (15 min/daily) for 5 days. After 5 days bronchoalveolar lavage (BAL) to analyses of cytokines release (TNF-α, IL-6, KC and IL-10) by ELISA. The trachea will be used to airway hiperresponsivines analyse by dose-response curve to carbachol (CCh; 0,01 – 100 μM) and potassium chloride (KCl; 10 – 80 mM). Right lung will be collected to morphological analysis such as lung inflammation score, bronchoconstriction index and count of inflammatory cells (macrophages and neutrophil) and left lung will be used to redox marks analyses (superoxide dismutase and catalase activity and, malondialdehyde levels).Statistical analysis were performed by Analysis Variance (ANOVA) followed by Tukey´s post hoc test. Significant difference when $p < 0.05$. The literature suggests that terpenes, such as carvacrol, has anti-inflammatory and antioxidant activity. Therefore, we expect that carvacrol will act reducing inflammation and oxidative damage, consequently being a promissory substance to treat the acute lung injury induced by cigarette smoke.
Keywords: Carvacrol, Antioxidant, Antiinflamatory
Supported by: UFERSA

CB.37 - PROJECT: Toxicological and antitumor evaluation of fabaceae family lectins

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Cancer is one of the major public health problems worldwide, accounting for about 10 million deaths per year. In Brazil, as malignant neoplasms are considered as the second cause of death among non-communicable diseases, which usually arise through a slow process, and it may take several years before they are diagnosed, however, anticancer drugs are often ineffective, present a number of side effects and drug resistance. In this context, more efficient scientific research in the search for drugs has grown considerably, and products of plant origin are a viable alternative. In view of the above, the objective of this work is to evaluate an antitumor and toxicological activity of vegetable lectins from Vatairea macrocarpa (VmL) and Dioclea violacea (DvL). The purification of the lectins will be carried out by affinity chromatography techniques. *In vitro* and in vivo techniques will be developed to evaluate possible mechanisms of cytotoxic actions, genotoxicity and oxidative stress markers. *In vitro* tests will be evaluated for cytotoxicity with cancerous and healthy cells, mechanisms of apoptosis, mitochondrial transmembrane potential, cell cycle, DNA fragmentation and levels of gene expression of proteases (caspases 2, 3, 6, 8, 9 and Apaf-1), in addition to evaluating damage caused to the cell's DNA, by the comet assay. The in vivo experiments will be carried out with *Drosophila melanogaster*, a promising invertebrate in tests to evaluate toxicological parameters, such as: cell viability, total thiols, non-protein thiols, lipid peroxidation, determination of free Fe2+ levels and nitrite levels. This research aims to contribute to the advancement of the knowledge of the therapeutic properties of lectins, that once these properties of the species under study have been proven, it will be possible to favor the development of a new anticancer substance.
Keywords: Cancer, Oxidative stress, Lectins

CB.38 - Cloning and expression of γBjPLI, a natural inhibitor of phospholipase A2 from *Bothrops jararaca* snake blood

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γBjPLI is a phospholipase A_2 inhibitor (PLI) present in low concentrations the *Bothrops jararaca* snake blood serum. This protein is classified as a gamma type based on its characteristic structural domains. γBjPLI has approximately 22 kDa and an inhibitory activity of enzymatic, edematogenic and myonecrotic activities, suggesting a role of this inhibitor for protection of these snakes against self-envenomation. Phospholipase A_2 acts in the cell membrane phospholipids resulting in fatty acids, lysophospholipids and deconstructing the cell membrane. This protein is commonly responsible for the local effect of snake envenomation, causing tissue inflammation in the affected area. To improve the yield in obtaining this inhibitor, this work aims to clone and express γBjPLI in its recombinant form. First, by PCR, a 543 bp fragment was amplified from a cDNA library produced from the liver of *B. jararaca* snake. The γBjPLI fragment was inserted into the pGEM-T easy vector, cloned using the DH5αstrain. The fragment was purified and introduced into a pET28a expression vector and cloned again into the DH5α strain. Plasmid pET28a+γBjPLI was isolated and sequenced to confirm the composition of the nucleotide sequence. Finally, by heat shock, pET28a+γBjPL was inserted into the expression strain Shuffle of *Escherichia coli*, the expression was induced with different concentrations of IPTG for 16 h at 37 °C, and analyzed by 15% SDS-PAGE. In the first step, 2 were positive for the pGEM-T+γBjPLI vector. In the next step, 4 were positive for the presence of pET28a+γBjPLI. The nucleotide sequence was confirmed, and the 4 colonies were able to express a 22 kDa band with IPTG 0,1 mM and 0,4 mM. In this work, the cloning and expression of γBjPLI in its recombinant form was carried out for the first time, using the pET28a vector and the SHuffle expression strain of the *E. coli* bacterial species.

Keywords: Snake plasma, Phospholipase A2 Inhibitor, Bothrops jararaca

Supported by: FAPESP

CC - Biotechnology and Biomaterial (Industrial Process)

CC.01 - Investigation of the use of xanthan gum as a controlled release system for doxycycline

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Xantham gums or Xanthans are biopolymers obtained by fermentation of bacteria from the Xanthomonas genus. They are biodegradable and biocompatible, being widely used by the pharmaceutical industry, especially as controlled drug release systems. Concerning their anionic feature, the present work aimed to investigate the formation of the complex spontaneously formed with the cationic antibiotic doxycycline (DOX), as well as its biological activity. The samples were characterized in solid state by FTIR and thermal analysis (TGA and DTA). The interactions between doxycycline and xanthan were characterized in solution by ITC, zeta potential (ZP) and rheological titrations. The antimicrobial action of the doxycycline/xanthan complex and its precursors was evaluated against Staphylococcus aureus 323886023, by determining the median lethal dose (LD50) and death curve. FTIR and TGA/DTA data confirmed the solid state interaction. ZP titrations showed the gradual neutralization of xanthans by DOX. The ITC data showed two distinct sites of interaction (one exothermic and the other endothermic). The rheological titration experiments showed a strong reduction in viscosity, suggesting that the DOX/xanthan interaction causes a collapse in the polymer structure, breaking the entanglements. The results obtained showed that the complexation led to an increase in antimicrobial activity, by reducing the LD50 and inhibition time, suggesting that the complex is promising for the development of new formulations for a antimicrobial drug controlled release.
Keywords: xanthan gum, controlled drug release, doxycycline
Supported by: FAPEMIG

CC.02 - Combination of biocatalysis and sonochemistry in the ethyl oleate production in a co-solvent free system

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Fatty acid alkyl esters (FAAEs) are a family of natural neutral lipids and can be produced cleanly and sustainably by esterification of free fatty acids (FFAs) with short chain alcohols using enzymatic catalysts. Recently alkyl esters have attracted much attention for their application in food, beverage, and chemical industries. Fatty acid methyl or ethyl esters are also widely used as biofuel in diesel engines. Due to inherent merits of enzymatic reactions, e.g., milder conditions, less pollution, etc., enzymatic production of fatty acid alkyl esters such as ethyl esters, have received much attention. In this study, it was evaluated the use of lipases in enzymatic esterifications of oleic acid with ethanol using the combination of biocatalysis and sonochemistry (ultrasound) in the absence of co-solvents. Reaction parameters, such as type of lipase, amount of enzyme, reaction time, alcohol hydration level and enzyme turnover were evaluated. Reactions were carried out in 5 mL Eppendorf® tubes containing 200 mg of oleic acid, 600 µL of ethanol, 20 mg (10%) of lipase and 20 mg of molecular sieves. The mixtures were incubated in ultrasound bath at 35ºC for 10 hours. For quantification, synthetic ethyl oleate (acid catalysis) was prepared and used as a standard. Eleven enzymes were initially tested and just C. antarctica lipase provided yields above 95% in less than 10 h with 10% (m/m) of catalyst. The use of hydrous ethanol (5% of water) showed a slight drop in yield but remained above 90% of ethyl oleate production and C. antarctica lipase showed no loss of efficiency even after 10 reaction cycles. The combination of biocatalysis and ultrasound radiation greatly increased the reaction yield, showing that this combination may be a good choice for ethyl oleate enzymatic synthesis.
Keywords: lipase, sonochemistry, ethyl oleate
Supported by: CNPq and CAPES

CC.03 - Production and application of prebiotic xylo-oligosaccharides obtained from agro-industrial residues using Thermomyces lanuginosus endoxylanase.

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Xylooligosaccharides (XOS) are short chain oligomers containing β-1,4-linked xylose residues that have prebiotic action and human health benefits due to stimulation of the growth of bacteria such as *Lactobacilli* and *Bifidobacteria.* The aim of this study was to evaluate the production of XOS from agro-industrial residues using *Thermomyces lanuginosus* endoxylanase, and to test these XOS in the growth of the probiotic *Lactobacillus acidophilus.* Hemicelluloses were extracted from corn straw and brewery bagasse after alkaline pretreatment with 6% hydrogen peroxide, and subsequently hydrolyzed with *T. lanuginosus* pure endoxylanase at 65ºC for 1h. Subsequently, the probiotic bacterium was grown in microplate containing basal MRS medium supplemented with XOS and incubated, at 37°C under anaerobic conditions for 24h. To monitor the growth of bacteria was measured by optical density at a wavelength of 600 nm at cultivation times 0, 4, 8, 12 and 24 h. The XOS released after the hydrolysis of each of the hemicelluloses by endoxylanase were mainly composed of xylobiose, xylotriose, xylotetraose and xylopentaose (X2-X5), and the yield of the XOS mixture was 4.06 mg / mL and 2.86 mg / mL for corn straw and brewery bagasse, respectively. The stimulus on the growth of the probiotic microorganism in the presence of XOS was verified after 12 h of incubation. Therefore, these XOS obtained from agro-industrial residues used as carbon source to probiotic bacterium culture were effective in promoting the growth of *L. acidophilus,* comparable to the control culture with glucose. Thus, these XOS obtained from agro-industrial residues show prebiotic and promising potential for biotechnological exploitation.

Keywords: agro-industrial residues, probiotics, xylooligossaccharides
Supported by: CAPES and Fundação Araucária

CC.04 - Production of pectinases by Aspergillus japonicus and Aspergillus sp. (M1) for juice clarification using agro-industrial waste as a carbon source

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Pectinases are a group of enzymes capable of degrading polysaccharides of the plant cell wall, facilitating the obtaining of carbon source by microorganisms as a result of cell fragmentation. Filamentous fungi are the most used for enzymatic production on an industrial scale, with application in the fruit juice clarification, among these are fungi of the genus Aspergillus. The goal was to evaluate the production and characterize pectinases produced by Aspergillus japonicus and Aspergillus sp. M1 from agro-industrial waste and apply these enzymes in the fruit juice clarification. Pectinase production was evaluated using different carbon sources in solid medium (5 grams) and the fungi were inoculated using 10 ml of a Salt Solution with spores. Optimal growth time was evaluated using Tereré Herb residue as carbon source in solid medium for 192h under stationary condition at 35°C. Enzyme activity was measured at temperatures between 30 and 70°C and McIlvaine buffer pH 3 to 7 using the DNS method. Juice clarification by pectinases was evaluated measuring transmittance according to Rosmine et al. (2017). Aspergillus sp. M1 had optimal production in Tereré Herb residue after 72h of growth and maximum activity at pH 4 (2.54 U/ml) and 60°C (4.05 U/ml). A. japonicus had optimal production in Tereré Herb residue after 48h and maximum activity with McIlvaine pH 4 buffer (1.09 U/ml) and at 60°C (2.16 U/ml). Pectinase from A. japonicus obtained maximum results in the clarification of guava juice at 50°C, with 49.3% ±1.8 clarification. The results showed that pectinases from both fungi have viable features for application in fruit juice clarification, whose tests with A. japonicus proved their potential for industrial application.

Keywords: pectinases, fruit juice clarification, Aspergillus sp.
Supported by: CNPq

CC.05 - Bioethanol production from pretreated sugarcane bagasse under optimised conditions using selected fungi

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Sugarcane Bagasse (SB), a waste of the sugar industry contains cellulose and hemicellulose which can be converted to bioethanol. However, its recalcitrant nature demands optimal pretreatment method to make sugar components available for enzymatic depolymerisation. This study was designed to optimally pretreat SB and to identify appropriate fungi for enhanced bioethanol yield. Yeasts isolated from SB dumpsite were screened based on their ability to convert pentose and hexose sugars to bioethanol using different nitrogen sources. Optimisation of pretreatment of SB at different concentrations of potassium hydroxide (KOH), temperature and treatment time was determined using Response Surface Methodology (RSM). Pretreated SB was hydrolysed using A. niger XY and a commercial hemicellulase mixture. Fermentation of pretreated SB hydrolysate with selected yeasts using Simultaneous Hydrolysis and Fermentation (SHF) as well as Simultaneous Saccharification and Fermentation (SSF) of pretreated SB were carried out. Bioethanol yield was determined by gas chromatography. Eleven yeasts grew on both glucose and xylose and were identified as Pichia kudriavzevii (7), *Saccharomyces cerevisiae* (1), and Candida tropicalis (3). All yeasts converted glucose to ethanol but only C. tropicalis Y5 converted xylose to ethanol (4.83g/L) with urea as the best nitrogen source. Optimum pretreatment conditions were: 150mg/g bagasse (KOH), 86oC and 120 minutes. Hydrolysis with hemicellulase yielded reducing sugars of 600 mg/g bagasse within 20 hours while hydrolysis with A. niger XY took a longer time (12 days) and yielded 18.8 mg/g bagasse. Bioethanol yield using SHF and SSF were 19 g/L and 30 g/L, respectively. Alkaline pretreatment followed by enzymatic hydrolysis gave a higher yield of total reducing sugars. Candida tropicalis Y5 converted both pentose and hexose to bioethanol and showed good prospect for its use in commercial fermentation of sugarcane bagasse.
Keywords: Bioethanol, Candida tropicalis Y5, KOH

CC.06 - PROJECT: Production, purification, biochemical characterization, and biotechnological application of amylase from the thermophilic fungus Humicola brevis var. thermoidea

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Starch is a polysaccharide commonly found in plants and, therefore, is a significant source in industrial applications. Amylases are enzymes capable of degrading starch and represent 25% of the world market for enzymes, of which several markets use them, such as in ethanol production and bakery. This enzyme belongs mainly to the class of hydrolases and cleaves starch in different regions depending on its classification of α-amylase, β-amylase, and γ-amylase. Microorganisms can produce amylases, such as bacteria and fungi, therefore, distinct means of production studied them for characterization of amylase to promote starch bioprocesses. In this context, previous studies have shown that the thermophilic fungus *Humicola brevis var. thermoidea* in solid culture medium is a good amylase producer. Hence, this work aims to produce, purify, and biochemically characterize the amylase obtained from *H. brevis* and its biotechnological application in the depolymerization of the different starch sources. Particularly, the native amylase will be purified from the crude extract obtained by solid-state fermentation (SSF). The maximal production will be performed using the one-factor-at-time method (OFAT) to determine the best source for overexpression of amylase activity. The amylolytic activity will be assayed using 1% starch, in optimal pH and temperature conditions, using the dinitrosalicylic acid method to reducing sugars to available starch hydrolysis. The homogeneity of purified enzyme will be available using electrophoresis in denaturing conditions SDS-PAGE. The biochemical characterization of crude extract and purified amylolytic activity will be realized by evaluating the optimal pH and temperature, stabilities, the effect of the presence of different ions, organic compounds, and detergent. Thus, it is expected to obtain the standardization of the expression of amylase, as well as the biochemical characterization and purification of the enzyme in the crude extract, so that can become an alternative for the starch liquefaction process. ***Keywords:*** *Amylase, Humicola brevis var. thermoidea, Starch*

CC.07 - Immobilization of fungi in polyurethane for production of phosphatases

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Phosphatase, an enzyme that catalyzes organic phosphorus, is involved in biotechnological processes, including bioremediation of domestic and industrial effluents, heavy metal removal, and plant fertilization. Enzyme catalysis can be optimized with simple methods of immobilizing cells in different matrices. The polyurethane foam matrix was applied in this work because it offers high porosity, good mechanical resistance and low cost. This work proposed the production of phosphatase by fungal immobilized cells *Aspergillus niger*, *Aspergillus flavus* and Trichoderma sp. entrapped in polyurethane foams. The isolated fungi was incubated in a minimum culture media containing potassium phosphate varied from 1g L^{-1} to 4g L^{-1} to induce phosphatase production. The commercial macroporous polyurethane foam (0.71 mm) was cut into 1.0 cm x 1.0 cm x 0.7 cm parallelepiped. Then, the biomass of *A. niger*, *A. flavus*, *Thichoderma sp.A* and *Trichoderma sp.B* were inoculated in minimal medium containing 0.5 g of previously autoclaved foam cubes. The microorganisms grew for 12 days and during this time the fungal biomass inside the foams was monitored for the appearance of the characteristic color of each isolate. The acid phosphatase activity with p-nitrophenyl phosphate was measured. Induction of phosphatase production occurred with KH2PO4 at 4 gL^{-1} for Aspergillus and at 1 gL^{-1} for Trichoderma sp. All fungi produced phosphatase, ie. *A. niger* for 12 days, followed by Trichoderma sp. B, 11 days, and 7 days for *A. flavus* and Trichoderma sp. The *A. niger* and *A. flavus* isolates had higher phosphatase activity (229 µmol/h), after 192 hours and 96 hours, respectively. The two Trichoderma sp. showed better enzyme activity in 72 hours, with 78 µmol/h for Trichoderma sp. B and 36.7 µmol/h for Trichoderma sp. A. Reusability of Trichoderma sp. A was 4 cycles and *A. flavus* in 2 cycles. Those isolated fungi are good candidates for phosphatase bioremediation.

Keywords: entrapment, biocatalysis, biorremediation
Supported by: CAPES; UEG

CC.08 - Expression and characterization of a thermostable endo-arabinanase

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Arabinan is an important constituent of plant biomass, this monosaccharide has several applications in the biofuel, food and pharmaceutical industries. Its hydrolysis by the enzymes endo-1,5-α-L-arabinanases and α-L-arabinofuranosidases results in L-arabinose releasing. Based on the evidences that no arabinase from Geobacillus sp. JS12 has been described, we characterized the recombinant enzyme endo-1,5-α-L-arabinanase. Produce and characterize the recombinant enzyme endo-1,5-α-L-arabinanase from Geobacillus sp. JS12. To develop this work, we first synthesized and cloned the putative enzyme gene, obtained from the CAZY database, in *E. coli* BL21. The recombinant enzyme was purified by affinity chromatography on a nickel-sepharose column. The amino acid sequence analyzes were performed *in silico*, the enzymatic activity analysis were performed in different synthetic and natural substrates. The influence of pH, temperature and additives, on the enzymatic activity, was verified by DNS method and the kinetic parameters were determined by Michaelis-Menten equation. The results showed that endo-1,5-α-L-arabinanase had greater activity at pH 7.0, at 70°C, on the specific substrate arabinan debranched. In tests with additives, the enzyme exhibited almost the same activity as previously, in the presence of EDTA and, its activity, was slightly improved in cobalt presence. It was found that the enzyme has good thermal stability at 70°C, especially in cobalt ion presence, after 48h. Also, the enzyme showed specific activity and kinetic parameters on debranched arabinan, what was similar to other endo-arabinanase already characterized. In this sense, the endo-1,5-α-L-arabinanase maximum activity, at high temperatures, and its thermostability turns the enzyme a potential candidate for obtaining and applying L-arabinose in industrial process.

Keywords: arabinan, glycosyl hydrolases, endo-α-1,5-L-arabinanase
Supported by: CAPES

CC.09 - Purification, characterization, and evaluation of the biotechnological potential of endo-xylanase from the thermophilic fungus Humicola brevis var. thermoidea.

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The biodegradation of lignocellulosic biomass, especially hemicellulose, depends on a group of enzymes (hemicellulolytic complex) produced mainly by fungi and bacteria. Among these hemicellulases, endo-xylanases have been widely used to enhance hemicellulose in bioprocess applications. In this context, this work aimed to purify, characterize and evaluate the biotechnological potential of endo-xylanase from *Humicola brevis* var. *thermoidea* in the saccharification of lignocellulosic biomass. For this purpose, the native endo-xylanase (XylHb) was purified from the crude extract obtained by solid-state fermentation. The biophysical characterization of secondary structures by circular dichroism indicated the predominance of the β-sheet, a typical characteristic of the GH11 family endo-xylanases, and the intrinsic fluorescence of the tryptophan analysis indicated that the protein has its tertiary folding with Trp residues immersed in a hydrophobic environment. The results of the biochemical and kinetic characterizations of XylHb indicated that the enzyme under study had a greater tolerance in a wide range of pH, temperature, NaCl, and ethanol, having a better performance in the hydrolysis of birch xylan at temperatures of 50 and 70 ºC in comparison with commercial xylanase (Cellic ® HTec2). The hydrolysis assays of hydrothermally pretreated sugarcane bagasse demonstrated that the supplementation of Cellic ® CTec2 with XylHb showed synergistic interactions and significantly increased the release of glucose and total reducing sugars compared to the individual Cellic ® Ctec2 commercial standard. Thus, a purified XylHb from the enzymatic extract of *H. brevis* var. *thermoidea*, due to its physical-active properties, performance, and synergism with commercial cellulase, which are important characteristics for application in an industrial environment, can contribute as a promising biotechnological alternative, application, and valorization of hemicellulose in the production of bioproducts.

Keywords: Endo-xylanase, Halotolerant enzyme, Sugarcane bagasse hydrolysis
Supported by: CAPES and CNPq

CC.10 - PROJECT: Obtainment and application of antimicrobial coating based on green banana starch for post-harvest fruit conservation

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Post-harvest losses of vegetables have been associated with the stages of transport to storage, highlighting the importance of developing appropriate packaging with the development of post-harvest technologies, with a view to optimizing transport costs, reducing losses and maintenance of fruit quality. In addition, the high perishability of post-harvest vegetables makes reference to the production of ethylene in climacteric fruits, providing rapid senescence of the vegetables, reducing their useful life even before reaching the consumer, generating damage to the small producer and the community. With this in mind, we have studied the production of biodegradable and low-cost coatings, obtained through natural polymers, with the insertion of antimicrobial systems as a way to increase the shelf life of post-harvest fruits, using active packaging technology. In view of this, this research project aims to produce an antimicrobial coating based on green banana starch and apply it to the conservation of postharvest papaya. The methodology applied will be to obtain and characterize the starch, evaluating its yield, composition, water solubility, gelatinization capacity and syneresis, in addition to producing the coating, applying to the fruits and analyzing their shelf life, through the evaluation of total soluble solids, titratable acidity, color, weight loss and mold and yeast count. The data will be evaluated by analysis of variance (ANOVA) with Tukey's means comparison test ($p < 0.05$ of significance). The statistical package SAS (Statistic Analysis System) version 9 will be used to carry out the analyzes. Expected results: In this work is expected to obtain a coating capable of increasing the shelf life of fruits compared to traditional methods of conservation.

Keywords: active packaging, biopolymers, food conservation

CC.11 - Characterization of lignocellulosic enzymes for hemicellulose degradation

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Environmental problems caused by the release of greenhouse gases (GHG) in the burning of fossil fuels have motivated the search for ecological and sustainable consumerism. One strategy is to exploit renewable and sustainable biomass resources. Lignocellulosic materials are renewable, low cost and abundantly available on the planet, and their product is widely used in various industrial sectors. One of the ways of converting this material is through the biochemical route, based on hydrolysis and fermentation processes. This process is carried out by several lignocellulolytic enzymes responsible for the degradation of lignocellulosic biomass in fermentable sugars. Some of this enzymes are α-L-arabinofuranosidases, β-xylosidases and xylanases, responsible for the degradation of hemicellulose. Therefore, this work seeks to characterize an α-L-arabinofuranosidase/β-xylosidase (TerARA) from termite intestine metagenoma and an endo-1,4-β-xylanase (PaeXYL2) from Paenibacillus sp. The putative enzyme genes were selected through the CAZy database, synthesized and cloned, and the recombinant proteins were expressed in *E. coli* BL21. Until this moment, the characterization of the TerARA enzyme is nearing completion and the PaeXYL2 enzyme has already been cloned, with initial production and purification tests underway. TerARA was purified by affinity chromatography on a nickel-sepharose column. *In silico* analysis of the amino acid sequence, analysis of enzyme activity in different synthetic and natural substrates, and the influence of pH, temperature and ions on the enzyme activity were carried out. The results show the bifunctionality of the enzyme to pNP-X and pNP-A, greater activity at pH 7.0 and temperature at 40 ºC. In tests with additives, the enzyme exhibited expressive inhibition effects in presence of Cu2+ and Fe2+ and EDTA. A slightly positive effects was exhibited in Ca2+ Mg2+ and Mn2+. The enzyme didn't show efficient thermal stability on the pNP-A substrate. The already results obtained show that TerARA has interesting characteristics for application in the deconstruction of plant biomass.
Keywords: hemicellulases, glycosyl hydrolases, bifunctional enzymes

CC.12 - High ethanol yield in mead fermentation after yeast immobilization and enzyme treatment of must

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Mead is an alcoholic beverage made from fermented honey. Publications about meads are growing in number since 2016 and several articles focus on fermentation problems due to lack of important nutrients, e.g. free amino nitrogen (FAN), in honey musts. Our work addressed a systematic review of mead literature and fermentation issues common to mead production, such as FAN and sugar availability. We hypothesised that sugarcane could be used both as juice for source of sugars and nutrients but also, its bagasse, as structural support to immobilize yeast for fermentation. In addition, commercial enzymes common to beer production were tested to improve nutritional characteristics of the honey must. A systematic search was done to understand all published scientific knowledge. Search in Google Scholar, PubMed and Web Of Science databases, choosing keywords: NMR (Nuclear magnetic resonance), HPLC (High performance liquid chromatography), Fermentation, Yeast, *Saccharomyces cerevisiae*, QA23, Honey, Mead, Beer, Wine, Cider. The results were extracted and submitted on organization software on-line Rayyan QCRI. 147 articles were found that describe the state of the art on the subject. FAN on musts treated or not with amylases, proteases and pectinases was measured by ninhydrin method. After protease only, FAN was increased by 55,5% and, after fermentation, final ethanol concentration was 49% higher. Morphology of sugarcane bagasse and yeast adhesion were analyzed by scanning electron microscopy. Bagasse pretreated with NaOH and heat resulted in greater cell adhesion, better cell dispersion per bagasse and higher fermentation yield. Our results shed light on the use of enzymes and support for yeast on the production of mead and provide strategies for this application on larger scale experiments. Despite the increase in publications, the literature is still incomplete and with many gaps and much still needs to be discussed.
Keywords: Mead, Fermentation, Free amino nitrogen
Supported by: faperj

CC.13 - Detection of Wheat stripe mosaic virus (WhSMV) using loop-mediated isothermal amplification.
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Wheat (Triticum spp.) is one of the most cultivated cereals worldwide. According to the national supply company (CONAB), Brazil is the sixteenth country in wheat production, yielding 5.2 tons per year according to the United States Department of Agriculture (USDA). In 2020, the southern Brazilian region was responsible for producing 90% of wheat, according to the Technical Office for Economic Studies of the Northeast (ETENE). The Wheat stripe mosaic virus (WhSMV) has been recently identified, whose infection leads to 50% wheat production loss. Our goal is to develop a point of care diagnostic test for the WhSMV virus using the Loop-Mediated Isothermal Amplification (LAMP) technique. The use of point of care strategies, such as the LAMP technique, allows the rapid identification of the pathogens in the field, improving decision-making, and expediting early treatment to reduce production losses.LAMP technique is a new DNA amplification method for pathogen detection that shows high speed, specificity, and efficiency, under isothermal conditions. Furthermore, the process is simple, low cost, and less sensitive to the presence of inhibitors. Our goal is to develop a new molecular test for WhSMV detection using LAMP. The rapid identification and early treatment of such a pathogen may improve grain production, thus making wheat and its derivatives more economically profitable for producers and millions of consumers worldwide.
Keywords: Wheat, WhSMV, LAMP
Supported by: PROMOP

CC.14 - Development and Stability study of Microemulsionate peelin of Papain
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The word “cosmetic” refers to products that amplify the skin´s appearance, instensify cleanliness and promote beauty.The dissemination of the increasing appreciation of appearance by the media, the cosmetic industry is expanding and little shaken by the economic crisis.Therefore, investiments in new product development have increased, and along with it, the appearance of new vehicles and used raw materials. Among them are the use of enzymocosmetics, an expanging area with promising results. To convey such products, several are used to improve their performance, and one of them is the conversion of regular materials into nanometric formulations. The objective of this work was developed and study the stability of a papain nanoemulsified enzymatic peeling. At first, a pseudoternary phase diagram was developed. The microemulsified systems were selected for delivery of 2% papain, followed by its characterization (size, zeta potential, PDI, conductivity, pH, preliminary stability, thermal stress.In the phase diagram, a wide region of transparent nanodisperse systems was identified, with PDI values below 0.2, and characteristic droplet sizes for this type of system. Formulation presented the average droplet size of 35.28. The values of PDI were 0.28. Macroscopic aspect of the formulation analyzed without separation of phases. In thermal stress, temperature generated large oscillations in the Zeta Potential values, but at values far from zero, which configures as main feature of ensuring system stability. PDI values have undergoing changes, as well as that of size, but still, if kept stable during the heating ramp. After conducting preliminar tests, the formulation becomes stable to deliver papain.Protein dosage tests, accelerated stability, and safety, should be performed to perform papaya delivery in this namoemulsified system
Keywords: Cosmetic, Nanotechnology, Papain
Supported by: FAPESB

CC.15 - Evaluation of Trim21 as a molecular tool for antibody purification

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The use of antibodies in therapeutics and diagnostic is an expanding segment of the pharmaceutical and biotechnology industries. Antibodies can be purified using chromatographic resins based on bacterial proteins A and G. However, these resins use acidic elution conditions, which may cause antibody denaturation. Therefore, improved methods of IgG purification are a need of the biotechnology industries. Trim21 is an intracellular protein that mediates intracellular antibody-mediated proteolysis and binds the Fc domain of IgGs and IgAs. The presence of increasing ionic strength weakens IgG binding. Therefore, Trim21 might be a good candidate for the development of chromatographic resins for IgG purification since elution conditions can occur at neutral pH conditions in the presence of salt. Previous studies from our group developed a chimeric protein consisting of the Fc binding domain of TRIM21 (PRYSPRY domain) linked to a streptavidin moiety to facilitate TRIM21 immobilization biotinylated matrices (PCT/BR2018/050310, 2018). Test the performance of the chimeric protein Trim21-streptavidin immobilized in biotinylated resins for the purification of G-type immunoglobulins (IgG). Trim21-streptavidin (Trim21-SA) was expressed in *E. coli* (BL21 (DE3) pLysS in the presence of 0.1mM isopropyl-β-D-thiogalactopyranoside (IPTG). The protein's inclusion bodies were denatured using 8M urea and refolded, followed by purification using Ni-NTA resin.Trim21-SA was immobilized in biotinylated resin, and the resultant resin was tested for IgG purification from human serum.Trim21-SA was efficiently immobilized in biotinylated resins, suggesting that the streptavidin moiety of the chimeric protein was efficiently folded. The resulting matrix was used to purify IgG from human serum. Neutral pH elution conditions are currently being tested. TRIM21-SA appears to be a promising tool in the purification of antibodies. This tool can expand the purification capacity of antibodies used in the manufacture of medicines, diagnostic, and research reagents, updating the biotechnological processes.
Keywords: Affinity chromatography, Biotin resin, Immunoglobulin G

CC.16 - EXPRESSION OF THE Cryptococcus flavus AMYLASE IN Pichia pastoris (Komagataella phaffii) FOR INDUSTRIAL APPLICATIONS

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The expression of recombinant proteins through heterologous systems is a promisiing alternative for the production of enzymes. The available models have adequate machinery to increase the production levels of a certain protein with the yield of expressed protein being higher than in native systems. The main goal of this study was to develop a recombinant *Pichia pastoris (Komagataella phaffii)* strain for the production of *Cryptococcus flavus* α-amylase (Amy1), using methanol as a sole carbon source. The nucleotide sequence encoding Amy1 was optimized with codon usage biased for expression in *K. phaffii*. The resulting recombinant plasmid was used to transform yeasts cells. Potential recombinant clones were then evaluated for their ability to degrade starch using FUWA method protocol qualitatively and quantitatively. The starch-degrading plate assay showed that Amy1 was successfully expressed in several clones and extracellular amylolytic activity increased during growth in fermentation, reaching a maximal value (5.45/5.64 U/mL) at 46h of incubation. These values were higher than those found in the literature. Preliminary results showed that the gene was successfully cloned and expressed in *K. phaffii* cells. However, additional studies on expression conditions need to be conducted. Therefore, this study was able to develop a recombinant *K. phaffii* strain with potential application in industrial starch degradation.
Keywords: Enzymes, Biochemistry, Recombinant
Supported by: CAPES, CNPq and FAPEMIG.

CD - Drug Discovery and Delivery

CD.01 - Details of the cooperative binding of piperlongumine with rat serum albumin obtained by spectroscopic and computational analyses

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Piperlongumine (PPL) has presented a variety of important pharmacological activities. Although PPL is present in the bloodstream, no information is found on the interaction between PPL and rat serum albumin (RSA). In this sense, the present study elucidated the mechanism of interaction between PPL and RSA, using in conjunction spectroscopic and computational techniques. The goal of present investigation is to elucidate the molecular biophysical mechanisms of the interaction between RSA and PPL. Two approach lines of the molecular biophysical field were applied: one experimental, based on spectroscopy; another computational, based on molecular docking and dynamics. Emission fluorescence was measured in an ISS PC1 spectrofluorometer with temperature controlled at 288, 298, 308 K.The sample was excited at 295 nm, and the emission spectrum was obtained in the region of 305-500 nm. Inner filter correction was applied for the excitation and for all the emission wavelengths. In the synchronous fluorescence experiments, excitation wavelength varied from 240 to 350 nm with the excitation and emission wavelengths interval (Δλ) set to 60 nm. The molecular docking was calculated with Autodock 4.2 and the molecular dynamics of the complex were executed by GROMACS/5.1.4, with GROMOS96/53a force field. Our work shows the importance of applying inner filter correction over the entire fluorescence spectrum prior to any conclusion regarding changes in the polarity of the fluorophore microenvironment. Thermodynamic parameters revealed that PPL binds to RSA spontaneously ($\Delta G < 0$) and the process is entropically driven. Interaction density function method (IDF) indicated that PPL accessed two cooperative sites in RSA, with moderate binding constants. The molecular docking described the microenvironment of the interaction sites, rich in apolar residues. The stability of the RSA-PPL complex was checked by molecular dynamics. The RSA-PPL complex was characterized by a molecular biophysical approach with the fluorescence technique and computational methods. **Keywords:** Spectrosocopy, Molecular dynamics, Protein-ligand interaction; **Supported by:** CAPES

CD.02 - Inhibitory effect of icetexane diterpenoids on Leishmania braziliensis old yellow enzyme

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Old Yellow enzymes (OYEs) are NAD(P)H flavin-dependent redox enzymes that promote the asymmetric reduction of activated α, β-unsaturated alkenes (e.g. as ketones, aldehydes, carboxylic acids). OYE are present in protozoan parasites such as *Leishmania braziliensis* (LbOYE) and could be a potential target for treatments of parasitic pathologies. Icetexane diterpenoid is a class of compounds from plants that have been reported with antiparasitic activity. In this study, we aimed to evaluate the inhibition of LbOYE activity by synthetic diterpene brussonol, a natural product first isolated from *Salvia brussonetti*, and two analogs AN-08 and AN-35. Recombinant LbOYE (rLbOYE) was produced in *Escherichia coli* cells and purified until homogeneity had its activity measured using N-ethylmaleimide (NEM) as substrate and NADPH as reductant. The kinetic experiments were performed following NADPH concentration decay at 340 nm in the presence of brussonol and analogs. The compound brussonol did not show significant inhibition while the analogs AN-08 and AN-35 did. Furthermore, the interaction of compounds with rLbOYE was carried out by fluorescence quenching with Kd on the order of μM. STD-NMR also demonstrates the interaction of rLbOYE and all ligands. The differences in the inhibition were better understood by molecular docking that showed differences of brussonol position in relation to the FMN group comparing to the analogs. These results demonstrate that icetexane diterpenoids modifications improved the inhibitory effects on the LbOYE activity.
Keywords: old yellow enzymes, Leishmania braziliensis, flavoenzyme. **Supported by:** FAPESP (2018/05576-1)

CD.03 - Antimicrobial activity of nanostructured lipid carriers prepared with natural oils and loaded with ciprofloxacin

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The fluoroquinolone ciprofloxacin (CIP) is a broad-spectrum antibiotic with activity against gram-positive and gram-negative bacteria of clinical interest such as Pseudomonas aeruginosa, Enterococcus faecalis, Staphylococcus aureus and Enterobacter aerogenes. Despite its low solubility, CIP inhibits the bacterial DNA gyrase, favoring degradation and blocking DNA replication, transcription, and DNA repair. Lipid-based drug delivery systems (DDS) such as nanostructured lipid carriers (NLC) have high upload capacity for lipophilic drugs such as CIP, and can be used to improve their bioavailability, chemical stability, and therapeutic action. Natural products can bring additional therapeutic properties if used as excipients of DDS. This study aimed to develop NLC composed of natural lipids with encapsulated CIP and evaluate its antimicrobial activity *in vitro*. The formulation was prepared using the emulsification-ultrasonication method, consisting of beeswax (7%), andiroba oil (3%), Pluronic F-68 (2.5%) and 3% encapsulated CIP. The optimized formulation presented 5.1 ± 0.2 x 1013 particles/mL, average diameters between 259-316 nm, low size polydispersity (< 0.20), negative zeta potentials (|28,6 - 35,3| mV), high encapsulation efficiency (94.6%) and shelf stability for 12 months of storage at 25 oC. In the antimicrobial susceptibility testing the diameters of the inhibitory halo of 10 bacteria strains (S. aureus - S.A., BEC9393, and RIB 1- S. Epidermidis, P. aeruginosa - 31NM, PA 26, PA-ATCC 25619 and 76JF - *E. coli* ATCC 129214 and K. pneumoniae KP230) treated with increasing concentrations of NLCCIP were compared to those of free CIP. The results showed that NLCCIP was effective against the 10 strains, and that encapsulation improved by 30-45% the antimicrobial activity of CIP. These results point out the potential use of NLC prepared with natural compounds – to join the intrinsic therapeutic properties of its lipid excipients to that of the fluoroquinolone antibiotics – in the treatment of bacterial resistant strains.
Keywords: Ciprofloxacin, nanostructured lipid carriers, Drug delivery. **Supported by:** FAPESP ; CNPq/PIBITI; CAPES

CD.04 - Peptide-conjugated silver nanostars as anticancer agent

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The challenge of bacterial resistance and the problems caused by the decreased efficacy of conventional antibiotics have led to the search new therapeutic strategies. Antimicrobial peptides (AMPs) have been identified as promising alternatives to the conventional molecules used nowadays against infection. Some of them have been shown to have dual activity, both as antimicrobial and anticancer peptides. Nanoparticles have been used as improved drug delivery systems and as diagnostic tools for the imaging of cancer cells. Silver nanoparticles (AgNPs) have high antibacterial activity, being a promising solution to treat intracellular infections. In addition to the use of AgNPs as therapeutic agent, their use as carrier, conjugating with molecules of biomedical importance, has been increasing in the last years. In the present work we conjugated the AMP PaMAP 1.9 with star-shaped silver nanoparticles (AgNSs) to improve the peptide efficacy against cancer cells. The peptide, AgNSs and conjugates were evaluated in terms of stability by the following methodologies: Nanoparticle Tracking Analysis, Transmission electron microscopy, Ultraviolet–visible spectroscopy, and Dynamic light scattering. Their anticancer activity was assayed through a XTT viability test. The biophysical characterization of the peptide-conjugated nanoparticles showed consistent size and stability. AgNSs induced cell death in MCF-7 cancer cells, at low concentrations, and the peptide itself exhibit cytotoxic activity on these cells. However, when combined with AgNSs, there was an enhancement of the percentage of cell death, showing that the anticancer activity of the peptide is amplified. The peptide and the nanoparticles were screened for cytotoxic effects in healthy cells as well. PaMAP1.9 has cytotoxic effects on MCF10-A cells above the IC50 of cancer cells. Furthermore, the peptide-AgNSs conjugate displays lower cytotoxicity to healthy cells in comparison with the peptide itself. Results indicate that PaMAP1.9 conjugated with AgNSs, provide lower toxicity and improve their anticancer effect. **Keywords:** antimicrobial peptides, silver nanoparticles, drug delivery, **Supported by:** Portuguese national funding agency for science, research and technology (FCT)

CD.06 - Articaine-loaded NLC functionalized with copaiba oil: Improvement of anesthetic effect in zebrafish and in murine inflammatory pain model

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Articaine (ATC) is the second most used amino-amide local anesthetic in dentistry. Due a peculiarity in its structure (an additional ester group), ATC can be used at higher doses, with lower systemic adverse effects that other anesthetics. However, like other local anesthetics agents, ATC is not effective on inflamed tissues. Articaine was encapsulated in nanostructured lipid carriers (NLC) prepared with copaiba oil (CO) as a functional excipient, to enhance its anesthetic and anti-inflammatory properties over inflamed tissues. After development and characterization, the NLC-CO-ATC formulation (particles size = 217.7 ± 0.8 nm, PDI = 0.174 ± 0.004, zeta potential = -40.2 ± 1.1 mV and ATC loading efficiency = 78.4 ± 0.1%) was tested regarding its anesthetic effectiveness using two in vivo models (Zebrafish larvae and murine). In Zebrafish larvae (5 days post fertilization), the NLC-CO-ATC formulation administered in the larvae medium was orally absorbed and promoted effects on the CVS (60% > bradycardia) and CNS (100% higher response to touch) than free articaine. In rats, nociception was measured by the electronic Von Frey test, using the carrageenan-induced inflammatory pain model and NLC-OC-ATC formulation increased by 30% the anesthetic efficacy compared to free ATC. Furthermore, NLC-CO-ATC prolonged the anesthetic effect until the end of the experiment (3 hours) while free ATC caused anesthesia for no more than 1 hour. Thus, the results found show that the encapsulation of ATC in NLC-OC is a promising strategy for the treatment of inflamed tissues, where the lower pH curbs the effectiveness of anesthetic agents. In addition, the formulation was found efficient to control postoperative pain (carrageenan-induced inflammatory pain model), probably due to ATC sustained release but also to the functionalization with CO, which is another advantage of this system.
Keywords: Articaine, NLC, inflammatory pain
Supported by: FAPESP (#17/15174-5, G.H.R.S. Ph.D scholarship and internship at Andres Bello University—Chile; E.P. grant #14/14457-5)

CD.07 - PROJECT: Study of neuroprotective potential of Amburana cearensis compounds in aminochrome *in vitro* model of Parkinson's disease

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Parkinson's Disease (PD) is a slowly progressing neurodegenerative disease with alarming epidemiological data, but without a cure. Some neuroprotective compounds derived from plants from the flora of the Brazilian caatinga have been prospected in order to inhibit dysfunctions and cell death in the pathogenesis of PD. Amburana Cearenses seed extracts have shown promising results in *in vitro* studies, as a potent inhibitor of neuronal death induced by glutamatergic excitotoxicity. In this sense, this work aims to study the potentially therapeutic effects of A. cearenses seed extract obtained by extraction with dichloromethane (EDAC), and its isolated coumarin. PD *in vitro* model will be induced by aminochrome. It is a molecule derived from the oxidation of dopamine and capable of inducing molecular alterations characteristic of PD, such as lysosomal and mitochondrial dysfunction, therefore, it has been used as an inducer in an experimental model of PD. Therefore, cell cultures of the PC12 lineage differentiated with nerve growth factor (NGF) treated with aminochrome and/or EDAC or coumarin will be used. In this model, cell viability will be evaluated by MTT, lysosomal acidification by staining with acridine orange and Lysosensor, as well as the evaluation of mitochondrial membrane potential by staining with 5.5', 6.6' tetrachlorine iodide 1 ,1,3,3' tetraethylbenzimidazolylcarbocyanine (JC-1) to investigate the association of aminochrome with lysosomal dysfunction and altered mitochondrial function. Thus, this work may contribute to the characterization of the neuroprotective effect of compounds from A. cearenses and will reveal potential pharmacological application of the plant and its constituents in the treatment of the disease.
Keywords: Amburana cearenses, neurodegeneration, neuroprotection

CD.08 - PROJECT: Evaluation of the antiproliferative and cytotoxic actions of Viridicatumtoxin A combined with antitumor drugs in 2D and 3D cell culture models.

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The viridicatumtoxins belong to a rare class of polyketide antibiotics of the tetracycline group produced by endophytic fungi of various species within the Penicillium, Aspergillus and Paecilomyces family. These toxins have positive functions in the pharmaceutical industry, acting as antibiotics. Furthermore, studies show their cytotoxicity in certain cancer cells, demonstrating that their use as a chemotherapeutic against cancer is a promising perspective. The objective of this proposal is to test the viridicatumtoxin A obtained from Penicillium, in human cancer cell lines (prostate,PC-03; kidney,786-0; colon,HT-29; MCF-7 breast and multidrug-resistant breast, MDA-MB-231) and a non-neoplastic one (fibroblast,HFF1) to obtain the selectivity index, in culture model 2D and 3D, since the three-dimensional model mimics the in vivo, where cells occupy the three dimensions of space, generating multicellular spheroid structures that exhibit aspects very similar to those observed in tumors in living organisms. In the 2D model, the antiproliferative activity in these strains will be evaluated, by staining with Sulforhodamine B, and then the strain with the highest antiproliferative activity will be selected by GI50 concentration for evaluations in the 3D model (spheroids) obtained by molds from a cell density of 1x106. These spheroids will be treated with viridicatumtoxin A and evaluated for apoptotic effect using fluorescent dyes and by flow cytometry for annexin V concentration, activation of caspase 3 and cell cycle arrest by 7AA-D. To estimate the oxidative stress action of the treatment, enzymatic and non-enzymatic markers will be evaluated. At the end of the study it is expected that it will be possible to determine the cytotoxic potential of the substance, as well as the selectivity index, cytostatic and cytocidal effect, and the possible pathways of antiproliferative activity from apoptosis and oxidative stress generated.

Keywords: cytotoxicity, viridicatumtoxin A, 3D cell culture

CD.09 - Combining Elmann's Colorimetric Assay and (STD)1H-NMR to Unravel Inhibitory Activity of a Ru(II) Polypyridyl Complex Towards human Acetylcholinesterase.

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Cholinergic hypothesis for Alzheimer's disease (A.D.) implicates neurotransmitter acetylcholine (ACh) cleavage by acetylcholinesterase (AChE) enzyme as the responsible event related to A.D symptoms. Anticholinergic drugs are the only available FDA approved treatment for A.D, although with several side effects and poor memory enhancement results. Ergo the search for acetylcholinesterase inhibitors remains important. Here, we report the inhibitory activity of cis-[Ru(bpy)2(EtPy)2].2PF6 (bpy = 2,2' bipyridine, EtPy = 4,2-ethylamino pyridine) against hAChE probed by classic Elmann assay and (STD)1H-NMR technique, that gives quantitative information about molecular orientation and surface interaction based on magnetic saturation transfer by NOE effect from enzyme to complex, enabling the fine tunning of the structure to enhance the results. Evaluate the cis-[Ru(bpy)2(EtPy)2].2PF6 inhibitory activity against hAChE by Elmann's assay and which ligand plays the key role in inhibition by (STD)1H-NMR. Reagents, solvents and hAChE were purchased from Sigma-Aldrich and used without purification. Elmann's assay was conducted in an Agilent 8453 Uv-Vis spectrophotometer using a Helma two window quartz cuvette (3 mL 1 cm length path) NMR experiments were conducted in a Bruker Avance 600 MHz. Michaelis-Mentem evaluation of Elmann's assay indicates that the complex strongly inhibits hAChE activity (inhibition constant Ki = 11,7 μM and IC50 = 39 μM) presenting a competitive mechanism. (STD)1H-NMR indicates that the aminopyridyl ligand interacts strongly with the enzyme surface (100% STD intensity) than bpy ligand (73%), probably due to structural resemblance between ethyl amino group and choline molecule. The complex presents strong inhibitory activity against hAChE as demonstrated by Elmann's assay. (STD)1H-NMR shows that the amino pyridine ligand is in close interaction to the surface of the enzyme, being most relevant to the result, which implies that structural changes in the other ligands may enhance the inhibitory activity of this complex.

Keywords: Acethylcholinesterase, Enzyme inhibition, Ru(II) complex
Supported by: CAPES (proc number 88882.332778/2019-01) and FAPESP (proc numbers 2018/16040-5, 2018/09145-5 and 2019/21143-0)

CD.10 - Role of residual Sb(III) in the therapeutic efficacy of topical meglumine antimoniate against cutaneous leishmaniasis

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Leishmaniasis is a global health problem and, in Brazil, the pentavalent antimonial meglumine antimoniate (MA) has been the drug of choice for the treatment of cutaneous leishmaniasis (CL) since 1943. This drug is used parenterally and is believed to act as prodrug, being reduced from Sb(V) into Sb(III) active form. Unfortunately, MA is highly toxic and the WHO strongly recommends the development of new therapeutic strategy. In this context, we investigate a topical formulation of MA for CL and the possibility of optimizing its efficacy through manipulation of the degree of polymerization of MA and the residual Sb(III) content. Three different forms of MA were used: synthetic MAs prepared from either SbCl5 (MA-SbCl5) or KSb(OH)6 (MA-KSb(OH)6); and commercial MA (Glucantime®). As demonstrated previously by our group, Glucantime® is more polymerized than the synthetic MAs and MA-SbCl5 exhibits the highest level of Sb(III) residue. These compounds were formulated as propylene glycol-containing hydrogels at 12%(w/v) Sb concentration. These formulations were applied daily for 30 days at the tail base of BALB/c mice after infection with *Leishmania amazonensis*. Control groups were treated topically with drug-free gel or remained non-treated. The efficacy of topical treatments was compared to that of intraperitoneal treatment with Glucantime® (200 mg Sb/kg/day). Treatment with the topical formulation of MA-SbCl5 promoted significant reductions in the lesion size and parasite load in comparison to controls, to a similar level as the parenteral treatment. On the other hand, Glucantime® or MA-KSb(OH)6 did not show significant efficacy. This study strongly supports the role of residual Sb(III) in the therapeutic efficacy of topical MA against CL. MA-SbCl5 also emerges as a promising chemical form of MA for the topical treatment of CL.

Keywords: antimony, topical formulation, speciation
Supported by: CNPq

CD.11 - Hot spots identification and molecular fragment screening as innovative approaches to development Leishmania major diidroorotate dehydrogenase inhibitors

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Although more than 1 billion people are at risk by neglected tropical diseases (NTD) and 35,000 deaths/day are due to such ailments worldwide, the R&D investment from both private and public sectors to fight NTD is disproportionally small. Among NTDs, cutaneous leishmaniasis (CL) is spread over 85 countries and causes up to 119,600 deaths, per year, in Brazil. The available drugs display high toxicity and low efficacy, highlighting the need for novel therapeutic alternatives to fight CL. In order to accomplish this goal, dihydroorotate dehydrogenase (DHODH), a key enzyme from the de novo pyrimidine biosynthetic pathway, has been exploited. In contrast to previous studies that target DHODH active site (orotate-BS), this work focus on a putative druggable pockets, identified with FTmap server (https://ftmap.bu.edu/). Aiming at identifying compounds that bind outside orotate-BS, the following screening strategy was designed: Fragments that shift DHODH Tm (melting temperature) in Thermofluor assays (DTm ≥ 1.0 ºC), but not in ThermoFMN were considered as promising compounds, since FMN binding site is close to orotate-BS. Thermal stabilization in ThermoFMN assays is highly correlated to competitive inhibitors, which bind to orotate-BS, whereas Thermofluor assay accounts for interactions to any hydrophobic surface that is exposed upon protein denaturation. Accordingly, compounds that are inactive in ThermoFMN, but active in Thermofluor might behave as non-competitive LmDHODH. Accordingly, a series 76 compounds had its effect over DHODH thermal stability investigated at a single concentration (0.5 mM). This strategy led to the identification of 15 hits. Currently, the concentration-response behavior of these compounds is being investigated by Microscale Thermophoresis to exclude false-positives and proceed with kinetic assays that support the non-competitive mechanism of action for those compounds.

Keywords: Dihydroorotate dehydrogenase, Fragment screening, Leishmania major
Supported by: FAPESP and CNPq

CD.13 - Production of metallic nanoparticles-polymeric hydrogels hybrid systems for tooth whitening applications

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The search for white teeth in aesthetic character by the world population pushed scientists to search for new technologies targeting at better whitening-process performances, allied to minimizing possible side effects such as teeth sensitivity and enamel wear. In this sense, new technologies have been developed associating different types of systems such as combining metallic nanoparticles and micellar hydrogels. This study aimed at the preparation and physico-chemical characterization of hybrid systems composed of metallic oxide nanoparticles and their further dispersion in thermosensitive hydrogels for tooth whitening purposes. Titanium nanoparticles (titanium oxide II, IV and isopropoxide, 5 mM) were prepared and dispersed into poloxamer 407-P407/10 or 90 kDa hydropxypropyl methylcellulose-HPMC (20/0.5 % m/v). Sol-gel transition temperatures (Tsol-gel) were determined by rheological analysis, with values from ~18.8 to 24.9 °C, according to titanium isopropoxide structures incorporation into 10 kDa HPMC-P407 hydrogels. Elastic (G') and viscous (G") modulii relationships were from 30 to 10-times for all systems. Ti-isopropoxide incorporation showed an influence on hydrogels structural organization considering the presence of P407 isolated or in association with 10 or 90 KDa HPMC. Scanning Electron Microscopy revealed an amorphous character with rough and porous surfaces for all formulations. FTIR analysis showed asymmetric and symmetric CH2 stretching (2922 and 2860 cm-1, respectively) and C=O stretching vibrations (1100 cm-1) suggesting an interaction between Ti nanoparticles and P407. The results revealed that all Ti nanoparticles were incorporated into PL-HPMC hydrogels and their differential influence on systems structural organization according to HPMC molecular weight, maintaining the hydrogels bioadhesive properties looking forward tooth whitening applications.
Keywords: Hydrogels, Metallic nanoparticles, Tooth whitening
Supported by: UFABC Multiuser Central Facilities; FAPESP (2019/20303-4; 2019/14773-8) and CNPq (307718/2019-0).

CD.14 - A new arylsulfanyl-benzo-2,1,3- thiadiazoles derivative has anti-amnesic effect on behavioral in mice

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Dementia is one the major complications involved memory loss, characterized by cognitive decline and it is commonly associated with behavioral disturbance in Alzheimer`s disease. Available treatments are based on symptom reduction, but they not stop the progression of cognitive disorder. This way, the search for new treatments is of great value. Faced with this, our research group showed recently the ability to inhibit *in vitro* acetylcholinesterase (AChE) activity in the cerebral cortices of mice by an arylsulfanyl-benzo-2,1,3-thiadiazoles derivative, 5-((4-methoxyphenyl)thio)benzo[c][1,2,5]thiadiazole (MTDZ). In this context, the aim of the present study was to investigate the binding affinity of MTDZ with AChE and effect anti-amnesic of compound in a scopolamine (SCO)-induced model in mice. The binding affinity of MTDZ with AChE was investigated by molecular docking analyses. For experimental model, male Swiss mice were treated with MTDZ (10mg/kg, intragastrically (i.g.)) or canola oil (10ml/kg, i.g.) and induced, thirty minutes after, with injection of SCO (0.4 mg/kg, intraperitoneally (i.p.)) or saline (0.9%, 5 ml/kg, i.p.). On days 6 and 7, mice were submitted to object recognition and location, and on day 8 were submitted to Y-maze task. We verified that MTDZ interacts with residues of the AChE active site, similar to donepezil. SCO caused amnesia in mice by reducing the exploratory preference for new object and for new location of object, as well as, by reducing the spontaneous alternation behavior. MTDZ treatment attenuated the behavioral changes caused by SCO, with actions similar to donepezil. In conclusion, MTDZ presented dual inhibition activity of AChE, presenting anticholinesterasic action and it ameliorated the SCO-induced behavioral changes in learning- and memory-impaired mice. **Keywords:** dementia, amnesia, thiadiazoles
Supported by: CNPq, FAPERGS, CAPES, UFPel

CD.15 - Outcomes of Se-DMC therapy for inflammatory response, nociception and oxidative stress in a mono-arthritis model in mice

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Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by inflammation of the synovial membrane, causing damage and destruction of the cartilage and bone tissue that form the joints. It is estimated that 1% of the world population is affected by RA. Pain is the most prevalent and debilitating symptom reported by RA patients and it is associated with inflammation of the peripheral joints. There is currently no cure for RA and its treatment aims to reduce pain and inflammation and improve the patients' quality of life. Despite major advances, not all patients achieve disease remission, and there is still an unmet need for new therapeutic approaches. Thus, the objective of the present study was to investigate the therapeutic potential Se-[(2,2-dimethyl-1,3-dioxolan-4-yl) methyl] 4-chlorobenzoselenolate (Se-DMC) on mono-arthritis model induced by complete Freund's adjuvant (CFA). The effect of Se-DMC and Meloxicam (5 mg/kg, oral route), both administered to animals daily for 14 days, on mechanical sensitivity (Von Frey test), inflammation (expression of tumor necrosis factor-alpha (TNF-α) and nuclear factor-κB (NF-κB) and paw histology) and oxidative stress (reactive species (RS) levels, non-protein thiol (NPSH) content and superoxide dismutase (SOD) activity) was evaluated in male *Swiss* mice exposed to intraplantar injection CFA (0.1 ml, 10 mg/ml). Se-DMC reduced the paw withdrawal threshold and mRNA relative expression levels of TNF-α and NF-κB induced by CFA. Paw histopathological results revealed the antiedematogenic potential of the compound, evidenced by the smaller amount of dilated lymphatic vessels when compared with the CFA group. In addition, Se-DMC reduced the RS levels and restored the SOD activity in paw of CFA mice. No alteration in the NPSH content was observed after treatments. Our results demonstrated that Se-DMC reduced inflammatory and nociceptive responses and exerts antioxidant actions, representing a new therapeutic approach for the treatment of the RA.

Keywords: arthritis, hyperalgesia, inflammation
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CD.16 - Potential Biomarkers with Quercetin and Lanthanides

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Flavonoids are ubiquitous plant compounds with great capacity as metal chelators and scavengers of free radicals. Some flavonoids such as myricetin have been shown to express antimicrobial activity by curbing the development of biofilms without killing bacteria. However, the molecular basis of the antimicrobial activity of this class of compounds is poorly known. The development of molecular probes to study the antimicrobial mechanism of flavonoids can provide useful insights into the action of these compounds. In this study, we have investigated the potential of quercetin and lanthanides ions (Eu^{3+} , Nd^{3+}) to coordinate to produce luminescent complexes. The flavonoid quercetin is a more affordable chemical variant of myricetin, and for this reason, has been chosen as a preliminary model. The synthesis of quercetin was obtained via hydrolysis of rutin in the presence of HCl. Subsequently, we have deprotonated the hydroxyl groups using NaOH, which was subsequently complexed with the Eu^{3+} and Nd^{3+} ions. The preliminary characterization of these complexes showed, via fluorescence spectrometry, luminescence intensification capacity of these complexes upon variations of the chemical environment. This observation points to the potential use of these complexes as biological markers that can respond to changes in the chemical environment in the cell, such as seen in the penetration in the cell membrane.

Keywords: Lanthanides, Markers, Quercetin. **Supported by:** FACEPE, CNPq and CAPES.

CD.17 - Aging aggravates acute oxaliplatin-induced peripheral neuropathy in mice through the increase of the oxidative stress and modulation of Na+, K+ - ATPase: Beneficial effects of 7-chloro-4-(phenylselanyl) quinoline

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Cancer is considered a major public health problem and its incidence increases with age. Chemotherapy remains one of the primary treatment approaches for patients with cancer. Despite the advancement in cancer treatment, several chemotherapeutic drugs, as oxaliplatin (OXA), promote peripheral neuropathy. Almost 90% of patients develop acute neuropathic pain immediately after OXA treatment. 7-Chloro-4-(phenylselanyl) quinoline (4-PSQ) is an organoselenium compound with potential antioxidant, anti-inflammatory, and neuroprotective actions. Thus, this study aimed to investigate the impact of aging on acute OXA-induced peripheral neuropathy in *Swiss* mice and the effect of 4-PSQ as a pharmacological strategy. Mice were divided into six groups: young; young+OXA; young+OXA+4-PSQ; old; old+OXA; old+OXA+4-PSQ. Male aged (20 months) and young (2 months) mice were intraperitoneally treated with OXA (10 mg kg^{-1}) or vehicle on days 0 and 2 of the experimental protocol. On day 2, half an hour after OXA administration, mice received 4-PSQ (1 mg kg^{-1}) or vehicle, by the intragastric route. The acetone drop test and the Von Frey test were performed 0.5 h after the 4-PSQ-treatment to access the OXA-induced thermal and mechanical sensitivities. Posteriorly, animals were euthanized and the sciatic nerve, spinal cord, and cerebral structures were collected to further assays. The reactive species (RS) and nitrate and nitrite (NOx) levels and their influence on Na^+, K^+-ATPase activity were also analyzed. It was observed that 4-PSQ reversed hypersensitivity induced by OXA and aging. 4-PSQ reduced the RS and NOx levels increased by OXA exposure in both ages. Na^+, K^+-ATPase activity was inhibited by aging and OXA. However, 4-PSQ reversed the inhibition of Na^+, K^+-ATPase activity OXA-induced, but not caused by aging. 4-PSQ might be a good prototype for the development of a more effective drug for the treatment of OXA-induced acute peripheral neuropathy.
Keywords: aging, Na+, K+ - ATPase, oxaliplatin
Supported by: FAPERGS, CNPq, CAPES, L'ORÉAL-UNESCO-ABC for Women in Science

CD.18 - Literature review of the therapeutic utility of *Campomanesia* ssp species focusing on the study of *Campomanesia adamantium*

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The *Campomanesia adamantinum*, popularly known as guavira, guabiroba or gabiroba-do-mato, is recognized as the symbol fruit of the state of Mato Grosso do Sul because it is a native plant with wide distribution of popular use as food and treatment, and its activities are attributed mainly by its phenolic components, flavonoids, chalcones, carotenoids and vitamins. The present work aims to perform a bibliographic survey in databases about biological activities related to species of the genus Campomanesia. The searches were made in the MEDLINE (PubMed) platform, with no lower period limit and no language restriction. The descriptors used in the data collection were Campomanesia; Myrtaceae; pharmacology. The search strategy used was Campomanesia and Myrtaceae and pharmacology. Forty-five articles were found, and 33 articles related to the theme were selected. The species C. xanthocarpa and C. adamantium were the most studied, with 17 and 7 studies. Other species cited were *C. guazumifolia, C. reitziana, C. velutina, C. pubescens* and *C. phaea*. Regarding the part of the plant used, 20 (60.6%) used leaves, 11 (33.3%) used fruits, used roots and used seeds. The species *C. xanthocarpa* and *C. adamantium* showed antioxidant, antilipemic and anti-inflammatory effects. *C. xanthocarpa* also showed trypanocidal, antiglycemic, antiplatelet and hepatoprotective activity and *C. adamantium* showed immunomodulatory and antidepressant activity. The other species showed anti-inflammatory, antioxidant and antinociceptive activities. And the leaf extracts of Campomanesia species presented pharmacological potential to be studied. Thus, it is concluded that the Campomanesia species have pharmacological potential to be studied.
Keywords: Campomanesia, Campomanesia adamantium, pharmacology

CD.19 - Acute toxicity assays of nanoencapsulated and free Colchicine in *Drosophila melanogaster*: Proposal for the repositioning of drugs for the treatment of Covid-19

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Much research has been conducted to develop possible treatments for Covid-19 and the repositioning of existing drugs is an opportune option. Colchicine is a promising drug to minimize the inflammatory conditions so characteristic of this disease, its administration through nanoencapsulated formulations may be able to optimize therapy and reduce its adverse effects. Therefore, the aim of our work is to evaluate the acute toxicity of nanoencapsulated (NCOL) and free (COL) colchicine in an alternative animal model such as *Drosophila melanogaster*. The flies were divided into 5 groups of different concentrations: Control, 0.001 mg/mL, 0.0025 mg/mL, 0.005 mg/mL and 0.010 mg/mL of NCOL and COL exposed for 48 hours. Afterwards, the survival rate, locomotor performance (open field test and negative geotaxis) and biochemical analyses (reactive species (RS) and thiobarbituric acid reactive substances (TBARS)) were analyzed. We obtained as results that both the NCOL and COL formulations did not affect the fly survival rate compared to the control group. Locomotor performance in the open field did not differ statistically for NCOL, but concentrations of 0.0025 and 0.010 mg/mL (COL) had reduced crossings compared to 0.001 mg/mL (COL). In the negative geotaxis test, there were no significant differences. COL showed an increase in RS levels at concentrations of 0.0025 and 0.005 mg/mL and TBARS levels were elevated in the 0.0025 mg/mL and 0.005 mg/mL groups. In NCOL, no statistical differences were found in RS, however TBARS were elevated by 0.001 mg/mL in relation to the control. It is concluded that acute exposure to COL induced oxidative damage, however the tested concentrations of NCOL proved to be safe and with low toxicity, so further analysis is needed to target it for therapeutic purposes against Covid-19.

Keywords: Nanoencapsulation, Repositioning, Toxicity
Supported by: CAPES

CD.20 - *In silico* studies on the interaction between bioactive ligands and DPP-IV: insights on potential candidates for the treatment of type 2 *Diabetes mellitus*

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Type II *Diabetes mellitus* is a metabolic disorder that involves a failure of insulin secretion by the pancreas and/or of action of insulin in target tissues. In 2019, the number of people with diabetes in the world reached the mark of 463 million and it is estimated that in 2045 we will have 700 million cases. It is known that a metabolic pathway involved in the control of diabetes is the inhibition of dipeptidyl peptidase-IV (DPP-IV), which regulates the degradation of GLP-1 (glucagon-like peptide-1) and GIP (gastric inhibitory peptide), which are responsible for maintaining the insulin levels and reducing the blood glucose levels. In this study, we employed molecular modeling strategies in a set of inhibitor compounds to understand the main molecular characteristics related to the biological activity of these ligands at the DPP-IV enzyme. For this study, a set containing 45 compounds was selected for all analyses. CoMFA (Comparative Molecular Field Analysis) method was used to understand the relationships between molecular features (electrostatic and steric) of the compounds and their biological data. The possible interactions that the set of DPP-IV inhibitors can perform at the active site of the DPP-IV enzyme were analyzed using the molecular docking method, implemented in the GOLD program. The models obtained from CoMFA presented significant values of internal (r2 = 0.988 e q2 LOO = 0.768) and external (r2pred = 0.986 and r2 m= 0.970) validations. Important interactions between the studied compounds and active site´s residues, such as Glu205, Tyr666, Arg125, Ser630, Phe357 and Tyr662, were also identified. Therefore, from the results obtained in this study, it is possible to propose molecular modifications in the structure of the DPP-IV inhibitors to improve their potential to treat type 2 diabetes.
Keywords: Diabetes, DPP-IV, molecular modeling. **Supported by:** FAPESP, CNPQ e CAPES

CD.21 - Virtual screening and *in vitro* assays of ligands with activity against dipeptidyl peptidase-4 (DPP-4) enzyme

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Diabetes is a metabolic disorder with hyperglycemia and vascular complications due to non-production of insulin or inappropriate use of insulin by the body. One of the treatment strategies is the inhibition of dipeptidyl peptidase-4 (DPP-4). Conduct virtual screening studies to search new bioactive inhibitors for the DPP-4 enzyme. This study involves a virtual screening with the AutoDock Vina software, in the crystallographic structure of DPP-4 (PDB 4A5S), using the "Diversity Set II" database available in the ZINC database. The applied filters were affinity energy, ligand efficiency, positioning at the three DPP-4 binding sites, molecular interactions and pharmacokinetic properties (ADME-Tox). The conformations of the ligands obtained from AutoDock Vina were analyzed using a consensus obtained by using two other algorithms (AutoDock and GOLD). For the biological assay, the "DPPIV-Glo™ protease assay" was used. The cytotoxicity test was performed using the following cells: MCF-10A, MCF-7, HeLa, A549 and GM07492A. The ZINC 1572309 molecule established π-stacking, π-π, T-stacking and cation-π interactions with important residues, such as Tyr547, Tyr662 and Tyr666; salt bridge with Arg125 and His740 and hydrogen bonding with Ser630. The results of the ADME-Tox prediction for the ZINC 1572309 molecule were compared with the drug sitagliptin. The ZINC 1572309 molecule presented TPSA outside the classification, representing limitations regarding the parameters of lipophilicity and polarity. The assessment of oral bioavailability confirmed the need for molecular modifications. The toxicity prediction of the mutagenic effects (AMES test) showed that both molecules are not toxic. Although the ZINC 1572309 molecule presents an available and accessible synthesis, the results of the ADME-Tox showed the need for structural changes. From *in silico* and *in vitro* studies, a molecule was promising as DPP-4 inhibitor that can structurally be optimized to achieve the suitable pharmacodynamic and pharmacokinetic profiles.
Keywords: Diabetes, DPP-4, Drug discovery

CD.22 - PROJECT - Analysis of The Effect of Flavonoid Rutin on Modulation of Glial Response and Neuroprotection In Amiotrophic Lateral Sclerosis Study Models

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Amyotrophic Lateral Sclerosis (ALS) is classified as a disease characterized by the degeneration of motor neurons and consequent loss of motor function. Among molecular and cellular alteration in ALS, mutation in SOD1 and gliosis seems to play a key role in the pathogenesis. It is shown that neuroinflammation associated with microglia and astrocytic reaction to SOD1 aggregates contribute to the motor neuron loss. In this bias, bioprospection of compounds with potential to modulate glial cells response is a strategy for drug discovery for ALS. Studies developed by the Laboratory of Neurochemistry and Cell Biology (LabNq) research group have already demonstrated that rutin has a regulatory effect on the activation of microglia and astrocytes, regulating tumor necrosis factors (TNF) and nitric oxide (NO) in cortical primary glial culture or microglia culture from rats. Therefore, our objective in this project is to evaluate if rutin have the potential to modulate glial cell function involved in ALS pathogenesis, preventing neuronal degeneration. The aim of this project is to evaluate the protective effect of rutin and modulation of glial response in ALS study model. *In vitro* studies will be performed in cortical primary culture of microglia from mutant SOD1G93A rats. To access the effects of rutin on glial activation and neuroinflammation, immunocytochemistry and westernblotting will be performed for GFAP, GS, IBA-1 and OX42, and qPCR will be performed for IL1b, IL6, TNF, IL10, TGFB1, ARG1. Additionally, in in vivo studies, neurodegeneration and glial activity will be evaluate using immunohistochemistry for GFAP, S100b, IBA-1, ChAT, CD206 and CD68 in spinal cord and nucleus hypoglassus of mutant SOD1G93A rats. This project is a collaborative work with Dr. Luis Barbeito from Pasteur Institut in Montevideo. We hope that the results obtained will contribute to the development of new therapeutic interventions for ALS.
Keywords: Flavonoids, Aminotrophic Lateral Sclerosis, neuroinflammation
Supported by: FAPESB and CAPES

CD.23 - Characterization of sub products from forced degradation of the antibiotic Cephalexin

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Cephalexin is a first generation & β-lactam antibiotic, largely used in the treatment of diseases caused by gram-positive bacteria such as those appearing in urinary and respiratory infections, being classified as an essencial medicine by the World Health Organisation (WHO). Guidelines for degradation studies of medicines have been stablished by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) to verify how changes on environmental physicochemical conditions influence the quality of pharmaceutical compounds. Through degradation analyses, it is possible to verify the intrinsic stability of the molecule under stressing conditions, and simulate conditions of incorrect storage. to identify byproducts from degradation of cephalexin under stressing conditions, and to investigate the antibiotic behavior of these byproducts against bacterial strains. Cephalexin solutions at 2 mg/ml were submitted to heating at 60 °C for 7 days or to UV irradiation to doses of 1.2 million lux-hours. The degraded solutions were studied by liquid chromatography, mass spectroscopy (MS) and nuclear magnetic resonance (NMR) spectroscopies to identify sub products. *In vitro* assays using *E. coli*, E. cloacae and B. subtilis as bacteria models have been performed to assess how minimum inhibitory concentrations and growth rates of colonies are affected by degradation of the antibiotics. Our data show that Cephalexin is highly susceptible to degradation under oxidizing and photolytic stress (> 1.2 million lux hours, UV and visible range). Acid and basic conditions appear to promote degradation to a lower extent, whereas very little formation of subproducts is observed in samples submitted to thermal stress (up to 60°C for 7 seven days) and exposure to metal ions. The main sub products generated from cephalexin degradation were cephalosoporanic acid, 7-ACA, and dimers. These species do not retain the antibiotic capacity of cephalexin.

Keywords: antibiotics, degradation, analytical chemistry

CD.24 - Expression and characterization of scFv antibodies against human tissue kallikrein 7

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Human tissue kallikrein 7 (hKLK7) is a serine protease associated with skin desquamation. The alteration in its activity is associated with several skin pathologies such as Netherton Syndrome, where high activity of hKLK7 leads to a severe desquamation process. Through phage display, three antibodies were selected against hKLK7 and aimed to be expressed as a single-chain fragment (scFv) in *E. coli*. Although numerous challenges of expressing scFv antibodies in *E. coli* lineages are known, such as aggregation in inclusion bodies, misfolding and existence of rare codons in prokaryotic organisms, this research purposes a study of the three scFv selected sequences, leading to a comparative expression experiments of them in different *E. coli* strains (BL21(DE3), Origami 2 and Roseta (DE3)), aiming to optimize recombinant scFv expression with different expression conditions (culture volume, concentration of IPTG and temperature). Bacteria were transformed and verified by PCR. Bacteria were inoculated in 10 ml flasks filled with LB medium. When OD_{600} reached 0.5, each flask was induced with different concentrations of IPTG (0.05 µM, 0.1 µM, 0.2 µM, 0.5 µM, 0.75 µM, 1 mM) at 30°C. After 2, 4 and 24 hours of induction, 1 ml of each culture was removed and harvested and pellets were loaded on 12% SDS-PAGE and analyzed by Western blotting. Positive results were then repeated in 100 ml of culture for antibody purification. There were none expressed scFv in BL21(DE3) and Origami 2 cultures. Low IPTG concentration (50 µM) at 30ºC in Rosetta 2(DE3) leaded to a great amount of recombinant protein. When repeated in 100 ml, most of the expressed scFv were in inclusion bodies, needing to be extracted with urea and refolded. Refolding leaded to a partial loss of scFv activity. The partial loss of scFv activity due to refolding is yet a challenge to be studied in this research.

Keywords: kallikrein, phage display, scFv
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CD.25 - Lidocaine- and synthetic curcuminoid-activated organogels towards dual drug-carrier formulations for topical applications

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Organogels (ORGs) are semi-solid systems, widely investigated for pharmaceutical and cosmetic applications. This work proposes the development of a dual drug-release system, considering the application of synthetic curcuminoid derivatives (CURs) and lidocaine (LDC) to treat topical inflammatory lesions. ORGs were prepared associating an aqueous phase (AP) of Pluronic F127-(30% w/v) and LDC hydrochloride (25 mg/mL) with 2 mL isopropyl myristate (IPM) with lecithin (LEC) 2% as organic phase (OP) , adding the CURs (1 mg/mL). The OP:AP ratio was 1:4 v/v. Physico-chemical characterization was performed by rheology, differential scanning calorimetry, and scanning electron microscopy. In addition, *in vitro* permeation assays were also performed by using vertical Franz-type diffusion cells and artificial skin-model (Strat-M®). Results revealed that the LEC-LDC-CURs incorporation into ORGs increased viscosity and elastic/viscous moduli (G'/G") ratio, favoring the ORGs structural organization. *In vitro* permeation assays through skin-model showed that LDC permeation flux was enhanced by the LEC/CURs (19,42 ± 1,47 μg·cm−2·h−1) when compared to formulations without LEC/CURs (14,64 ± 1,07 μg·cm−2·h−1). The LDC permeation coefficient was also observed to enhance, from 1,22 ± 0,02 (without additives) to 1,62 ± 0,03 cm·h−1 (with additives) after 48h. Moreover, latency time was also increased, i.e., LDC-release starts after longer times in formulations containing the LEC/CURs additives. Porcine ear skin epidermis (EP) were analyzed by Infrared Spectroscopy (FTIR), where the C-H symmetric and asymmetric stretching bands and the splitting of the CH2 scissors band were studied in detail. The lipids from matrix into the stratum corneum were observed to have an orthorhombic structure, which tends to disorganize after 4 and 24 hours after treatment with formulations. Perspectives include Optical Coherence Tomography studies to concept the interaction between the ORGs and the stratum corneum lipidic matrix. In summary, results pointed ORGs as promising formulations for skin-delivery with potential pharmacological application in topical lesions. **Keywords:** Organogels, drug-release, skin. **Supported by:** FAPESP (2019/14773-8, 2019/20303-4), CNPq, CAPES

CD.26 - Structure-based virtual screening identifies Novobiocin, Montelukast and Cinnarizine as TRPV1 modulators with anticonvulsant activity in-vivo.

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Transient Receptor Potential Vanilloid 1 (TRPV1) is a nonselective cation channel modulated by endogenous and exogenous ligands, pH, temperature, and voltage. In the last few years, it has been proposed as a promising target to develop novel anticonvulsant compounds. However, thermoregulatory effects associated with channel inhibition have hindered the way towards TRPV1 antagonists becoming marketed drugs. We conducted a structure-based virtual screening (VS) campaign to repurpose TRPV1 inhibitors among approved drugs, which are known to be safe and thermally-neutral. Initially, three homology models of the hTRPV1 were constructed and refined with Rosetta, representing biologically relevant states of the channel: unliganded, open agonist-bound, and closed antagonist-bound. Then, several docking conditions were evaluated to find the best docking model, in terms of pose and score accuracy, able to identify compounds interacting with the capsaicin binding site. Top scoring hits were evaluated *in vitro* using the patch-clamp technique and, in vivo on the maximal electroshock seizure (MES), the 6 Hz psychomotor (6 Hz) and pentylenetetrazole (PTZ) mice tests. Among top scoring hits from the VS campaign, Novobiocin, Montelukast, and Cinnarizine were selected for biological testing. The interaction between the selected compounds and the TRPV1 channel heterologously expressed in HEK293 cells was evaluated by their ability to reduce currents induced by 250 nM capsaicin using the patch-clamp technique. All tested compounds showed inhibitory effects on capsaicin-induced TRPV1 currents measured at -100 mV (% inhibition: 60±7 (n=10), 62±8 (n=8) and 40±6 (n=6) for 0,1 μM Novobiocin, 0,1 μM Montelukast and 1 μM Cinnarizine, respectively). Finally, their *in vivo* anticonvulsant profile was completed, showing promising anticonvulsant activity mainly against maximal electroshock seizure test. Our results further support the modulation of TRPV1 channels as a promising strategy to develop novel antiepileptic drugs. **Keywords:** docking, epilepsy, TRPV1. **Supported by:** PICT 2016-0165 (ANPCyT), Argentina

CD.27 - Development of nanoparticle-based DNA vaccine against SARS-Cov-2.

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A safe and effective vaccine with long-term protection against SARS-CoV-2 and variants is an urgent global health priority. Nucleic acid vaccines have emerged as a promising alternative to conventional vaccine approaches. Here, we developed a platform of nanoparticle-based DNA to provide safe and effective delivery of DNA to directly induce antigen presenting cells (APCs) to express antigens implicated in immune recognition of SARS-CoV-2. Therefore, our objective is to develop a nanoparticle-based DNA vaccine for potently neutralizing responses against SARS-CoV-2. Lipid nanoparticles (LNPs) were formulated via rapid microfluidic mixing and characterized with regard to size, zeta potential, pKa and encapsulation efficiency. To assess the immunogenicity and protection against SARS-CoV-2, BALB/c mice were immunized intramuscularly with LNPs containing DNA coding for GFP (Control Group), spike protein (S), nucleocapsid protein (N), or spike protein plus protein nucleocapsid (S+N). Two vaccine schedules were carried out, one in a single dose, while the other received a second dose 3 weeks after initial immunization (prime+boost). To assess cell-mediated immune response, splenocytes from vaccinated mice were incubated in culture with SARS-CoV-2 proteins and evaluated by flow cytometry. To assess neutralization titer, plaque reduction neutralization test was performed. Single dose and prime+boost schedule were able to induce lung resident memory. In two doses schedule, an antigen-specific CD8+ T cells significantly responded to SARS-CoV-2 proteins (S and N) in animals immunized with S+N-LNPs. In addition, immunizations with S+N LNPs in two doses was able to neutralize significantly Wuhan and P1 variants infections. We developed a LNP platform capable to provide effective delivery of DNA to APCs to express antigens implicated in immune recognition of SARS-CoV-2 and variants. Clinically, S+N-LNP, which induced robust systemic humoral and cell-mediated immune responses, hold promise as a vaccine candidate for preventing SARS-CoV-2 and variants infection.
Keywords: nanoparticle-based DNA, vaccine, COVID-19. **Supported by:** CNPq, PRPq-UFMG, CAPES and FAPEMIG

CD.28 - Nanostructured lipid carriers prepared with olive oil shows anti-inflammatory activity, as seen in zebrafish acute inflammatory model

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Oleocanthal, a component of olive oil, has a poor bioavailability but anti-inflammatory properties comparable to those of ibuprofen. Nanostructured lipid carriers (NLC) are drug delivery systems composed of a blend of solid and liquid lipid stabilized by surfactants; they are able to encapsulate insoluble drugs, increasing their bioavailability. In here, olive oil was used as the liquid lipid of NLC stabilized with Pluronic F68 (NLC-OO-P) or Tween 80 (NLC-OO-T). The characterization of these particles revealed for NLC-OO-P: size= 236.4 ± 0.5 nm, polydispersity index (PDI)= 0.163 ± 0.03, zeta potential (ZP) = -31.5 ± 0.7mV and 6.08±0.70 x 10∧13 particles/mL. For NLC-OO-T: size = 225.7 ± 0.6 nm, PDI = 0.205 ± 0.02, ZP= -22; 26.5 ± 0.1mV and 4.90 ± 0.21 x 10∧13 particles/mL. The aim of this work is to verify the anti-inflammatory activity of these nanoformulations. Zebrafish larvae (5 days after fertilization, dpf) were used to determine drug absorption through the intestinal mucosa, with both formulations. In the two cases, the lethal dose was >1012 particles/mL. In a well stablished acute inflammation model, which involved caudal fin transection of the 5 dpf Tg (BACmpx:GFP)i114 larvae, the animals were incubated in suspensions with the different NLC formulations for 1 h prior to the caudal fin transection. Neutrophils recruitment to the affected area was analyzed at 3 hours post-damage. The results obtained for the control group showed an average of 15 neutrophils infiltrated in the damaged zone. The number of neutrophils decreased to 8 and 6, for NLC-OO-P and NLC-OOT, respectively. These results demonstrate that NLC prepared with olive oil have a significant anti-inflammatory effect. In addition, the different surfactant composition did not interfere with the anti-inflammatory activity of the oil. Thus, the use of olive oil as a functional excipient is an interesting approach to achieve anti-inflammatory activity in pharmaceutical formulations. **Keywords:** drug delivery, nanostructured lipid carrier, anti-inflammatory effect
Supported by: FAPESP, CNPq/Brazil

CD.29 - Evaluation of structural changes of benzocaine-loaded, optimized nanostructured lipid carriers using SANS and Raman imaging approaches

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Local anesthetics are substances that reversibly block the nerve-impulse conduction, alleviating pain without loss of consciousness. Benzocaine, a poorly soluble local anesthetic, is an ester of para-aminobenzoic acid. Several strategies of formulations can be used to improve bioavailability and decrease adverse effects of benzocaine. In this study nanostructured lipid carriers (NLC) were employed. These lipid-based drug delivery carriers have a lipid core composed of a blend of solid and liquid lipids, and a shelf of non-ionic surfactant. The main aim of this work was to optimize benzocaine-loaded NLC and to investigate structural changes in these nanoparticles, under different temperatures. The ratio of excipients (cetyl palmitate, Capmul® PG-8 NF and Pluronic®F68) and benzocaine in the NLC was optimized using a 2^3 factorial design with respect to the following parameters: particle size, polydispersity index (PDI) and zeta potentials. The interactions between the factors were found relevant to determine particle size and PDI. Using desirability function, the best formulation conditions were found. Structural changes in optimized NLC were observed with Small-Angle Neutron Scattering (SANS) and Raman imaging, in samples at 27, 37 and 40º C. SANS pointed the formation of lamellar structures inside the NLC, which interlamellar distances increase at higher temperature. Raman imaging showed that the incorporation of P68 and benzocaine in-between the lipids increased at higher temperatures, explaining the changes in Q values (SANS). This work shows how different scattering techniques can provide complementary information and be used together to characterize and understand the physical, chemical, and structural changes on the organization of pharmaceutical carriers in drug delivery system.

Keywords: factorial design, local anesthetic, pharmaceutical formulations

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CD.30 - Docetaxel-loaded lipid nanoparticles prevent the growth of murine melanoma

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Melanoma is the most aggressive type of skin carcinoma. Considering its low response and resistance to chemotherapy, nanotechnology brings new therapeutic options for melanoma treatment. Nanostructured lipid carriers (NLC) are ideal drug-delivery systems to encapsulate hydrophobic drugs such as docetaxel (DTX). The aim of this work was to develop an innovative treatment for melanoma. For this, we developed an optimized NLC formulation containing myristyl myristate (65% w/v), Mygliol 812® (25% w/v), Pluronic F68® (3% w/v) and docetaxel (1% w/v). The formulations were developed by factorial design (2^4) using the ultrasonication method. Subsequently, the formulations were characterized by: Dynamic light scattering(DLS), Nanoparticle Tracking Analysis(NTA), Cryo-electron microscopy(Cryo-EM), Small-Angle Neutron Scattering(SANS), Raman imaging, and encapsulation efficiency (%EE) by HPLC. The orthotopic model of murine melanoma was used for preclinical trials. Tumor regression was assessed by tumor volume quantification and microPET/CT image analysis. Possible adverse effects were considered. The physicochemical characterization revealed particles with average sizes of 214.0 ± 10.9 nm, low polydispersity (0.09 ± 0.01), negative zeta potential (-24.2 ± 0.30 mV) and spherical morphology (Cryo-EM) at pH 6.12 ± 0.24, with high DTX-encapsulation (%EE= 97.29 ± 2.64) and stable at long-term storage (12 months). SANS revealed that incorporation of DTX turned the surface of NLC rough and induced the formation of hydrophobic clusters. Miscibility tests by Raman imaging indicated that DTX favored the interaction between excipients (solid/liquid lipids and the surfactant). In vivo tests, indicated that the intratumoral treatment with NLC-DTX was effective, inhibiting the tumor volume rate by 97.9%, in comparison to the commercial formulation (94.9%). The general therapeutic evaluation of NLC-DTX was better than that of the commercial formulation, with lower adverse effects and 100% survival rate. These results endorse such NLCDTX as a promising formulation for the treatment of melanoma. **Keywords:** docetaxel, melanoma, nanostructured lipid carriers. **Supported by:** FAPESP (# 2019-17784-0), CAPES (L.D.M. fellowship)

CD.31 - Using a fragment based strategy to identify compounds that intectact with Mycobacterium tuberculosis' MurE ligase.

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New therapy strategies against tuberculosis are necessary and the enzymes from the cellular wall biosynthesis pathway are interesting targets since they are involved in many pathogenic resistance mechanisms. Accordingly, this work targets MurE, an essential enzyme for peptidoglycan biosynthesis in *M. tuberculosis* (Mtb). In this work, we intended to accomplish the identification of fragment-like compounds that interact with Mtb MurE using a fragment library composed of about 600 compounds through biophysical techniques . We also intend to observe ligand interaction using protein crystallography and molecular docking strategies. MurE has been cloned in pET28a, expressed in LB media and purified using affinity and gel filtration chromatographies. Thermal shift assays (DSF) were performed and docking experiments simulations were effected using GOLD and Autodock vina softwares. At the moment, MtbMurE was successfully expressed, purified in a soluble fraction, and used in two Thermal Shift screening strategies, leading to promising fragments. These molecules still need further confirmation through other biophysical techniques such as RMN, ITC, protein co-crystallization. Molecular docking was performed with the most promising compounds in order to predict the interaction with MtbMurE. Since MtbMurE was not prone to crystallization without its substrate, UAG, we were not able yet to confirm the binding mode of the fragments and we intend to use *M. thermoresistibile MurE* as a surrogate model. The integration of several biophysical techniques will provide key information about the identification of new lead molecules against this target and help in the discovery of new Mtb inhibitors.
Keywords: Mycobacterium tuberculosis, Fragment-Based Drug Discovery, ATP-dependent ligase
Supported by: FAPESP

CD.32 - Molecular interaction of neocuproine and its copper(II) complex with model membranes

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Metal complexes are a tool in the search of new drugs to improve current cancer treatments and have been used since the discovery of Cisplatin. The aim of this research is the development of new copper complexes with antitumor activity. To reach that goal a series of ternary complexes were synthetized and characterized. The ternary complexes [Cu(phenolic acid)(diimine)] were synthetized using 1,10-phenanthroline (phen) and neocuproine (neo) because they are intercalating agents to DNA. As a second ligands we selected phenylacetate acid and phenylpropionic acid because they stabilize ternary copper complexes by the presence of the phenolic ring. Affinity to DNA was studied by UV, determining the Kb for each complex and the binding to DNA was characterized by circular dichroism and variation of viscosity. Although many studies have been focused on the interaction of diimines and their metal complexes with DNA as a biomolecular target molecule for cytotoxic action, little attention has been paid to the interaction with lipidic membranes. The interaction of the complexes with membranes models has been studied by differential scanning calorimetry (DSC) and EPR. The DNA Kb values ranged from 3x10e3 to 7x10e3, characterization studies have shown that these complexes could act as partial intercalator or binding by the grooves. For complex [CuCl(fenilpropanoato)(neo)] in the thermogram obtained by DSC, a new peak can be observed, a behavior that had not been previously recorded, and in the EPR it was observed that the copper remains completely rigid within the membrane at room temperature, both studies suggests a strong interaction of the complex with the DPPC/DPPS membrane. The complexes are potent cytotoxic agents on cancer cell lines (IC50 in the low micromolar range). Their cytotoxicity was modulated by the diimine: phen complexes < neo complexes.
Keywords: copper, cytotoxity, membranes

CD.33 - Applications of computational biochemistry techniques in the discovery of drug candidates: *in silico*, *in vitro*, and in vivo studies

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This work used computer simulations to prospect new antifungal, hypoglycemic and antiviral drug candidates and investigate their interaction with the target proteins. Firstly, the target protein structures were modeled and subjected to energy minimization procedures. Then, molecular docking protocols were validated for large-scale virtual screening (VS) and molecular dynamics simulations (MD) were performed to calculate the $\Delta G_{binding}$ by MM-PBSA method, aiming to verify the system behavior involved in the stabilization of protein-ligand complexes. The theoretical results were later confirmed by *in vitro* and *in vivo* assays. The antifungals were discovered using two targets: homoserine dehydrogenase and chorismate synthase. Four drug candidates were selected by VS and then evaluated against strains of *P. brasiliensis*, where MICs/MFCs ranged between 8-128 µg.mLµ1 and low cytotoxicity in mammalian cells were found. One of those molecules (CaCS02) demonstrated a strong synergistic effect in combination with amphotericin-B. The *in vitro* studies using recombinant *P. brasiliensis* CS showed an IC50 of 29 µM for CaCS02. Regarding the discovery of hypoglycemic compounds, the crystallographic structure of human pancreatic α-amylase was used as target. The hydrolyzed (HTN) and Condensed (CTN) tannins were selected by VS from a library containing different tannins and also the reference ligands acarbose (ACA) and amylose substrate. Theoretical $\Delta G_{binding}$ calculations were performed and indicated that HTN, ACA, and CTN have a high affinity for α-amylase, where their relative affinities are in agreement with the experimental evaluation. Finally, an antiviral against BmNPV, a virus that infects the silkworm targeting the viral cathepsin was discovered. $\Delta G_{binding}$ calculations indicated that Bm5 compound has a high affinity for the enzyme. The in vivo assays using *B. mori* caterpillars infected with B*m*NPV and treated with B*m*5 increased silkworm survival by 84%. The techniques used in this work selected candidate compounds for applications in human health and agricultural technology.
Keywords: computer simulations, virtual screening, drug discovery
Supported by: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and Fundação Araucária (FA)

CD.34 - Development of new copper coordination compounds as possible drugs to fight cancer
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Metal containing coordination compounds emerged as anticancer drugs since the discovery of Cisplatin´s antitumor activity, whose use in clinic started in 1978 bringing cure to some malignant tumors. Consequently, an intense research aimed at expanding the pharmacological arsenal to fight cancer with coordination compounds started. Our goal is to develop new copper complexes with cytotoxic activity. To that end several series of compounds were synthesized and characterized. We started using [Cu-dipeptide] complexes, which are very stable systems where a second ligand can be introduced. DNA intercalating agent such as a planar diimines were used as ligands, making [Cu(L-dipeptide)(diimine)] complexes series. The diimine used were: phenanthroline (phen), 5-NO2-phenanthroline (5-NO2-phen), neocuproine (neo), 3,4,7,8-tetramethyl-phenanthroline, (tmp) and bathophenanthroline (batho). Their cytotoxicity was evaluated on different cancer cell lines. Affinity to DNA was assessed as Kb (determined by UV) and the binding to the DNA was characterized by circular dichroism and paramagnetic electronic resonance (EPR). Recently, the cellular membrane has emerged a possible target or site of accumulation for metallic complexes. Therefore, we explored the interaction of the complexes with membrane models by calorimetry and Spin-label EPR. We found that the compounds were highly potent cytotoxic agents on cancer cell lines (IC50 in the low micromolar range). Their activity was modulated mainly by the diimine attached, following the general tendency: 5-NO2-phen complexes ≤ phen complexes ∠tmp complexes ∠ neo complexes≅ batho complexes. Their DNA Kb value ranged from 1x103 to 1x106, structurally the binding occurs through partial and/or groove binding. No direct relation towards the observed cytotoxicity could be determined. The first insights into the interaction of [Cu(L-dipeptide)(phen)] with membranes suggest that it depends both on the dipeptide and phenanthroline ligands, being more marked in DPPG than in DPPC membranes. The complexes present an adequate activity to be tested in vivo as antitumor drugs.
Keywords: copper, cytotoxicity, membranes
Supported by: CSIC, PEDECIBA and ANII (Uruguay), FAPESP and CAPES (Brazil)

CD.35 - APE1 endonuclease inhibition study by modeling and molecular dynamics methods
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Apurinic/apyrimidinic endonuclease 1 (APE1) is a multifunctional protein responsible for removing lesions in the DNA molecule and to perform about 95% of the incision activity of the Base Excision Repair pathway. The overexpression of APE1 and other DNA repair proteins in cancer cells is associated with greater resistance to treatment using drugs that induce damage to genetic material. This makes these proteins target of studies for pharmacological inhibition and the increase of the efficiency of chemotherapy treatments. In this work, modeling and molecular dynamics methods were used to characterize the APE1 protein and to optimize ligands proposed in the literature as inhibitors of DNA repair activity. For this, the mapping of the protein surface and characterization of the cavities, description of pharmacophore properties, simulations of Molecular Dynamics (MD), clustering of conformations, analysis of the hydrogen bonds of the complexes, and the free energy of binding were performed. For the 13 cavities identified on the surface of APE1, the active site showed the higher druggability score, thus it was chosen as the site for performing molecular docking of the mc43 and mc47 ligands. Subsequently MD simulations of complexes were performed. The inhibitor mc47 showed a higher prevalence of hydrogen bonds over time and its pharmacophore properties were obtained to optimize the interaction with the enzyme. Through simulations with mixed solvent (ethanol/water), decomposition of the free energy of binding by residues, and computational mapping by probes, the hotspot residues Asn 174, Asn 212, Tyr 171, His 309, Arg 156 and Asp 210, were selected. Ligands modification suggestions were made based on the information obtained from this study.
Keywords: APE1, cancer therapy, molecular dynamics
Supported by: CAPES

CD.36 - Nanostructured Lipid Carriers loaded with docetaxel for the treatment of breast cancer

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Breast cancer is the cancer with the highest incidence in women worldwide. Docetaxel (DTX) is a taxoid antineoplastic agent which mechanism of action involves inhibition of microtubule formation and angiogenesis and induction of apoptosis, among others. Technological strategies can improve the effectiveness of chemotherapy by promoting sustained release or directing the drug to tumor cells, thus reducing its systemic toxicity. Nanostructured lipid carriers (NLC) are capable of encapsulating hydrophobic drugs in their blend-of-lipids matrix, which imperfections prevent early expulsion of the drug and increase the colloidal stability of the nanoparticles. The aim this work is to developed and characterize NLC encapsulated DTX, by factorial design and physicochemical analysis. In this work, a formulation of NLC consisting of beeswax (solid lipid), copaiba oil and Miglyol® (liquid lipids), Pluronic F-68 (surfactant) and containing 0.5%DTX was developed. The stability of the formulation (NLCDTX) and its control without DTX (NLCCTL) was evaluated for 3 months regarding size, polydispersity index (PDI), zeta potential (ZP), and particle concentration, besides pH and DTX encapsulation efficiency (%EE). The sustained release of docetaxel encapsulated in the NLC was compared to that of the reference drug using Franz-type cells. The optimized formulation had particles with relatively homogenous distribution (PDI< 0.200) of sizes (170-240nm) negative ZP (|25–35|mV), ca. 5.1013particles/mL, acidic pH(~5.5), and %EE of almost 100%. The kinetic experiments revealed that the encapsulated DTX was sustained released compared to the reference, the equilibrium being reached after 34 and 10h, respectively. In the search for a less toxic and more effective treatment for breast cancer, the optimized formulation was able to promote sustained release (diminishing the serum levels/systemic toxicity) and its natural excipients (beeswax, copaiba oil) have intrinsic anticancer and analgesic properties, which are valuable in oncology. These pharmacological properties are now being tested over normal and breast cancer cell lines.
Keywords: Breast cancer, Docetaxel, Drug delivery, **Supported by:** CAPES, FAPESP

CD.37 - Drug repositioning in treatment of cryptococcosis

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Cryptococcosis is a systemic mycosis caused by pathogenic yeasts of the *Cryptococcus* genus. The cryptococcal meningitis causes 180.000 deaths per year, being the second biggest cause of mortality among individuals with HIV/AIDS. Currently, there are few treatments available, which presents high toxicity and antifungal resistance. Drug repositioning is a strategy for identifying new uses for approved or investigational drugs that are outside the scope of the original medical indication. This strategy is promising in the development of antifungals drugs and can reduce costs, risks and research time. Evaluate the antifungal activity of 28 drugs with privileged chemical structures, outside its original indication, alone and in combination with fluconazole (FLZ) against *C. neoformans* (ATCC H99). Values of minimum inhibitory concentrations (MICs) were determined using the methods proposed by CLSI M-27-A3. The combinatory effects between drugs and FLZ was measured by checkerboard assay with the fractional inhibitory index (FIC) and by synergism scores in SynergyFinder web application through models Bliss, Loewe, HSA and ZIP. Albendazole, fenbendazole, flubendazole and mebendazole showed promising anticryptococcal activity with a MIC between 0.047µM and 3.125µM. None of the drugs evaluated had an ICF ≤ 0.5, therefore no synergism between the drugs and FLZ was observed. However, additive effect was observed between FLZ + finasteride (FIN) and FLZ + hydroxyzine (HID) with FIC > 0.5 and ≤ 1.0, respectively. The combination FLZ/HID has mean scores by model Bliss (12.05), HSA (11.35) and ZIP (12.14) and FLZ/FIN combination with average scores by the Bliss (1.06), HSA (4.81) and ZIP (2.29), both suggestive of synergistic or additive effects. Benzimidazoles antiparasitic drugs are promising candidates for repositioning treatment of cryptococcosis, as well as the FLZ/FIN and FLZ/HID combinations. In the future, the phenotypic impacts of these combinations on *C. neoformans* will be evaluated.
Keywords: Drug repositioning, cryptococcosis, drug combination, **Supported by:** PROPP/UFJF

CD.38 - Peptide and antibody phage display: a platform for antibody engineering
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Antibodies are important biopharmaceuticals due to their specificity and high affinity for targets. Nevertheless, one of the challenges to generate antibodies with clinical potential is the immune system itself, which avoids targeting highly conserved domains in proteins such as binding sites. Combinatorial techniques (i.e., antibody phage display) may help mitigating these difficulties but it still rely on the immune system to generate the repertoire of complementarity-determining regions (CDR) in order to produce high diversity antibody libraries. Our goal is to by-pass this limitation by combining peptide and antibody phage display technologies. Design and optimize a platform using peptide, antibody and yeast display to graft bioactive peptides into the CDR of human globulins to facilitate the discovery of high affinity antibodies with clinical potential. We have engineered plasmid vectors to build peptide libraries within the CDR of a human IgG1 to select scFv antibody fragments with specificity toward selected targets. To validate our platform, we have engineered an anti-Tie1 antibody. To increase affinity toward its target, we are now combining peptide grafting with yeast display to optimize the remaining CDRs. We have engineered the variable region of human IgG1 gene to carry restriction sites flanking the heavy and light chain CDR3.Using oligonucleotides encoding the sequence of a Tie1 binding peptide, we have built an artificial antibody phage display library and selected scFv that bind specifically to Tie1. Currently, a new scFv human library is being used to optimize the remaining CDRs to increase affinity towards Tie1. By combining combinatorial methodologies (phage and yeast display), one might overcome an important limitation in biopharmaceutical production, our own immune system. If successful, this work might generate a new anti-angiogenesis antibody and pave the way for a new platform for rational antibody discovery.
Keywords: Angiogenesis, Antibody Engineering, Phage Display
Supported by: FAPESP, CNPq

CD.39 - Characterization of nonionic cubosomes in thepresence of model proteins: a structural approach
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One area of research that has gained much attention in recent years is nanomedicine, with particular attention to drug delivery systems. Among the various nanoparticles used for this purpose, we highlight the systems formed by lipids and polymers, such as liposomes and cubosomes. The main objective of this research project is to build nanostructured systems capable of acting as antimicrobial systems. These systems will be composed of cubosomes in the absence and presence of the enzyme and will be analyzed using several biophysical techniques such as: small angle X-ray scattering (SAXS) and dynamic light scattering (DLS), potential -ζ besides essays in vivo. This cubosomes will be obtein in a equipment, developed by our research group so that we could reproducibly obtain cubosomes. This equipment uses *Arduino* type electronics, made in a 3D printer, and our objective will be to characterize, in terms of size and polydispersion, the cubosomes formed using different injection speeds. The results obtained in this research project will be presented at national scientific events (such as the meetings of the Brazilian Biophysical Society) and published in international scientific journals with arbitration. Due to the progress of the research, there are no conclusions about what was initially proposed.
Keywords: lysozyme, cubosomes, nanostructured systems
Supported by: FAPESP, CNPq and CAPES

CD.40 - Investigation of the trypanocidal activity of naphthoquinone-derived analogues as possible therapeutic agents for leishmaniasis.

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Leishmaniasis is caused by protozoa of the genus Leishmania spp. and it affects millions of people, with an increasing number of new cases each year, as well as being neglected by health agencies around the world. The drugs used in leishmaniasis are Glucantine and Pentostam, with Amphotericin B and Pentamidine as a secondary approach in some cases. Such procedures cause adverse effects, which lead to treatment withdrawal, and severe liver damage. Thus, the search for new therapeutic agents is essential to ensure a better quality of life and a higher cure rate. In this context, the project proposes the investigation of promising selective compounds: naphthoquinone analogues. Determine the IC50 of 2TIONQ for *L. amazonensis* with viability assay using the MTT method; Investigate the cellular stress caused in proliferative forms of *L. amazonensis* treated with 2TIO-NQ, quantifying changes in cellular oxygen consumption and production of reactive oxygen species; Investigate the cellular stress caused in proliferative forms of *L. amazonensis* treated with 2TIO-NQ, quantifying alterations in the mitochondrial membrane potential; Investigate the drug potencial against infection on animal cell lines. The current proposal was to deepen the biochemical investigation of the action of 2TIO-NQ compounds in *Leishmania amazonensis*, which suggest similar results in regard of ROS measurement, mitochondrial depolarization and cell cycle *in vitro* assays under treatment. Thus, positioning the 2TIO-NQ as a pharmacological perspective in combined treatments in Chagas disease and leishmaniasis. It is expected similar results as seen in the *T. cruzi* model, with higher detection of ROS and depolaryzation of mitochindrial membrane upon drug treatment. Cell cycle assay suggets to be arrested on low quantities of drug. The work is still in progress, but show promising end towards the drug being used in further tests. We expect trying other methods to know more about its mecanisms and then proceed to in vivo tests.

Keywords: Leishmania, leishmaniasis, naphtoquinones
Supported by: FAPERJ; IFRJ

CD.41 - Interaction of chaperone proteins with biomimetic systems of membrane: a structural and spectroscopic approach

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Chaperone proteins, also known as heat shock proteins (HSPs), are present in several cell locations, being responsible for maintaining the cell proteostasis. Studies of chaperone proteins have been growing in recent years, mainly due to the great potential to unveil cell protection mechanisms. However, there is still a lot to be analyzed regarding interaction and mechanisms of functioning of these proteins. In particular, it is known that chaperone proteins from HSP70 family can interact with plasma and mitochondrial membranes. In this sense, this research project has as character the study of the interaction of chaperone proteins (HSPA9 and BIP) with biomembranes, investigating how this interaction occurs, which domains interact, in addition to verifying how the protein is internalized in the membrane. To do this, it will be performed a structural characterization study of liposomes before and after the interaction with chaperone proteins. Techniques such as Dynamic Light Scattering will be used (DLS), Isothermal Titration Calorimetry (ITC), Cryo Electron Microscopy of Transmission (Crio-TEM) and Small Angle X-ray Scattering (SAXS), emphasizing for the new possibility of SAXS resolved in time, which will be made available in the Sirius laboratory soon. *Corresponding author E-mail:lbarbosa@if.usp.br

Keywords: chaperone, proteins, biophysics, membrane
Supported by: FAPESP, CNPq and CAPES

CD.42 - Identification of novel class of VEGF inhibitors using virtual screening

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Inhibition of angiogenesis, the formation of new blood vessels from pre-existing ones, is a reality and an important therapeutic option for patients suffering from oncological and ocular diseases. Most angiogenesis inhibitors target the VEGF pathway, the main factor responsible for initiating and maintaining the neovascularization process. Although effective, there are challenges to anti-VEGF therapy, such as side effects and drug resistance. Our group has shown that small molecules targeting the VEGF receptors might be an important alternative for a novel class of VEGF inhibitors (Michaloski, et al., Sci Adv, 2016). Here, we show that small organic compound mimetic of this peptide identified by virtual screening (VS) inhibit angiogenesis and might be an important drug lead for the development of novel angiogenesis inhibitors. Crystal structure of VEGFR-1 complex with VEGF (PDB 1FLT) was used to dock a pre-filtered subset of the ZINC database ($7.8x10^{6}$ molecules) with FRED (v. 3.3.03, OpenEye Scientific). Docking poses with good fit were further minimizaded and visually inspected using VIDA (v. 4.1.1, OpenEye Scientific). Most promising compounds were purchased, and their anti-VEGF activity evaluated using *in vitro* and *in vivo* angiogenesis assays (VEGF induced-cell proliferation/migration; aorta-ring and oxygen-induced retinopathy — OIR). The VS campaign suggested 29 possible VEGF inhibitors. Three of them were purchased and tested as anti-VEGF inhibitors. One compound (V2) selectively inhibits the VEGF-induced proliferation of endothelial cells over epithelial ones. V2 also inhibits two tumor cells responsive to VEGF (CAKI-1 and U87). We also analyzed V2 using two angiogenesis models: aorta-ring and OIR. V2 inhibits the neovascularization in both models. Finally, V2 showed low acute toxicity in mice. Altogether, these results suggested that V2 might be an important drug lead for the development of novel anti-VEGF inhibitors.

Keywords: Angiogenesis, Drug Discovery, Virtual Screening
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CD.43 - Optimization of Lipid Nanoparticle Formulations for DNA delivery in cardiomyocytes

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Gene therapy is a promising approach to be applied in cardiac regeneration after myocardial infarction and gene correction for inherited cardiomyopathies. One of the greatest challenges faced is the gene delivery vector. Here, we developed a library of lipid nanoparticles (LNPs) containing plasmid DNA (pDNA) for enhanced transfection efficiency in cardiomyocytes. Identify an optimized LNP formulation to enhance gene expression in cardiomyocytes *in vitro* and *in vivo*. pDNA encoding GFP was encapsulated in LNPs consisting of varying lipid molar ratios via rapid microfluidic mixing. LNPs were characterized using DLS, zeta potential and cryo-TEM. pKa of LNPs was assessed via fluorescent reagent 6-(ptoluidinyl) naphthalene-2-sulfonic acid. pDNA concentration was determined using a NanoDrop and encapsulation efficiency was obtained through Qubit dsDNA HS Assay Kit. Primary culture of cardiomyocytes was treated with LNPs at pDNA dosages of 0.00625-0.8µg *in vitro*. C57/BL6 mice were treated via tail vein injection with LNPs at a dose of 10 µg total of pDNA to determine in vivo GFP expression. Cell viability was assessed by resazurin reduction method. GFP fluorescence was carried out in confocal and Cytation 5. 90-120nm LNPs containing pDNA were formulated via microfluidic mixing. Encapsulation efficiency varied from 71% to 94%. Lead LNP induced higher than 60% transfection efficiency after 24h and 80% after 48h, which remained until day 8. LNPs formulated with higher DOPE and lower cholesterol molar ratio exhibited enhanced GFP expression in cardiomyocytes. In addition, LNP with pKa closer to endosomal pH has shown higher GFP expression and cellular uptake, without cell toxicity. *In vivo*, lead LNP was able to induce significant gene expression in heart tissue 7 days after intravenous injection, with negligible toxicity. Collectively, the use of our LNPs holds promise to improve pDNA delivery and transfection efficiency for the treatment of cardiovascular diseases.

Keywords: Lipid nanoparticle, DNA delivery, Heart, **Supported by:** CNPq, CAPES and FAPEMIG

CD.44 - PEGylated liposomal formulation of amphotericin B for improved treatment of cutaneous lesihmaniasis

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Cutaneous leishmaniasis (CL) is caused by the parasite Leishmania and found in the tropics, subtropics, and southern Europe, and can generate social and psychological stigmas due to the permanent scars generated by injuries. The few drugs available exhibit toxicities, decreased patient compliance and increased risk of resistance. Liposomal amphotericin B (AmB) or Ambisome® is the most effective and safer therapeutic agent currently available against visceral leishmaniasis (VL), but its clinical efficacy is limited in CL and HIV/VL co-infections. The aim of this work was to develop a formulation of AmB in long-circulating PEGylated liposomes and compare it to Ambisome® regarding physicochemical characteristics and efficacy in a murine model of CL. Formulations of AmB in conventional and PEGylated liposomes were developed and characterized for particle size and morphology by DLS and cryomicroscopy, drug encapsulation efficiency by HPLC and AmB aggregation state by circular dichroism (CD). Those were evaluated and compared to Ambisome® in *Leishmania amazonensis*-infected BALB/c mice for their effects on the lesion size growth and parasite load as determined by qPCR. The conventional and PEGylated formulations showed hydrodynamic diameter of 125 and 158 nm, with polydispersity indexes of 0.1 and 0.25, respectively. The drug encapsulation efficiencies were higher than 95%. CD spectra demonstrated the interaction of AmB with the membrane mainly under the monomeric form, and differed from that of Ambisome®. Following IV administration in the murine model of CL, only PEGylated liposomal formulation significantly reduced the lesion size growth and the parasite load in comparison to the control group receiving empty liposomes. This work demonstrates for the first time that PEGylation improves the efficacy of a liposomal formulation of AmB in a murine model of CL, presumably through prolonged blood persistence and passive targeting to the skin lesion.
Keywords: Amphotericin B, Liposome, Leishmaniasis. **Supported by:** CNPq, CAPES

CD.45 - Dihydroorotate dehydrogenase: A potential target against schistosomiasis

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Trematode worms of the genus Schistosoma are the causing agents of schistosomiasis, a parasitic disease responsible for a considerable economic and healthy burden worldwide. It is our interest to exploit the enzyme dihydroorotate dehydrogenase (DHODH) as a target for the development of new therapies against schistosomiasis. Combination of structural, biophysical and biochemical studies of dihydroorotate dehydrogenase from Schistosoma mansoni (SmDHODH) demonstrated that SmDHODH is a member of class 2 DHODHs and catalyzes the oxidation of dihydroorotate into orotate using quinone as an electron acceptor by employing a ping-pong mechanism of catalysis. SmDHODH crystal structure showed the presence of all structural features reported for class 2 DHODH enzymes and reveal the presence of an additional protuberant domain that folds as a flexible loop and is absent in the other known class 2 DHODHs. Molecular dynamics simulations showed that the ligand-free forms of SmDHODH and HsDHODH undergo different rearrangements in solution. Well-known class 2 DHODH inhibitors were tested against SmDHODH and HsDHODH and the results showed that the variable nature of the quinone-binding tunnel between human and parasite enzymes, as well as the differences in structural plasticity involving rearrangements of the N-terminal α-helical domain can be exploited for the design of SmDHODH selective inhibitors. In our experiments, the antimalarial drug and class 2 DHODH inhibitor atovaquone was found to selectively inhibit SmDHODH in the nanomolar range. Similarly, different series of antimalarial compounds, and their fragments designed based on the selective inhibition of plasmodium spp DHODH were tested and allowed the identification of potent and selective inhibitors. The best inhibitors were tested against against all schistosome stages (cercariae, schistosomule and adult) and revealed potent antischistosomal activity, in particular to the juvenile stage. **Keywords:** Dihydroorotate dehydrogenase, schistosomiasis., drug gtarget

CD.46 - Drug delivery with lipid nanoparticles and cosmetic applications

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lipid nanoparticles (LNP) are inspired by their natural homologous, the "virus", microscopic agents with a great capacity to deliver RNA or proteins in cells of different species. Knowing the current state of the drugs that use LNPs in the market by reviewing specialized literature provides valuable information for future developments. Objectives: Analyze scientific articles in the area of administration of drugs made with lipid nanoparticles to offer an overview of the forms of application in the human body and the therapeutic objective for which it is currently being used, showing the versatility of technology as a releasing agent of drugs. Materials and methods: Scientific articles from the Scopus and CAS Content Collection databases were investigated and filtered between the years 2000 to 2021.Results: LNPs supplies DNA, RNA, proteins and other agents for pharmacological are used in the treatment of various health problems. The ability of the LNPs to deliver various therapeutic agents is the key to the localized and spatialized delivery of drugs that remain protected and stabilized within the limits of the LNPs to release their contents at a desired time. The versatility of LNPs in their design and assembly has provided simple liposomes that have evolved into next-generation LNPs, such as solid lipid nanoparticles, nanostructured lipid carriers, cubosomes, and cationic lipid-nucleic acid complexes. Databases such as Scopus an CAS Content Collection show the growing interest in publications as evidence that different branches of science seek a better understanding of new generations of LNPs to be applied. Currently about 29 LNPs drugs are registered as approved in the world market. These drugs are distributed in 45% dedicated to cancer treatments, 2.1% in delivery of amino acids for vaccines, 1.3% to antifungal treatment, 1% in analgesics and later they are distributed in hormonal therapies, immunosuppressants and respiratory therapies, etc. Recent FDA approvals for cancer therapies and the well-known Covid-19 vaccine are a product of the versatility of encapsulation and open the opportunity to better understand the importance of technology, exhibiting more complex architectures and stabilities. LNPs have shown versatility in the application routes, where the parental path stands out with 43 %, oral 20 %, topical/transdermal 17 %, nasal 5 %, rectal 4 %, ocular 3 %, pulmonary 3 %, vaginal 2 % and 3 % other routes. In the cosmetic area, applications of LNPs are reported in applications of: sunscreen 22%, antiaging & antiwrinkle 17%, skin moisturizer 12%, shampoos 11%, etc. Finally, the prospects for finding better and more efficient LNPs preparation/application systems are growing the more these modern drug delivery systems are studied. Conclusions: It highlights that the applications of drugs that use lipid nanoparticle technology are applied parentally and are directed in genetic treatments against cancer. Vaccine, hormonal and cosmetic applications are also mentioned which shows the potential of the technique. The interest of scientists and companies is growing in the study and development of new and better lipid nanoparticle formulations.
Keywords: Lipid nanoparticles, Drug delivery, LNPs application routes

CD.47 - Hybrid Nanoemulsions for Migraine Treatment

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Migraine is a painful, disabling and often chronic disease, with no descriptions of cure so far. Intranasal sumatriptan succinate (SMT) is the gold standard drug in the treatment of migraine symptoms. Conversely, its efficacy by intranasal route is restrained by poor mucoadhesion. The preparation of hybrid nanoemulsions (NE) with copaiba oil nanoparticles and xanthan, pectin or alginate biopolymers, for the intranasal sustained release of SMT (2%; w/v). The blend of SMT and biopolymer matrices were encapsulated in copaiba oil nanoparticles, providing different hybrid NE. Structural characterization was performed by FTIR-ATR, DSC and TEM and the physicochemical stability was followed up a year (25 °C). The efficiency of encapsulation and SMT *in vitro* release profile were carried out. The nanotoxicity of the alginate-based NE (ALG-NE/SMT) was evaluated in vivo by measuring the mortality, spontaneous movement, morphological changes and cardiotoxicity of Zebrafish larvae. The hybrid NE exhibited physicochemical stability over a year of storage at 25 °C, with size in the range of 120 nm, low polydispersity (~ 0.2), zeta potential around -25 mV, and concentration of ca. 2.1014 part/mL. The hybrid systems showed excellent SMT encapsulation (41-69 %), prolonging the *in vitro* SMT release for ca. 24 h, at 37 o C. The structural analyses confirmed the morphology of the particles and details on the molecular arrangements of the hybrid systems. The most suitable formulation (ALG-NE/SMT) was tested in Zebrafish larvae, showing no nanotoxicity in any of the evaluated parameters, after 48 h of treatment. These assays point out the ALG-NE/SMT system as a promising candidate for the treatment of migraine.

Keywords: hybrid nanoemulsion, vegetable oils, biopolymers

CD.48 - The role of frugal chemoprophylaxis against COVID-19: Possible benefits for the populace

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Since the outbreak of Covid-19 identified around December 2019 which is rapidly expanding with confirmed cases in over 210 countries. Global incidence is on a ravaging increase and with new cases reported daily which has imposed threat to global health and economy. Some of the notable symptoms include dry cough, cold, fever and respiratory distress alongside inflammation. In order to curtail the community spread and reduce the incidence of new cases of Covid-19 we reviewed some medicinal foods, leaves and spices that has chemoprophylaxis and potent pharmacological activities. The anti-inflammatory, anti-viral, anti-bacterial, antioxidant, anti-pyretic and inhibition of viral replication properties against the SARS-COV-2 (Severe acute respiratory syndrome coronavirus 2) symptoms and elucidate their bioactive compounds with their mechanism of action and suggest possible preventive roles they play in the abating the spread of the Covid-19 and reduce the cases globally in a bid of raising the immune system function and possible abate the development of symptoms and general health function. In this research, we presented the preventive potentials of some medicinal foods, natural products, and their role in abating the community spread of Covid-19 globally as well as the enhancement of immunity.

Keywords: Frugal Chemoprophylaxis, COVID-19, Medicinal foods

Supported by: CAPES

DA - Protein Studies and Interactions

DA.01 - The cold shock domain of the glycine-rich protein AtGRP2 shows sequence selectivity and folds upon binding its cognate DNA

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AtGRP2 (*Arabidopsis thaliana* glycine rich protein 2) is a glycine-rich, RNA-binding protein that plays key roles in abiotic stress response and flowering time regulation in *Arabidopsis thaliana*. AtGRP2 consists of an N-terminal cold shock domain (CSD) and two C-terminal retroviral CCHC-type zinc knuckles interspersed with glycine-rich regions. Despite the wealth of information on AtGRP2 function, the molecular mechanisms underlying its biological role is largely unknown. Thus, the objective of this work was to investigate the structure and systematically evaluate the binding selectivity of AtGRP2-CSD. A combination of biophysical technics was employed, including NMR. A set of 25 different 7-mer DNA oligonucleotide sequences was used for the binding specificity analysis through fluorescence spectroscopy. 1D ^{1}H NMR spectra exhibited features of a folded protein, including a large amide chemical shift dispersion. In contrast, 2D [^{1}H, ^{15}N] HSQC spectra revealed the presence of an unfolded state in equilibrium with the native, folded protein with exchange kinetics in the slow time regime. Using multidimensional, triple resonance NMR, we unambiguously assigned 95% of the folded state and 68% of the unfolded state resonances. On the fluorescence experiments, it was observed that increasing concentrations of oligonucleotides led to suppression of fluorescence spectra, suggesting that Trp37 is part of the binding site. DNA binding occurred with affinities ranging from low nM to µM. Remarkably, AtGRP2-CSD bound to cognate DNA with a Hill “n” coefficient of 0.4, suggesting negative cooperativity arising from folding upon binding events. NMR titration results identified aromatic residues at strands β2 and β3, as well as loop β3β4 as critical for DNA binding. In addition, we show that full-length AtGRP2, but not AtGRP2-CSD, phase separates into a gel-like condensate. Therefore, these results suggest that AtGRP2 selectively interacts with DNA oligonucleotides with, preferably, T-rich sequences. The phase-separation of full-length AtGRP2 has potential functional implications.
Keywords: AtGRP2, Structure, NMR
Supported by: FAPERJ, CNPq and CAPES

DA.02 - The gill (Na+, K+)-ATPase from the swamp ghost crab *Ucides cordatus* is regulated by protein kinases and the FXYD2 peptide

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Ucides cordatus is a mangrove crab known as the swamp ghost crab. While its osmoregulatory ability is well characterized, little is known of the biochemical processes underlying gill ion transport. After 10-days acclimation to different salinities we investigated the crab's osmotic and ionic regulatory ability, kinetic behavior of the posterior gill (Na^+, K^+)-ATPase and its regulation by protein kinases A and C, the calcium/calmodulin-dependent kinase and the FXYD2 peptide. For enzyme activity estimation see Leone et al. (2020) Comp. Biochem. Physiol. 250B: 110507. Salinity acclimation had no effect on hemolymph osmolality, which was very strongly hyper-/hypo-regulated; [Cl^-] was well hyper/hypo-regulated although [Na^+] was less so, becoming iso-natriuremic at high salinity. An unusual, notable decrease in (Na^+, K^+)-ATPase activity on acclimation to lower salinities suggests that osmolytes other than Na^+ and Cl^- sustain hemolymph osmolality in dilute media. Crabs acclimated to low salinities exhibit a single family of ATP binding sites that obey Michaelis-Menten behavior. At salinities above 18 ‰S, high-affinity ATP binding sites appear and correspond to 10-20% of total (Na^+, K^+)-ATPase activity; both the low- and high-affinity sites exhibit allosteric behavior. Salinity acclimation affected the enzyme's specificity constant ($kcat/K_M$), inducing a direct increase in catalytic efficiency under saturation conditions. The endogenous protein kinases A and C inhibited (Na^+, K^+)-ATPase activity while the FXYD2 peptide stimulated activity in a salinity dependent manner. The endogenous calcium/calmodulin-dependent kinase exhibited regulatory phosphorylation of the gill ATPase and represents a novel finding in the regulation of the crustacean (Na^+, K^+)-ATPase. These data reveal that *U. cordatus* exhibits a suite of osmoregulatory and enzymatic adjustments to salinity acclimation that sustain its osmotic and ionic homeostasis, an ability particularly important given its challenging, variable salinity environment.

Keywords: Gill (Na^+, K^+)-ATPase, Protein kinases, FXYD peptide

Supported by: FAPESP, CNPq, FAPEAM, INCT ADAPTA II.

DA.03 - BoophilinD1 Phage Display Library: Selection for Zika virus NS2b-NS3 protease

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Arboviruses are a worldwide concern due to increased human mortality and incidence. Outbreaks of different arboviruses have been stimulated the search for drugs and vaccines for viral control. Zika virus translates a single polyprotein, containing structural and non-structural proteins. Our interest is the only serine protease (NS3), a chymotrypsin-like, present in the genome. The complex NS2b (cofactor)-NS3 is responsible for the polyprotein processing, together with host enzymes therefore, being essential to viral cycle. Our objectives were the construction of a phage display library using Boophilin domain1 (D1), thrombin inhibitor of Kunitz family, as a template, and selection of Boophilin D1 mutants for the Zika virus NS2b-NS3 protease. Therefore, we produce a BoophilinD1 library containing random mutations at positions P1-P4' with a title of $2.9x10^{6}$ (cfu). The library was selected for purified Zika NS2b-NS3 protease by three selection cycles. 17 clones were sequenced, and the results showed: 47% of RALHA (P1-P4') and 11.8% for RASWA, SMRPT or KALIP (wt). The wild type, mutant 12 and 14 containing the sequence KALIP, RALHA and RASWA (P1-P4'), respectively were expressed and purified. They presented Ki values for Zika protease of 0.046, 0.409 and 0.06 nM, respectively. The inhibitors were also able to inhibit DENV2 protease with Ki values in nM range as well. Docking data indicate two possible interactions between enzyme and inhibitor for mutant 12. The protease has an open and shallow pocket, in addition to an allosteric site, which makes stable interaction and selection of compatible inhibitors challenging. Boophilin D1 mutant 14 selected for Zika protease showed low Ki in the same order of Boophilin D1 wt. Our results suggested that those inhibitors are the strongest Zika inhibitors present in the Boophilin D1 phage display library and this library can be used to select inhibitors for other flavivirus proteases. **Keywords:** phage display, inhibitor, NS2b-NS3 Zika. **Supported by:** CNPq

DA.04 - Production and Characterization of the Recombinant Human Proteins TRAP-1 and CyP-D and *in vitro* Detection of their Interaction

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Proteins are molecules responsible for several biological functions, including maintaining homeostasis within the cell. Molecular chaperones such as the mitochondrial HSP90, TRAP-1, are fundamental to this process due to their ability to ensure correct protein folding and protect the cell from apoptosis. TRAP-1 is a crucial protein in this process and is responsible for maintaining mitochondrial integrity and playing a protective role against reactive oxygen species. Another important protein in this context is Cyclophilin-D (CyP-D), which acts as a co-chaperone, regulating the activities of other proteins. Both proteins are heavily studied as targets for therapies, as imbalances in their functionality are related to diseases, such as cancer. The main objective of this project was to produce and characterize the two recombinant proteins, as well as detect and assess the *in vitro* interaction between them. Both proteins were produced in *E. coli* BL21(DE3), and protein expression was induced by isopropyl-β-D-thiogalactoside (IPTG). Purification was achieved through immobilized metal affinity (IMAC) and size-exclusion (gel filtration) chromatographies. The efficiency of the methods was verified by polyacrylamide gel electrophoresis (SDS-PAGE) and protein concentration was determined by spectrophotometric measurements. A *pulldown* assay was performed to detect the interaction, and both proteins were submitted to analytical gel filtration for characterization. Both proteins were successfully expressed and purified, with a high degree of purity and homogeneity. They were both characterized by the analytical gel filtration and the *pulldown* assay was able to detect their interaction. The results show that both proteins can be successfully produced *in vitro*. The characterization showed that TRAP-1 was an asymmetric dimer in solution, while CyP-D was a globular monomer. The *pulldown* assay confirmed the physical interaction between both proteins under all tested conditions, which justifies further and more detailed investigation of this interaction.
Keywords: Molecular Chaperone, TRAP-1, CyP-D. **Supported by:** CNPq e FAPESP

DA.05 - macrophage migration inhibitory factor of *Leishmania major* (LmMIF) and C-terminal truncated mutants: structural stability and migration inhibitory activity

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The macrophage migration inhibitory factor (MIF) is an important proinflammatory and immunoregulatory protein and participates in both innate and adaptive immune responses. MIF orthologues were identified in several parasites, including *Leishmania*, and have been suggested a possible modulating function of the host immune response to benefit the development of parasites. In this work, the structural characterization and the migration inhibitory activity of *L. major* MIF2 (LmMIF2), C-terminal truncated Delta5 (W108FDelta5) and Delta10 (WtDelta10) mutants were performed in solution. The recombinant LmMIF2, Delta10 and Delta5-C-terminal mutants were expressed in *E. coli* and purified by affinity chromatography. The solution stability of the LmMIF2 and mutants were evaluated by circular dichroism, Intrinsic Fluorescence Tryptophan Emission and SEC-MALS analysis. The inhibition of the macrophage migration was evaluated by wound healing assay. Far UV CD analyzes showed secondary structure is stable between pHs 4 and 10 and that the C-terminal region is important only extreme pHs. The decreased fluorescence emission at acidic pH indicated a tertiary structure adjustment that was most effective in the mutants at pH < 4. ANS binding revealed a molten globule state for LmMIF2 and mutants and chemical denaturation confirmed a decreased stability of WtDelta10 mutant, indicating the importance of the C-terminal portion for the structure. The SEC-MALS experiments revealed a trimeric structure for LmMIF2 and mutants at pH 7 and pH4 indicating that C-terminal portion is not essential in the maintenance of the trimeric quaternary structure. The wound healing assay showed that the macrophage migration inhibitory activity of the C-terminal mutants was preserved. The stability of the solution structure of LmMIF2, resulting from a robust secondary structure associated with tertiary structure adjustments of a trimeric state, allows its migration inhibitory activity independent of the C-terminal region.

Keywords: Protein , Stability, Leishmania
Supported by: FAPESP, CNPq, Capes, PRP-USP

DA.06 - The tyrosine-rich repeat in chlamydial TarP is intrinsically disordered

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TarP (Translocated actin-recruiting phosphoprotein) is a chlamydial effector protein essential for host cell invasion. It is injected into the host cell in the beginning of the infection process via type III secretion. Once inside the host cell, multiple regions in TarP interact with host proteins in different ways to reorganise the actin cytoskeleton and bring chlamydia into the host cell. Some of these regions are common to all chlamydia species, such as the WH2-like region that is capable of directly binding actin monomers and nucleating the formation of new actin filaments, while other regions are absent in some species. Chlamydia trachomatis is the species responsible both for the most commonly diagnosed sexually transmitted disease in the UK and the most common cause of preventable blindness in the world. Unlike some other chlamydia species, its TarP contains a variable number of tyrosine-rich repeats, which get phosphorylated upon injection into the host cell. Once phosphorylated, they interact with SH2 domains in signalling proteins, disrupting cytoskeleton signalling. To determine the folding status of the Tyr-rich repeats in TarP, we have expressed and purified a single Tyr-rich repeat from C. trachomatis TarP, and characterized it using NMR and Synchrotron Radiation Circular Dichroism spectroscopy. The NMR chemical shifts and the SRCD spectrum showed mainly characteristics of a random coil. The resulting spectra are characteristic of a mostly intrinsically disordered protein, with some residual helical content.

Keywords: chlamydia, intrinsically disordered proteins, NMR

DA.07 - Biochemical and Biophysical studies of the *Neurospora crassa* AAA+ RVB-1/RVB-2 protein complex and its novel function in stress response

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The RVB protein complex, composed of two paralogs (RVB-1 and RVB-2 in *Neurospora crassa*), belong to the AAA+ (ATPases Associated with various cellular Activities) superfamily present in archaea and eukaryotes. They are highly conserved proteins and can interact with many proteins forming multimolecular complexes involved in many biological processes, such as gene expression, cellular differentiation, chromatin remodeling and tumorigeneses. The objective of the present work is to characterize the *N. crassa* RVB-1/RVB-2 complex in the context of stress response and structural properties. To characterize the protein complex we used functional assays, and biochemical and biophysical approaches. Growth of the heterokaryotic mutant cells is severely impaired in the presence of stressors and both proteins are overexpressed under stress-induced by salt and temperature. In addition, both are light-regulated proteins exhibiting opposite expression modulation, while RVB-1 is activated, RVB-2 is repressed by light. Both proteins show a perinuclear location, and their nuclear expression are increased under stress. RVB-1 co-immunoprecipitations assays of cell extracts exposed to osmotic and heat stresses led us to identify components of several chromatin remodelers and the shugoshin-like protein, a protein described as involved in stress response in mouse cells. Biochemical and biophysical studies were also performed with the RVB-1/RVB-2 recombinant proteins. SEC-MALS analysis revealed that the complex predominantly exists as a dimer in solution and that nucleotides (ATP/ADP) influence the oligomerization state, while ATP favors hexamers assembly, ADP favors the formation of multimeric states, likely dodecamers. Molecular Dynamics simulations showed that nucleotides promote a structural rearrangement of the complex, more likely influencing the cooperativity between the monomers. The protein complex binds to dsDNA fragments and exhibits ATPase activity, which is strongly enhanced in the presence of DNA. Our findings show that some properties of this protein complex are fungus specific.**Keywords:** SEC-MALS, co-immunoprecipitation, stress response.
Supported by: FAPESP, CAPES e CNPq

DA.08 - Myristoylation and its effects on the Golgi Reassembly and Stacking Protein 55

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GRASP55 is a myristoylated protein localized in the medial/trans-Golgi faces and involved in the Golgi structure maintenance and the regulation of unconventional secretion pathways. It is believed that GRASP55 achieves its main functionalities in the Golgi organization by acting as a tethering factor and, when bound to the lipid bilayer, its orientation relative to the membrane surface is restricted to determine its proper trans-oligomerization. Despite the paramount role of myristoylation in GRASP function, the impact of such protein modification on the membrane-anchoring properties and the structural organization of GRASP remains elusive. Here, an optimized protocol for the myristoylation in *E. coli* of the membrane-anchoring domain of GRASP55 is presented. Optimization of a myristoylation protocol in GRASP55 in *E. coli* and analysis of its biophysical properties and interaction with model membranes. Protein expression and purification in E.coli, size exclusion chromatography (SEC), liposome electrophoretic mobility assay (LEMSA), near and far-UV CD, fluorescence, MD simulations. The biophysical properties of the myristoylated/non-myristoylated GRASP55 GRASP domain were characterized in a membrane-mimicking micellar environment. Although myristoylation did not cause any impact on the protein's secondary structure, according to our circular dichroism data, it had a significant impact on the protein's thermal stability and solubility. Electrophoresis of negatively charged liposomes incubated with the two GRASP55 constructions showed different electrophoretic mobility for the myristoylated anchored protein only, thus demonstrating that myristoylation is essential for the biological membrane anchoring. Molecular dynamics simulations were used to further explore the anchoring process in determining the restricted orientation of GRASPs in the membrane. Less soluble and more stable protein, differences in electrophoretic mobility of liposomes incubated with myristoylated protein, a successful anchoring in model membranes. **Keywords:** GRASP, Membrane interaction, Myristoylation. **Supported by:** CAPES

DA.09 - *Vibrio cholerae* YaeO is a structural homologue of RNA chaperone Hfq that inhibits Rhodependent transcription termination by dissociating its hexameric state

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Efficient and accurate transcription termination is required for the correct regulation of bacterial gene expression. In bacteria, two main mechanisms of transcription termination have been described, Rho-independent and Rho-dependent termination. In Rho-dependent termination, Rho binds to the rho utilizing (rut) sites on the nascent RNA and translocates in the 5' to 3' direction using the ATP hydrolysis energy eventually dislodging the RNA-polymerase from elongation complex [1]. The Psu and YaeO are two established inhibitors of Rho-dependent termination [2,3]. Crystal structure of Psu, solved by our lab, demonstrates novel fold and uniquely knotted dimer [2]. Structure of Escherichia coli YaeO (EcYaeO), solved by NMR, exhibits similarity with Hfq protein [4]. Detailed sequence and phylogenetic analysis demonstrate that *V. cholerae* YaeO (VcYaeO) is significantly distinct from its *E. coli* counterpart. VcYaeO causes significant growth defect upon in vivo expression and inhibits *in vitro* functions of the Rho. Structure of VcYaeO solved at 1.75 Å resolution, the first crystal structure of a YaeO protein, demonstrates a beta-sandwich fold distinct from the NMR-structure of the EcYaeO. Through various biophysical techniques like SEC, DLS and chemical crosslinking we have demonstrated that VcYaeO disrupts the oligomeric state of the VcRho. Interestingly, VcYaeO has structurally more resembles to the Hfq protein and like the latter it exhibits ssDNA/RNA binding properties whereas EcYaeO is unable to do so. Through docking studies and ssDNA/RNA binding properties we propose that VcYaeO inhibits the function of the Rho protein via disruption of the latter's hexameric assembly and also likely by sequestering the RNA from the Rho primary binding sites. References: [1] T. Platt, Mol. Microbiol. 11 (1994) 983–990. [2] R. Banerjee. et al. J. Biol. Chem. 287 (2012) 44667–44675. [3] S. Pichoff. et al. Mol. Microbiol. 29 (1998) 859–869. [4] P. Gutiérrez. et al. J. Biol. Chem. 282 (2007) 23348–23353. **Keywords:** X-ray crystallography, anti-termination, disruption of Rho hexamer

DA.10 - Structure and antibacterial properties from BMOOLAAO-II, A L-Amino acid oxidase isolated from *Bothrops moojeni*.

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The snake venom is composed of a mixture of macromolecules with a wide variety of biological effects. Due to their pharmacological properties, many components call attention to the treatment of various human diseases, such as L-amino acid oxidases from snake venoms (SV-LAAOs). The present study aimed to (i) isolate an L-amino acid oxidase (LAAO) isoform from *Bothrops moojeni* called BmooLAAO-II; (ii) evaluate the bactericidal effect of the toxin against Gram-positive and Gram-negative strains acquired from the American Type Culture Collection and multidrug-resistant clinical isolates; and (iii) investigate the molecular structure to clarify the biological function. The purification of the toxin was performed using three chromatographic steps including molecular exclusion, hydrophobic interaction, and affinity. The antibacterial activity was determined by the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (CBM). The structure analysis was performed by molecular modeling, molecular docking, and molecular dynamics. Purified BmooLAAO-II showed to be a homodimeric acidic glycoprotein with molecular weight around 60 kDa under reducing conditions in SDS-PAGE. The enzyme exhibited high specificity catalysis on amino acid L-Leucine, 4,136,683 U/mg/min. After incubation, the toxin demonstrated a strong bactericidal effect against *Staphylococcus aureus* and *Escherichia coli*, additionally the toxins show bacteriostatic action against *Enterococcus faecium*. Sequence analysis indicates a high sequence identity among SV-LAAOs. Structures studies show highly conserved structural pattern of SV-LAAOs composed by FAD-binding domain, substrate-binding domain, and helical domain. In this sense, BmooLAAO-II demonstrated promising pharmacological properties. These results are important to direct our research for the development of new and more effective antibacterial agents, including the control of resistant bacterial infections. Furthermore, the structure-functional relationship contributes to clarify the mechanism of action and assist the research of potential molecules with biotechnological applications. **Keywords:** Bothrops moojeni, L-amino acid oxidase, Pharmacological properties. **Supported by:** CNPq

DA.11 - Fibrillation of alpha synuclein probed by surface methylation

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The nature and size of the accessible surface area (SASA) of the polypeptide chain plays a key role in protein folding and complex formation. SASA can be probed with diazirine (DZN), a tiny precursor of the extremely reactive methylene carbene (:CH2). Sparse methylated sites left on the polypeptide provide revealing signs on the conformation. The metric extent of methylation (EM) derived from mass spectra discriminates between native and alternate states. Human alpha synuclein (AS) aggregates into oligomers and amyloid fibrils, constituents of Lewy bodies, a cytosolic hallmark of Parkinson disease. DZN labeling proves particularly fit to analyzing the conformational plasticity inherent to this intrinsically disordered protein. Unlike folded proteins where EM differs markedly between native and unfolded states, AS in buffer or in 6 M GdmCl displays a similarly enhanced value, revealing high solvent accessibility under normal physiological conditions. Strikingly, AS fibrils display an enhanced EM value compared to the monomer, as a consequence of the organization of a hydrophobic interface. In addition, location of methylated sites by multidimensional NMR (1H15N-HSQC) opens a vast panorama. The extent of: CH2 reaction across the surface of AS defines sequential intensity profiles. Differential labeling signs point to the involvement of the N-terminal domain in fibril formation. We anticipate that the combined picture derived from MS and NMR data will serve to elucidate the contribution to fibrillation of the different structural moieties of AS, allowing the appraisal of species belonging to the monomeric ensemble, the lesser-known oligomers, and the fibrillar aggregates.
Keywords: Synuclein, Methylation, Diazirine
Supported by: UBACyT, CONICET and ANPCyT

DA.13 - Investigation of New Therapeutic Targets in *Trypanosoma cruzi* Genome Using Different Computational Approaches

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Trypanosoma cruzi is a protozoan that belongs to the Trypanosomatidae family and an etiologic agent of Chagas disease. It's transmission to insect vector *Rhodnius prolixus*. About 6 million to 7 million people worldwide are estimated to be infected with *T. cruzi*, the parasite that causes Chagas disease. The Benzonidazole is used to treat infection in Brazil, which has no guarantee of the treatment's total effectiveness yet. The objective of this work is to analyze the hypothetical proteins predicted in the genome of the *Trypanosoma cruzi*, unveiling their functionalities and interactions using *in silico* tools aiming at the search for new therapeutic targets for the treatment of Chagas disease. We used data of the *Trypanosoma cruzi* [PRJNA15540] with 19,607 proteins. We separate hypothetical proteins and make a structural prediction through comparative 3D modeling using the MHOLline platform. The 3D models were selected by analyzing the identity-coverage between the alignments, RMSD between model-template and Ramachandran's plot generated by the PROCHECK software. We search for structural similarity using Kpax, CATH and ScopE. Molecular Function was predicted using Gene Ontology. It was possible to build 808 three-dimensional models from the 19,607 proteins predicted in the *T. cruzi* genome. After analyzing the favorable-prohibited regions through the Ramachandran's plot, of these 360 proteins to predict function and resulting in 27 target proteins for the next step. It was possible to build 3D models for 27 proteins from interesting groups previously predicted like hypothetical proteins. The next steps will be to analyze the metabolic pathways of these proteins in KEGG (Kyoto Encyclopedia of Genes and Genomes) to direct these proteins like possible therapeutic targets.
Keywords: *Trypanosoma cruzi*, Chagas disease, predict function
Supported by: CNPq and CAPES

DA.14 - Effects of the endocannabinoids AEA and 2-AG on single channel gramicidin A conductance and membrane capacitance of lipid bilayer

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AEA and 2-AG are endogenous cannabinoids molecules lipid messengers with amphiphilic character that exercise regulatory functions in nervous system by binding to cell membrane receptors CB1 and CB2. They also exert direct actions, without receptor mediation, regulating the function of ion channels. The regulation model by endocannabinoid specific binding to its target does not promptly explicates its activity modulation of different classes of ion channels. Our research propound an alternative endocannabinoid mode of regulation of protein ion channel based on a nonspecific and receptor-independent mechanism involving the adjustment of the membrane/protein hydrophobic coupling, which arises from drug-induced local changes in bilayer elastic properties that alter the energetic coupling between the protein and its host bilayer. The regulatory action of both AEA and 2-AG on gA ion channel in planar lipid bilayers composed of DOPC and DPhPC was determined using single channel conductance and membrane capacitance measurements together with techniques of continuous theories of elastic deformation of bilayers. The endocannabinoids do not change the membrane capacitance or the amplitude of single channel current, but produce increase in the channel's appearance frequency and its open state average lifetime. The observed effects are more intense in bilayer of DOPC then of DPhPC due to higher hydrophobic mismatch of DOPC. The insertion of a protein into the hydrophobic environment of the lipid bilayer has an energy cost related to the adjustments of the bilayer to the membrane protein hydrophobic portion. The correlation between channel kinetics and energetics shows that the action of the endocannabinoids on gA channel has a linear rate-equilibrium relation that provides strong support for the hypothesis that their effects are mediated by a more general and nonspecific modulation mechanism associated with locally adjusting the bilayer hydrophobic thickness to match the channel length. **Keywords:** bilayers, channels, endocannabinoids. **Supported by:** FAPESP and CNPq

DA.15 - Preliminary over view of the interaction features between the recombinant disintegrin, Jararacin, and platelets: NMR studies

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Desintegrins are small cysteine-rich peptides found in snake venom. They have modulating activity over a broad range of Integrins, which are heterodimeric receptors that have a key role in mediating physiological and pathological processes, such as cell adhesion, migration, apoptosis, and platelet aggregation. Integrins, thus, have become a therapeutically target and disintegrins can provide new information about physiopathology activities, such as thrombosis and angiogenesis. The objective of this study was to express and analyze the activity of a recombinant disintegrin, jararacin (rJarc), over platelet aggregation. To complete our objective, we expressed the disintegrin on a Pichia pastoris system. The yeast was transformed with the vector pPIC9 containing the synthetic gene of rJarc. The disintegrin was expressed and secreted in the cultured media and then purify by molecular exclusion chromatography. Also, 15N-rJarc was expressed and purified as described. We confirmed the molecular mass and internal sequence by mass spectrometry and its correct folding by 1H nuclear magnetic resonance spectra and circular dichroism. Then, we performed biological assays to evaluate the activity of rJarc over platelet aggregation. rJarc yield was 30 mg/L approximately. Also, rJarc was capable of inhibiting platelet aggregation induced by ADP, collagen, and thrombin. Also, presented an inhibitory activity over the adhesion of platelets to collagen (by 500nM) and fibrinogen (by 150 nM) under continuous flow. In addition, preliminary NMR studies, in the presence or absence of platelets, revealed changes in rJarc structure and the residues involved in the platelet-desintegrin interaction. In conclusion, rJarc was expressed in a correct folding and inhibit platelet aggregation in a similar way to the native protein. NMR data shows that rJarc is a useful tool to understand the interaction of disintegrins with platelets and integrins.
Keywords: Disintegrin, Integrin, Platelet aggregation. **Supported by:** FAPERJ, CNPq and CAPES

DA.16 - The phosphoprotein of respiratory syncytial virus, a protein of low structural complexity and still an essential player of the viral polymerase

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The phosphoprotein (P) is an essential co-factor of the polymerase of Mononegavirales order viruses. We focused on the phosphoprotein of respiratory syncytial virus (RSV), which is a major pathogen responsible for pneumonia in children and the archetype of the Orthopneumovirus genus, Pneumoviridae family. The RSV P phosphoprotein is an essential co-factor of the viral RNA polymerase complex. Its primary function is to tether the catalytic subunit of the polymerase (L) to the ribonucleoprotein genome by specific interactions with the viral nucleoprotein (N), by binding to the L protein on the one hand and to the N protein on the other hand. More generally, the RSV P protein acts as a hub protein for the viral RNA polymerase complex by assembling its different components. For example it recruits additional factors like the RSV transcription factor M2-1 and the cellular phosphatase PP1. We used a combination of biochemical, byophysical and NMR approaches to gain structural and dynamic insight into the RSV P protein. We showed that it contains large N- and C-terminal intrinsically disordered regions (IDRs), the only domain with well-defined structure being a small tetramerization domain. We analyzed the full protein and fragments. By NMR we showed that the IDRs display transiently structured regions, some with high helical propensity. The combination of intrinsic disorder and tetrameric organization allows RSV P to bind to multiple partners and endows it with adaptor protein-like properties. We identified several binding regions that correspond to different partners and complexes AND/or that drive compaction of this protein. Finally, we showed that the RSV N-P protein-protein interaction could be targeted by small compound inhibitors to design new antiviral therapeutics.

Keywords: nuclear magnetic resonance, intrinsically disordered protein, viral polymerase complex
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DA.17 - Human TMED 1 GOLD domain: Thermodynamic and structural analysis

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TMEDs are eukaryotic transmembrane proteins located in all subcompartments of the initial secretory pathway. Although essential during bidirectional transport between the endoplasmic reticulum and the Golgi, information about their structure, oligomeric state and how they anchor the transport cargo is still lacking. To fulfil this gap, we performed a detailed biophysical characterization of the GOLD domain of human TMED1. The recombinant TMED1 GOLD protein was purified in two steps and analysed through: Circular Dichroism, Size Exclusion Chromatography with Multi-Angle Light Scattering, Size Exclusion Chromatography, Differential Scanning Calorimetry, X-ray crystallography, Phylogenetic analyses, Molecular dynamics simulations and MM/PBSA free energy calculation. The circular dichroism data showed that, after purification, the protein was folded and its secondary structure content was predominantly formed by β-sheets (~44%). The high-resolution structure was determined by protein crystallography and showed a structural organization in a β-sandwich composed of two four-strand antiparallel β-sheets and a conserved disulphide bond. The protein formed a dimer structure in the crystal organization, further confirmed experimentally in solution and showed to be salt-dependent. The proposed dimeric structure was further analysed using molecular dynamics simulation and the residues important for oligomerization were mapped. A model for the TMED docking to the membrane was proposed.

Keywords: TMED, P24 family, Early Secretory Pathway
Supported by: CNPq, CAPES and FAPESP

DA.19 - Quantitative evaluation of the binding of the scFv antibody NUsc1 to Alzheimer's Disease-Associated Aβ oligomers using Surface plasmon Resonance

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Alzheimer disease (AD), the leading cause of dementia worldwide, is characterized by the brain accumulation of protein aggregates, including those formed by the β-amyloid peptide (Aβ). Although the molecular mechanisms underlying the pathogenesis of AD are still not completely understood, mounting evidence implicates soluble Aβ oligomers (AβOs) as key players in disease onset and progression. Here we have used surface plasmon resonance (SPR) to evaluate the binding of a scFv antibody fragment previously selected by our group, named NUsc1, which has been shown to neutralize the neurotoxicity of AβOs, to *in vitro*-prepared AβOs. Evaluate the binding of the scFv antibody NUsc1 to soluble Aβ oligomers. A SPR CM-5 chip was functionalized with the anti-c-myc IgG 9e10. Next, purified c-myc tagged-NUsc1 was immobilized this chip via interaction with c-myc tag. The interaction of synthetic AβOs with this chip was analyzed by SPR. The immobilization of NUsc1 to the chip via myc tag, and the binding of AβOs to immobilized NUsc1 were clearly observed. The binding of AβOs at different concentrations revealed that AβOs binds to NUsc1 with high affinity, in the low nanoM range. We also observed the selectivity of NUsc1 binding comparing its binding to AβOs to the response obtained with lysozyme oligomers, which was significantly lower. We have established an efficient strategy to quantitatively evaluate the binding of the scFv antibody NUsc1 to Aβ oligomers. Using this assay, we have determined the high affinity of NUsc1 to AβOs, which is in the low nanoM range. This SPR assay may be used in the future to quantify the affinity of other high-affinity conformation-sensitive antibodies to AβOs, with implications to diagnostic and therapeutic applications in AD.

Keywords: AβOs, Alzheimer, SPR
Supported by: FAPESP, CNPq, CAPES, FAEPA

DA.20 - Biophysical characterization of the RNA-dependent RNA polimerase (nsP4) protein of Chikungunya virus

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The alphavirus Chikungunya virus (CHIKV) is the causative agent of Chikungunya fever, an arboviral disease transmitted to humans by the bite of mosquitoes from the Aedes genus. The symptoms include rash, fever, edema, and joint and musculoskeletal pain. The lack of an effective treatment, associated with the debilitating period caused by joint pain, creates the need to intensify studies that may lead to new drugs or vaccines against this virus. The CHIKV belongs to Togaviridae family and is a positive-sense single-stranded RNA virus , whose genetic material encodes two polyproteins (structural and non-structural), which after cleavage give rise to nine mature proteins. Among them, nsP4 is the RNA-dependent RNA polymerase (RdRp) and plays a crucial role in the viral RNA replication. Due to this notorious importance, the nsP4 emerges as a promising target for the search for drugs against CHIKV. So far, its three-dimensional structure has not been elucidated, then biophysical methods can be useful for obtain structural and dynamic information about this protein. Here, the CHIKV nsP4 was cloned, expressed in heterologous system and purified. Differential Scanning Fluorimetry, Circular Dichorism and Differential Scanning Calorimetry were performed to assess stability, secondary structure content, thermal/chemical denaturation profiles and thermodynamic parameters of the transition process. As results, the CHIKV nsP4 showed a predominance of helical content, which is a common feature of viral polymerases. The chemical denaturation showed a non-cooperative process and the thermal denaturation occurs cooperatively, presenting well defined state transition and this process is kinetically controlled. Based on these findings, studies of ligand interactions will be carried out to search for compounds able of interact with nsP4, aiming the development of novel antiviral drug candidates to treat CHIKV infection.

Keywords: Chikungunya virus, nsP4, thermal denaturation
Supported by: FAPESP

DA.21 - Structural, Functional and Evolutionary aspects of Calpain/Cactus complex action in Toll pathway modulation

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Calpain A is a cysteine protease regulated by Ca++ able to cleave Cactus (IKB) resulting in modulation of Toll signaling. However, structural and biophysical requirements involved in the interaction of Calpain A and Cactus remains unknown. Here, we report the molecular structures and conformational dynamics of Calpain A-Cactus complex and functional analysis of Calpain in D. melanogaster and *Rhodnius prolixus*. All-atom molecular dynamics of Calpain A-Cactus complex were carried out with AMBER14 using AMBER (ff14SB) force field followed by 350 ns simulations. Molecular dynamic simulations revealed that the N-terminal region of Cactus undergoes wide conformational rearrangement indicating that acts as a modulator in the cleavage process, by approximating the Cactus cleavage site to the catalytic site of Calpain A. Our results also indicate that the complex depends on electrostatic interactions, involving negatively charged regions in Cactus ankyrin repeats and positively charged regions in Calpain A domains PC1 and PEF. Furthermore, the Calpain A-Cactus complex is sustained by salt-bridges formed along the Cactus alpha helix anti-parallel ankyrin repeats and Calpain A EF hands 2, 3, 5 and CBSW domain of Calpain A. Next, we interrogated the conservation of Calpain in modulate Toll signaling in a phylogenetically basal insect R. prolixus. We found amplification of Calpain system in Rhodnius genome. Calpains in R. prolixus revealed differential architecture, with differential EF-Hand domain and containing key mutations at the catalytic residues of CysPc domain of Calpain A/B like, yielding catalytic dead-Calpains. Functional assays by pRNAi revealed phenotypes at oviposition for CalpA1, CalpA4 and CalpC. Interesting, in situ hybridization after catalytic-dead-CalpA2 knockdown indicated disruption of Twist and SoxN territories resulting at fail of germ band elongation. These evidences reveal the structural requirements involved in Calpain modulation of Toll signaling, as well as a different domain-architecture of Calpains including catalytic-dead that influences critical early stages of Rhodnius development.

Keywords: CalpainA-Cactus complex, NFkB, protein-protein interactions
Supported by: CAPES, CNPq, FAPERJ, INCT-EM

DA.22 - Exploring the Energy Landscape of Chromosomes

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Significant efforts have been made to obtain the genome's three-dimensional structure and understand how chromosomal organization may affect gene regulation and expression. Chromosome conformational capture techniques such as Hi-C have been essential in uncovering the quantitative information needed to determine chromatin organization. Complementing these experiments, co-polymers theoretical methods are necessary to determine the ensemble of three-dimensional structures associated with Hi-C maps. In addition to the structural information, these theoretical advances also shed light on the underlying mechanisms governing genome assembly and function. We perform chromatin dynamics simulations using a maximum entropy approach by applying the MiChroM (Minimal Chromatin Model) energy function to generate the 3D ensemble of chromosome structures. The energy function is transferable between chromosomes, cell lines, cell cycle phases, and different organisms. MiChroM energy function is now available in a fast and scalable software version, which can perform chromosome simulations using GPUs via OpenMM Python API, called Open-MiChroM. Open-MiChroM and 3D ensemble of chromosomal structures are available at the Nucleome Data Bank - https://ndb.rice.edu.

Keywords: Genome Organization, Chromosome Modeling, Chromatin Dynamics

DA.23 - Structural and biochemical characterization of LsfA, a 1-Cys Prx involved in *Pseudomonas aeruginosa* virulence

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Pseudomonas aeruginosa is a ubiquitous, gammaproteobacteria that is the main cause of nosocomial infections and some its strains present multiple antibiotic resistance. Macrophages and neutrophils release oxidants in response to pathogen infection. In turn, *P. aeruginosa* contains antioxidants such as LsfA, a Cys-based peroxidase of the peroxiredoxin family of enzymes. LsfA belongs to the Prdx6 sub-family and as most of its members displays the 1-Cys Prx mechanism. Prdx6 enzymes are still poorly characterized. Additionally, LsfA is involved in *P. aeruginosa* virulence. Therefore, the aim of the present work is the structural and biochemical characterization of LsfA. We investigated the kinetics of LsfA by analyzing redox dependent changes in its intrinsic fluorescence and also by a competitive assay, employing heme-dependent peroxidases. Thereby the second order rate constants for the reactions of LsfA with: H2O2 (107 M-1.s-1), tert-butylhydroperoxide (106 M-1.s-1) and peroxynitrite (107 M-1.s-1) were determined. Furthermore, rate constants of LsfA hyperoxidation by H2O2 (230 ± 2.31 M-1.s-1) and by tert-butylhydroperoxide (286.9 ± 11.27 M-1.s-1) were also determined by the fluorimetric approach and confirmed by western blotting. LsfA is rapidly oxidized by several peroxides, but its hyperoxidation rate constants are lower than for other peroxiredoxin enzymes. Notably, this is the first description of hyperoxidation rate constants for enzymes of the Prdx6 sub-family. Furthermore, two crystallographic structures of LsfA were elucidated in distinct oxidative states (reduced and sulfonic acid), both in the dimeric state; at 2.6 and 2.0 Å resolution, respectively. The native quaternary structure (dimer) was confirmed by SAXS analysis. LsfA presents distinct topology and substrate accessibility in the active site in comparison to human Prx6. Thus, relevant structural and biochemical insights were obtained on an antioxidant enzyme involved in P. aeruginosa virulence that might enable the identification of a specific inhibitor.
Keywords: Peroxiredoxin, antioxidant defence , kinect characterization
Supported by: FAPESP/CNPq

DA.24 - Effect of hydrogen sulfide on protein tyrosine phosphatase A from Mycobacterium tuberculosis

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Tuberculosis is caused by the bacterium *Mycobacterium tuberculosis*, in 2019, this disease was the world's 13th leading cause of death. To survive within the host macrophage, *M. tuberculosis* secretes the protein tyrosine phosphatase A (PtpA), which contributes to the inhibition of phagosome-lysosome fusion. PtpA activity is modulated by nitric oxide (NO), the S-nitrosylation of cysteine 53 decreasing 50% of its activity. Hydrogen sulfide (H_2S) is also involved in the posttranslational modification of cysteine residues. Thus, we aimed to investigate *M. tuberculosis* PtpA susceptibility to persulfidation. PtpA and its heterologous cysteine mutants (C16A, C53A and C16/53A) were expressed and purified. To perform the analyses, the proteins were incubated in the presence or absence of 1 mM NaSH (H_2S donor) for 30 min at 25 ºC. PtpA WT and C53A specific activities, using *p*-nitrophenyl phosphate as substrate (*p*NPP), were significantly reduced by persulfidation, 2 and 3.5 times, respectively (one-way ANOVA, $p<0.001$), while for the Cys16 mutants no difference were observed with the treatments. Using circular dichroism (CD), we observed that the overall far-UV spectra of the proteins were similar, indicating that posttranslational modification of PtpA and mutants do not affect the secondary structure profile. Also, no difference in the thermal denaturation profile of PtpA and mutants were detected in the presence of NaSH. Altogether, our preliminary results indicate that PtpA persulfidation may occur at Cys16 residue and that the posttranslational modification inhibits PtpA activity but does not decrease protein thermal stability.
Keywords: Bacillus of Koch, posttranslational modification, S-sulfhydration
Supported by: CAPES

DA.25 - Identification of novel substrates for the E3 ubiquitin-ligase SCF(Fbxl17) complex by protein microarrays.

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The SCF(Fbxl17) complex is a human E3 ubiquitin-ligase responsible for substrate recognition and ubiquitination leading them to either proteasomal degradation or modulating its function. Fbxl17 protein has a LRR domain (Leucine Rich Repeat) for substrate interaction and a F-box domain to interact with SKP1 and assemble the complex. Previously, a CGH-array (Comparative Genomic Hybridization) was performed in 746 cancer cell lines, and it was found breaks in *FBXL17* in 3 lines of breast cancer (BT-474, HCC38 and HCC1395) and in 1 line of esophageal/gastric cardia adenocarcinoma (OE-19). These breaks led to the generation of Fbxl17 containing the LRR domain truncated. Surprisingly, these truncations impaired Fbxl17 association with the other components of the SCF complex decreasing its activity. The consequences of LRRs truncation regarding substrate binding and ubiquitination were not investigated yet. Moreover, substrates identification for SCF(Fbxl17) remain poorly described. Identification of potential substrates and the effects of those truncations in substrate interaction and ubiquitination. We performed a large-scale *in vitro* ubiquitination assay using SCF(Fbxl17), SCF(Fbxl17-Δ3LRR) complexes or Fbxl17-ΔF-box purified from HEK293T cells and protoarrays as substrate source. Targets differentially ubiquitinated by the wild-type complex were selected through statistical analysis and submitted to validation assays. A total of 194 candidates were ubiquitinated by SCF(Fbxl17) and SCF(Fbxl17-Δ3LRR), among them, 92 targets were positive only for the wild-type complex. Functional analysis identified that most targets were involved in RNA metabolism and cell cycle modulation. Several possible substrates were submitted to validation assays, and we obtained promising results with DDB1, a protein that is involved in DNA damage repair and protein ubiquitylation. According to our results, DDB1 interacts with Fbxl17 and is ubiquitinated by SCF(Fbxl17). Mutation in the LRRs domain affect SCF(Fbxl17) activity. DDB1 interacts with Fbxl17 and is ubiquitinated by SCF(Fbxl17).

Keywords: DDB1, SCF(Fbxl17), Ubiquitination. **Supported by:** CAPES, FAPESP and CNPq

DA.26 - Biochemical characterization and structure elucidation of a new cytochrome P450 decarboxylase

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Alkenes have an economic appeal, especially in the biofuels field, since they are precursors for drop-in biofuels production, which have similar chemical and physical properties to the conventional fossil fuels, with no oxygen in their composition. After the discovery of the first P450 CYP152 $OleT_{JE}$ in 2011, reported with its unique property of decarboxylating fatty acids (FA), by using hydrogen peroxide as a cofactor and producing 1-alkenes as the main product, the scientific and technological interest in this family of enzymes vastly increased. In this context, the present work presents a new decarboxylase ($OleT_{RN}$) with low similarity with $OleT_{JE}$ (32%), its biochemical characterization, and the structure elucidation. As main results, $OleT_{RN}$ presented a high yield of expression and purity, optimum reaction conditions at 35 °C and pH from 6.5 to 8.0, and higher specificity for oleic acid. Besides that, structure-guided mutations were performed, and, according to the functional characterizations, it was observed that some mutations presented different specificity and chemoselectivity by varying the chain-length of FA substrates from 12 to 20 carbons. These results are extremely interesting from a biotechnological perspective as those characteristics could diversify the applications and contribute to designing better cytochrome P450 decarboxylases. Considering that peroxygenases have the potential activity of decarboxylating and hydroxylating fatty acids and that the elucidation of the intriguing mechanistic involved in the decarboxylation preferential from $OleT_{JE}$ is still a challenge, the elucidation of $OleT_{RN}$ structure and the functional characterizations of $OleT_{RN}$ and its mutants contribute with new information about CYP152. Besides that, the work also contributed to the discovery of a new decarboxylase with a different selectivity profile from $OleT_{JE}$, which allows a wide range of applications.

Keywords: P450, decarboxylases, alkenes. **Supported by:** FAPESP, SBBF

DA.27 - The crystal structure of the exonucleolytic domain of the ribonuclease RRP44 of *Trypanosoma brucei* reveals details of the catalytic mechanism

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Parasitic trypanosomatids, such as *Trypanosoma brucei*, *T. cruzi* and Leishmania sp. present unique features compared with other eukaryotes regarding RNA processing and maturation. For instance, *T. brucei* ribosomes contain specific rRNA expansions and the 60S subunit is composed of eight rRNAs molecules instead of the three rRNAs found in most eukaryotes. The role of specific endo- and exonucleases in the maturation of the unusual rRNA precursor of trypanosomatids remains largely unknown. One of the nucleases involved in rRNA processing is Rrp44, a member of RNase II/RNB exonuclease family and an exosome associated ribonuclease in yeast, which is involved in several metabolic RNA pathways. Here we investigate the structure of *T. brucei* RRP44 and its exonucleolytic domain. Recombinant full-length with mutations at the catalytic sites and a truncated form of TbRRP44 containing the RNB domain (TbRRP44_CSD1-S1) were overexpressed in *Escherichia coli* and purified by chromatography methods before co-crystallization assays with RNA oligonucleotides. The structures of TbRRP44_CSD1-S1 in the apo and holo forms were determined at 3.1 Å and 2.6 resolution respectively. The overall folding of the RNase II/RNB family is conserved in TbRRP44, however comparative analyses indicate a distinct path for the substrate entrance, comparing to the human orthologue. The crystallization of TbRRP44 in its active form revealed an intermediate state of the catalytic cycle, between substrate cleavage and product exit, pointing out the role of two conserved arginine residues in catalysis. Our structural analysis of this essential *T. brucei* protein provides detailed information on the enzyme mechanism and reveals specific features that could be exploited for structure-based drug design.
Keywords: Trypanosoma brucei ribonuclease RRP44, crystallographic structure, catalytic mechanism
Supported by: CNPq, CAPES, COFECUB, FIOCRUZ, Synchrotron SOLEIL

DA.28 - Synergistic Effects of Xylanase-Arabinofuranosidase Chimers Against Minimally Pre-treated Lignocellulosic Biomass

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Lignocellulosic biomass is an abundant and renewable resource for the production of biofuels, which as an agroindustrial by-product does not compete against food crops. Arabinoxylan is a major constituint of the hemicellulose of cereals and grasses and is composed of a xylopyranoside backbone (β-1,4) substituted by α-L-arabinofuranosyl residues. The release of reducing sugars from arabinoxylan is performed by a series of glycosyl hydrolases, including the endo-β-1,4-xilanase (EC 3.2.1.8), which cleaves internal bonds in the xylan backbone, and arabinofuranosidase (EC 3.2.1.55), responsable for the release of the arabinose moiety. In this work insertional chimerogenesis between the B. subtilis GH11 endo-β-1,4-xilanase A (XynA, BSU18840) and the GH43 arabinoxylan arabinofuranohydrolase (AXH-m2,3 XynD, BSU18160) was used to create a bifunctional enzyme (AraXyl) for the degradation of arabinoxylan. The coding sequences of both enzymes and the chimera were cloned in pET28a, expressed in *E. coli* BL21 (DE3) Arctic Express and purified by metal affinity chromatrography. Biochemical characterization of both Araxyl and individual enzymes was assayed using wheat arabinoxylan and synthetic substrates. Biochemical characterization against synthetic substrates revealed that the xylanase and arabinofuranosidase KM values of the paretnal enzymes were generally lower than AraXyl. However a synergistic effect of equimolar mixtures of XynA and XynD and AraXyl against wheat arabinoxylan as compared to individual XynA and XynD was observed. Analysis of arabinose release measured by HPAEC-PAD, however, show that the chimeric enzyme possessed a 6-fold higher activity against sugar cane bagasse alchohol insoluble residue when compared to individual enzymes and XynA/XynD equimolar mixtures. These results indicate that the arabinoxylan conformation in the context of the plant cell influences its susceptability to enzyme hydrolysis, and that protein engineering may be used to generate enzymes that are suitable for releasing sugars from minimally pre-treated lignocellulosic biomass.
Keywords: biomass, biofuels, enzymes. **Supported by:** CAPES, FAPESP, CNPq

DA.29 - Criteria for the Development of Structurally Functional Humanized Antibody Molecules

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Envenomation can be broadly defined as the result of the exposure to a venom or toxin caused by the bite or sting of a venomous animal. In this context, Loxosceles spider envenomation remains an important health concern worldwide, and of medical relevance, especially in the Americas. To this day, there is no established treatment for Loxoscelism, albeit horse polyclonal antivenoms are currently employed. Amongst all Loxosceles venoms components, the Phospholipases D are widely known as the main toxic constituents, reproducing most of the cytotoxic effects observed in Loxoscelism. Considering this, molecules able to bind these venoms components would be interesting suitors in the proposition of alternative, effective, specific, and safer treatments. In this context, we introduce LimAb7, a monoclonal antibody capable of binding and neutralizing Loxosceles intermedia PLDs and its derived recombinant antibody fragment counterparts. We have previously tried to humanize this antibody and produce it in the scFv and diabody formats, however our results indicated loss in fragment affinity, stability and neutralization leading us to infer that molecule size and format might be of importance to the maintenance of parental antibody features. Aiming to develop evolved, more structurally stable antibody fragments, we report for the first time the design of humanized antibody light and heavy V-domains produced and purified as Fab fragments (Fab') and whole IgG1 molecules. 16 humanized Fab variants of LimAb7 were produced and screened as to their biophysical features. The best construct was considered our lead molecule, and thus produced in the IgG1 format. Improvements in the constructs were observed in humanized Fabs and IgG1 with respect to thermal and pH stability, production yield, as well as target binding affinity. Overall, our findings shed light on the criteria to be considered when developing functional humanized antibody molecules. **Keywords:** antibody humanization, recombinant antibodies, venoms
Supported by: CAPES-PRINT

DA.30 - Biophysical characterization of human 71 kDa heat shock cognate protein (HSPA8/hHsc70)

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Molecular chaperones are critical for cell proteostasis, with functions related to protein folding and refolding, trafficking and directing poorly folded proteins for degradation. 70 kDa heat shock proteins (Hsp70s) are a family of structurally conserved chaperones with different expression levels and subcellular locations. These proteins are composed of two domains connected by a hydrophobic linker: a nucleotide binding domain (NBD, responsible for ATP binding and hydrolysis) and a protein binding domain (PBD, which binds to client proteins), which can be in an open or closed conformation if bound to ATP or not. Human 71 kDa heat shock cognate protein (HSPA8, or hHsc70) is an essential cytosolic Hsp70 that, unlike other cytosolic Hsp70s, is not induced by heat shock and is present in other cellular functions, such as cell signalling and antigen processing. These features draw attention to this chaperone as a possible therapeutic target for pathologies such as neurodegenerative diseases and cancer. To better understand this chaperone's structure, we've characterized recombinant HSPA8 by means of techniques such as circular dichroism (CD), instrinsic tryptophan fluorescence, small angle x-ray scattering (SAXS), isothermal titration calorimetry (ITC) and chaperone activity assays, which allowed probing its overall biophysical and biochemical features. Our studies have shown that HSPA8 was purified with high α-helical and β-sheet content, and having at least one Trp residue buried within its hydrophobic core. Chaperone activity and kinetics assays highlighted its specificities in ATP hydrolysis rate and aggregation prevention, while ITC has shown some differences regarding its interaction with nucleotides in comparison with other human Hsp70s. SAXS has allowed low resolution envelope calculation and flexibility studies, showing that HSPA8 is a slightly elongated monomer which seems to remain mostly in a compact state. We hope these results help in a better understanding and guiding future studies on the Hsp70 chaperone family. This work was supported by Conselho de Aperfeiçoamento de Pessoal de Nível Superior (PROEX – 0487, grant #88887.508861/2020-00), by CNPq (grants #471415/2013-8, #303129/2015-8, 420567/2016-0, #141986/2017-4 and #303262/2018-4) and by FAPESP (grants #2011/23110-0, #2012/50161-8, #2014/07206-6, #2014/16646-0, #2015/15822-1, #2016/22447-1, #2017/07335-9 and #2017/26131-5). **Keywords:** Protein folding, Chaperones, Heat shock proteins

DA.31 - The double-edged influence of guanidinium chloride on protein aggregation

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Due to its ability to form micelle-like clusters, trifluoroethanol (TFE) promotes conformational changes that can trigger amyloid formation. Unlike classical denaturants, TFE favors protein aggregation mainly at moderate concentrations (~30% v/v). Accordingly, our protein model IFABP (intestinal fatty acid binding protein) shows amyloid-like aggregation above 15% v/v. As a straightforward correlation has been generally postulated between stability and amyloid propensity, our aim was to address the influence of sub-aggregating TFE concentrations on IFABP stability. Urea or guanidinium hydrochloride (GdmCl)-induced unfolding transitions were monitored by fluorescence and circular dichroism (CD) spectroscopy. Insights on the process arise from the comparison of the shape and intensity of the full set of CD spectra obtained from thermal ramps performed with a Chirascan V-100 (Applied Photophysics). With urea, IFABP can be assimilated to a 2-stage system and its stability remains unchanged. At variance, with GdmCl, the appearance of amyloid-like aggregation becomes more evident as TFE concentration increases. Temperature-induced denaturation profiles show that both additives diminish stability. Whereas a concomitant increase of amorphous aggregation occurs upon heating in the presence of TFE, no aggregation takes place with GdmCl. Conversely, when both additives are present, amyloid-like aggregation initiates immediately. In summary, two aggregation pathways might happen: amorphous or amyloid-like. Upon heating, low TFE concentrations promote the first. If GdmCl is also present the second route takes over. The explanation must reconcile the effects of additives on both the protein and solvent structures. Briefly, TFE desolvates the protein, a process further reinforced by heat. Although GdmCl might prevent thermal aggregation by solubilizing non-native states, this same phenomenon could favor amyloid aggregation. On top of that, the electrolytic-induced segregation of TFE might contribute to the development/stabilization of TFE clusters that might reach a large enough size to act as nucleation-inducing interfaces, thus leading to the observed amyloid aggregation.

Keywords: aggregation, guanidinium, trifluoroethanol
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DA.32 - SARS-CoV-2 Orf9b-host protein-protein interaction: molecular and biophysical characterization

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The emergence of Covid-19 pandemic, caused by Severe Acute Respiratory Syndrome (Sars-Cov-2) remains a great threat to the global health despite the early development of vaccines. However, researchers have continued to intensify studies into understanding the molecular characteristics of the viral proteins during infection, replication and host immune evasion. Orf9b an important accessory protein of the Sars-Cov-2 virus is identified to play a critical role in the viral host interaction, targeting a member of the mitochondrial translocase of outer membrane complex, Tom70. This orf9b and Tom70 assembly is implicated in disrupting the mitochondrial antiviral signaling, leading to immune evasion. Here, we described the recombinant expression, purification, and characterization of orf9b using molecular and biophysical techniques. We expressed Orf9b singly and co-expressed the two proteins (Tom70-Orf9b) in an *E. coli* expression system, followed by purification by affinity and size exclusion chromatography. Structural characterization and protein-protein interaction studies was further accessed. The 97 amino acid protein purified as a homodimer with an approximate molecular weight of 22 kDa as determined by SEC-MALS. Folding and conformational studies using circular dichroism showed that the protein exhibit a random conformation and stable upon 90C. This conformation is believed to be altered when forming a complex with Tom70. Therefore, a series of pull-down experiments are underway to characterize the Tom70-Orf9b complex. Our results may contribute to understand the role that Sars-Cov-2 orf9b protein has on virus infection.

Keywords: SAR-CoV-2, Orf9b, Tom70
Supported by: FAPESP, CAPES

DA.33 - Resolving the fine structure in the energy landscapes of repeat proteins

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The Ankyrin (ANK) repeat is a recurrent tandem sequence motif that mediates protein-protein interactions in a diversity of biological functions. Ankyrin repeat-containing proteins have an elongated non-globular shape and a complex folding mechanism that has not been fully elucidated. In this work, we investigated the energy landscape of proteins consisting of 3, 4, and 6 ANK repeats using the Energy Landscape Visualization Method (ELViM).1 For that, we have used biased and unbiased coarse-grained AWSEM simulations to sample conformations along the folding pathway. Combining the ELViM structure-based phase space with energy information from simulations, it is possible to reconstruct a 3-dimensional energy surface, which is funneled towards the native structure. On this surface, it is possible to indicate the existence of on-pathway and off-pathway intermediate states, suggesting the most favorable folding routes. Our results show that folding of all proteins initiates forming internal repeats, whereas a terminal repeat undergoes an unfolding/refolding process along the pathway. Overall, the results are consistent with a cooperative folding, and its pathway may be inferred from the concise ELViM representation.v[1] A.B. Oliveira Jr. et al, J. Chem. Theory Comput., 15, 6482-6490 (2019).

Keywords: Protein folding, Protein folding, Protein folding
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DA.34 - The stressed life of a lipid in the Zika Virus membrane

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Protein-lipid interactions modulate a plethora of physiopathologic processes and have been the subject of countless studies. However, this kind of interactions in the context of viral envelopes have remained relatively unexplored, partially because the intrinsically small dimensions of the molecular systems escape to the current resolution of experimental techniques. However, coarse-grained and multiscale simulations may fill that niche, providing nearly atomistic resolution at an affordable computational price. Here we used multiscale simulations to characterize the lipid-protein interactions in the envelope of the Zika Virus, a prominent member of the Flavivirus genus. Comparisons between the viral envelope and simpler molecular systems, indicate that the viral membrane is under extreme pressures and asymmetric forces. Furthermore, the dense net of protein-protein contacts established by the envelope proteins creates poorly solvated regions that destabilize the external leaflet leading to a decoupled dynamics between leaflets. These findings lead to the idea that the flaviviral membrane may store elastic energy, playing an active role in the membrane fusion process. On the other hand, we would like to show the developed an optimized pipeline for building and simulating enveloped virus-like particles (VLP). Detailed structural analysis of the protein envelope also shows very good agreement in root-mean-square deviations and B-factors with the experimental data. The level of details attained shows for the first time a possible role for anionic phospholipids in stabilizing the envelope. Combining an efficient and reliable setup procedure with an accurate coarse-grained force field provides a valuable pipeline for simulating arbitrary viral systems or subcellular compartments, paving the way toward whole-cell simulations.

Keywords: Zika Virus VLP Flavivirus, SIRAH force field, Coarse-grained mol dynamics simulation

DA.35 - Effect of C-terminal and N-terminal dimerization and alanine scan on antibacterial activity of the analogs of the peptide p-BthTX-I

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The study of relationship between peptide structure and antimicrobial activity has been mandatory on the search for novel antimicrobial agents. On previous study, peptide (pBthTX-I)2 and the analogue des-Lys12/Lys13-(p-BthTX-I)2 showed activity against bacteria, indicating a potential specificity uppon prokaryotic cells. The present study amis to analyze the antimicrobial and hemolytic activities of different dimeric analogs of p-BthTX-I. In addition, we evaluated the importance of each cationic and aromatic amino acid on the antimicrobial and hemolytic activities of des-Cys11,Lys12,Lys13-(p-BthTX-I)2K, the most active analog. Solid phase peptide synthesis (SPPS) was performed manually using the Fmoc protocol. Minimal Inhibitory Concentration (MIC) was determined by the technique described in the Clinical & Laboratory Standards Institute: CLSI Guidelines. In this work, the peptide des-Cys11,Lys12,Lys13-(pBthTX-I)2K [(KKYRYHLKPF)2K] was synthesized and dimerization was performed with a Lys instead of Cys residue, beginning the synthesis with a Fmoc-Lys(Fmoc)-OH. This change was important to avoid Cys oxidation, decreasing one step on original peptide synthesis and obtaining a smaller peptide. This peptide showed antimicrobial activity similar or superior to (pBthTX-I)2. Additionally, in order to evaluate the impact of linker position on peptide dimerization, the peptide E(pBthTX-I)2 [E(KKYRYHLKPFCKK)2] was synthesized, using a Fmoc-Glu-OH in the end of the synthesis. The antibacterial activity of this N-terminal dimeric peptide was worse than the original peptide, showing that free N-terminal is important to antimicrobial activity of the (pBthTX-I)2. Additionally, the alanine scan was also performed. Alanine scanning is used in order to identify specific amino acid residues responsible for a peptide's activity.Interestingly, removal of any single amino acid resulted in lower antimicrobial activity. In this work, we demonstrated that the des-Cys11,Lys12,Lys13-(p-BthTX-I)2K analog, which is shorter and synthesized by an easier process leading to a more stable peptide, is the most antibacterial active peptide against multidrug-resistant bacteria.
Keywords: p-BthTX-I, Antimicrobial peptides, (pBthTX-I)2

DA.36 - Single Molecule Investigation of Conformation and Dynamics of Macromolecules

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Studying how macromolecules fold correctly and undergo conformational changes is crucial to understand the underlying biological mechanism of how diseases arise. Single-molecule force spectroscopy represents an ideal tool to study these molecular phenomena because of its unique capability to isolate individual biomolecules and observe conformational transitions as they happen in real-time. Here, we introduce the high-resolution C-Trap® optical tweezers technology and its application in directly observing conformational changes of individual proteins by measuring their length with sub-nm precision. A mutant of AdK was designed as previously described, where cysteine residues were inserted and used for attaching DNA handles to the enzyme. DNA handles were biotin and digoxigenin modified at their respective ends, and coupled to beads surface functionalized with streptavidin and anti-digoxigenin to form a dumbbell configuration for the optical tweezers assay. The setup used was a high-resolution dual-beam optical trap to detect conformational dynamics of the enzyme at the nanoscale level and to determine and quantify the mechanism of action of small molecule inhibitors. To investigate the equilibrium dynamics of the conformational transitions of Adk, we monitored bead displacement under constant force. We observed that in the absence of kinase inhibitors, conformational transitions of AdK were undetectable as the force load applied stretches and locks AdK in an extended (open) state. However, in the presence of nanomolar concentrations of the AdK inhibitor, AP5A, the two extended and contracted states of AdK were observed over time in a distance range of 1.5-1.7 nm. This agrees with previously described crystal structures and data published. Our data show high-resolution measurements of protein conformational dynamics at the nanoscale level using the C-Trap. Importantly, this feature can be used to determine the mechanism of action of candidate drugs on enzymatic activity relating to conformational changes of the enzymes.
Keywords: Dynamic Single Molecule, Protein/Enzyme Dynamics, Optical Tweezers Technology

DA.37 - Molecular docking studies of the interaction of the Rose Bengal dye with human serum albumin

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The Human Serum Albumin (HSA) protein is a transport protein. Thus there are several possible binding sites for complex formation. The interaction between biological macromolecules (proteins and DNA) with drugs and/or other ligands is one of the research areas of great importance in the field of life sciences. These studies allow us to understand the formation of complexes such as: the proposition of interaction and reaction mechanisms, and molecular level recognition studies of binding sites. In the study carried out using molecular docking to form complexes between the (HSA) and the rose bengal dye (RB), crystallographic structures of HSA-ligands complexes, FA free, obtained from the PDB (Protein Data Bank) were considered. The selected complexes in the PDB were pdb code 2BXE (HSA-diflunisal) and 2BXF (HSA-azapropazone). With the two crystallographic structures, it was possible to investigate whether RB can accommodate the DS1 and DS2 (Sudlow's nomenclature), FA1 and FA6 sites. Docking was performed with the Gold 2020.20 Program, using the crystallographic ligand as the center of the calculations, adding a radius of 6 Å and the GoldScore adjustment function. As a result, poses (orientation, conformation and position) of the RB were obtained at the sites indicated and selected by the output pattern. The results showed that most HSA sites studied can be occupied by RB. DS1 Site: Lys199:HZ1-RB:O3 2.21 Å, Glu292:OE2–RB:H1 1.86 Å; His242–RB:Cl33 2.79 Å, Ala292:HB–RB 2.28 Å. FA1 site: Arg117:HH12–RB:O4 2.11 Å; Tyr138:HH–RB:I4 2.48 Å, Arg145:HD1–RB:Cl33 2.50 Å, Arg186:O–RB:Cl36 3.15 Å, Ser192:HB2–RB:I3 3.01 Å. FA6 site: Arg209:HH11–RB:I2 2.40 Å, Lys351:HZ1–RB:O1 1.58 Å, Asp324:OD2–RB:H49 1.97 Å. More possibilities are still under investigation. At least 3 HSA sites as described above. More possibilities are still under investigation.
Keywords: docking molecular, human serum albumin protein, rose bengal

DA.38 - Structural prediction of proteins related to lesions in the cerebral cortex

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Cellular and molecular alterations resulting from focal lesions cause distinct effects on behavior, tissue response and neuroplasticity. The objective of this work is to analyze proteins from the perilesional cortical region seven days post-ischemia in the primary sensorimotor cortex, to search for new molecular targets in ischemic lesions. The methodology included the creation of a multifast file for proteins with increased expression (UP) and for decreased expression (DOWN) compared to the control group of proteins - with normal expression level - containing 184 proteins in the UP group and 191 proteins in the DOWN group. Both groups were analyzed using Uniprot and Gene Ontology (cellular component, molecular function and biological process). The MHOLline program was used to build three-dimensional protein models, with identity and coverage validation; RMSD between template and model and Ramachandran plot. Behavioral, tissue and neuroplasticity groups were selected: antioxidant activity, cellular anatomical entity, immune system process, behavior and response to stimuli. Of the 184 UP proteins analyzed, 172 had defined cellular functions and components and 163 biological processes were identified. 7 proteins were classified with antioxidant activity with 3 constructed structures, 167 proteins related to the cellular anatomical entity and 37 structures, 28 proteins in the immune system process (2 structures), 9 proteins in behavior (1 structure) and in response to stimuli 69 proteins and 27 structures. In DOWN the results showed that of the 191 proteins analyzed, 166 had defined molecular functions, 171 cell components and 170 had identified biological processes. It was possible to generate 153 models for UP and 152 models for DOWN, with 69 models excluded in UP and 74 in DOWN according to the validation criteria. It was possible to build 3D models for 37 UP proteins and 24 DOWN proteins in selected groups. Further analysis needs to be performed to relate these results to ischemia.
Keywords: structural prediction, cerebral cortex, ischemia

DA.39 - Comparative study between different techniques to obtain affinity between biomolecules

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Biophysical methods have been increasingly used as complementary approaches to traditional ones to increase the knowledge of molecular mechanisms. Techniques applied to the study of protein/peptide or protein/protein interaction provide important information to understand the binding process. There are several techniques that theoretically or experimentally predict the affinity between two or more molecules. The comparison between affinities values obtained by different physical principles need to be used with criterion because these values are not numerically equal, and the objective is to understand the specificities and similarities of one technique in relation to the other. In this work, isothermal titration calorimetry (ITC), fluorescence spectroscopy, molecular docking and molecular dynamics simulation were used to study the affinity of nuclear transport protein importin-alpha and nuclear localization sequences (NLS) from cargo proteins. The experimental techniques studied allowed to calculate similar values in order of magnitude, which indicated the accuracy of the proposed methodology for fluorescence spectroscopy. The computational techniques, on the other hand, differed considerably from the ITC, used as an experimental reference value, as docking calculates affinity from the best orientation of the peptide in the protein and molecular dynamics considers the movement of the peptide in the complex, these dissociation constant values are a little overrated. In conclusion, despite the difficulty of comparing results obtained by different techniques, it is possible, knowing each calculation principle, to relate these results and choose the most adequate for your study, as experimental techniques have their limitations, the use of computational models is very welcome in the understanding of protein/peptide binding.

Keywords: affinity assays, isothermal titration calorimetry, fluorescence spectroscopy
Supported by: FAPESP, CNPq and CAPES

DA.40 - Three-Finger Toxins from Brazilian Coral Snakes: From Molecular Framework to Insights in Biological Function

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Brazilian Micrurus venoms are mostly predominant in Three-finger toxins (3FTx), which belong to a family of small non-enzymatic proteins constituted by 58 to 90 amino acid residues. In all members of the family, the protein fold is based on three loops of beta strands that reassemble “fingers" extending to a globular and hydrophobic core, stabilized by four conserved disulfide bonds. Studies on 3FTxs around the world are showing the amazing diversity in these proteins both in structure and function. In Brazil, we have not realized the broad variety of their amino acid sequences and probable diversified structures and targets. In this context, this work aims to conduct an *in silico* systematic study on available 3FTxs found in Micrurus species from Brazil. We elaborated a specific guideline for this toxin family. First, we grouped them according to their structural homologue predicted by HHPred server and further curated manually. For each group, we selected one sequence and constructed a representative structural model. By looking at conserved features and comparing with the information available in the literature for this toxin family, we managed to point to potential biological functions. In parallel, the phylogenetic relationship was estimated for our database by maximum likelihood analyses and a phylogenetic tree was constructed including the homologous 3FTx previously characterized. Our results highlighted an astonishing diversity inside this family of toxins, showing some groups with expected functional similarities to known 3FTxs, and pointing out others with potential novel roles and perhaps structures. Moreover, this classification guideline may be useful to aid future studies on these abundant toxins.

Keywords: Three-finger toxins, Sequence variability, Structure-function classification
Supported by: Faperj, Capes, CNPq, Inbeb

DA.41 - DNA translocase repositions a nucleosome by the lane-switch mechanism

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Nucleosome is the basic unit of chromatin and contributes to package eukaryotic genome DNA into a nucleus. During DNA transactions such as DNA replication, transcription, and DNA repair, various translocases (e.g., DNA/RNA polymerases, replicative helicases, and exonucleases) unidirectionally move along the genomic DNA. Therefore, these translocases inevitably collide with the nucleosomes. In previous studies, single-molecule experiments have shown that a translocase causes downstream nucleosome repositioning after the collision. However, the molecular mechanism of this repositioning has not been identified. This study proposes the molecular mechanism of nucleosome repositioning by a DNA translocase. In this study, we performed molecular dynamics simulations to observe the molecular details of the collision between a translocase and a nucleosome. We also verified the lane-switch mechanism by restriction enzyme digestion assays and deep sequencing assays. From these simulation results, we proposed the lane-switch mechanism in which a translocase unwraps nucleosomal DNA up to the site proximal to the dyad, the remaining wrapped DNA switches its binding lane to vacant one, and the downstream DNA rewraps, completing the downstream repositioning. The restriction enzyme assays show that T7 RNAP causes downstream repositioning of a nucleosome after unwrapping nucleosomal DNA up to the site proximal to the dyad. The deep sequencing assays show that the repositioning distance is ~100 base-pairs, which is consistent with the estimation from the simulations. The molecular mechanism shown here may improve the understanding of the DNA transactions on nucleosomal DNA.

Keywords: Nucleosome, Molecular dynamics simulation, Biochemical experiment

DA.42 - Plasma Membrane Calcium ATPase (PMCA) activity is modulated by the biophysical properties of the surrounding bilayer

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PMCA is a P-ATPase involved in the regulation of the cell calcium homeostasis transporting Ca^{2+} from cytoplasm towards the extracellular medium. PMCA like other integral membrane proteins operates surrounded by a complex and dynamic lipid bilayer, and its activity largely depends on the lipids. Aluminium (Al^{3+} and other soluble species) is environmentally ubiquitous, providing human exposure and neurotoxic effects in humans and animals. The mechanisms proposed to explain aluminium toxicity are linked to changes in the cellular calcium homeostasis. In previous works, we demonstrated that aluminium inhibits PMCA activity preventing the dephosphorylation of the pump. The aim of this work is to understand the effect of the surrounding bilayer on the PMCA activity and on the effect of the aluminium. Aluminium would have distinct effect depending on the lipid composition of the cell membrane where the PMCA is located. To characterize this effect, mixed micelles of phospholipids and detergent ($C_{12}E_{10}$) were formed at different molar fractions, and we measured how PMCA activity varied. To evaluate other biophysical changes in the lipid bilayers we performed small Angle X-ray scattering (SAXS) experiments for studying how the lipidic environment was changing alongside different molar fractions and also in the presence of aluminium. In turn, we evaluate changes in the membrane phase properties using two fluorescent probes, laurdan and merocyanine 540. The results show a biphasic effect of activation and inhibition of the pump that depended on the molar fraction of phospholipids. SAXS measurements indicate that biophysical changes of the bilayer at different molar fractions could explain the difference in enzymatic activity. Moreover, we demonstrated how aluminium interacts with the micelles and influences the biophysical properties that in turn, affected the PMCA activity. PMCA activity largely depends on the biophysical properties of the surrounding bilayer that affects the activity and also the aluminium effect on the pump.

Keywords: ATPase, bilayer, SAXS
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DA.43 - EfTenA: a protein involved in thiamine biosynthesis in Enterococcus faecalis

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Bacterial resistance to antibiotics in therapeutic usage is a worldwide concern. The World Health Organization highlights, among other efforts, the urgency of finding new compounds to tackle multidrug resistance. Thiamine biosynthetic pathway (TBS) has been proposed as a source of drug targets for human pathogens, as this could be a pathogen-specific pathway and is absent in humans. TenA is a bifunctional enzyme with aminohydrolase and thiaminase II activity involved in the thiamine salvage pathway; however, the physiological function of this enzyme is not entirely clear. Here, we aim to structurally characterize and investigate the function of the TenA enzyme from *Enterococcus faecalis* (EfTenA). *E. faecalis* is related to healthcare-associated infections, and it is a relevant human pathogen within the context of antibiotic resistance. The recombinant protein was overexpressed in *E. coli*, purified by affinity and size-exclusion chromatography steps, and submitted to biophysical, biochemical, and structural experiments. Differential scanning fluorimetry assays showed a binding affinity of EfTenA to thiamine > thiamine monophosphate (TMP) > and thiamine pyrophosphate (TPP). The thiaminase II activity, measured by the thiocrome colorimetric method, revealed that EfTenA can hydrolyze thiamine and TMP but is inactive on TPP. After crystallization screening, crystals were submitted to an X-ray diffraction experiment. Two crystallographic structures were solved by molecular replacement. The first one shows a molecule of imidazole bound to the active site of ETenA, while the second structure shows an HMP ring (4-amino-5-hydroxymethyl-pyrimidine) bound to the active site. The HMP ring is the catalysis product of a TMP molecule by EfTenA crystals. Taken together, these results show that the tolerance of the active site decreases with the addition of phosphate groups to the thiamine molecule. As it is accepted that TMP is not a substrate for TenA, our findings can help to understand the TenA role in the TBS pathway.
Keywords: Bacterial resistance, Thiamine biosynthetic pathway, Enterococcus faecalis
Supported by: CAPES and FAPESP

DA.44 - *Trypanosoma evansi* interactome with mouse hippocampal neuronal cell (HT22)

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Trypanosoma evansi is the agent of "surra," a trypanosomosis endemic in many areas worldwide being responsible for a major economic problem in rural regions due to the loss of animals.*T. evansi* has the ability to migrate to other tissues, especially the Central Nervous System (CNS). Few studies have been carried about the interactions between the parasite and the CNS, despite the knowledge of some alterations resulting from this possible contact. This work consists of identifying which proteins secreted by *T. evansi*mediate the interaction with mouse hippocampal neurons cells (HT22) and may be related to the invasion of the parasite in the CNS. *T.evansi* soluble protein extract at concentrations of 100µg/mL and 200 µg/ml of were incubated with cells of HT22 strains in DMEM 10% FBS culture medium, 100 U/ml penicillin and 100 mg/ml streptomycin, and kept in a 5% CO_2 incubator at 37 ˚ C for one, three and five hours. Then, protein extraction was performed from co-culture cells subjected to ten cycles of freezing and thawing at -80 ˚ C and 37 °C, and sonicated in an ice bath. Soluble protein supernatant was subjected to SDS-PAGE. Western blot will be used to analyze the soluble *T. evansi,* proteins that bind to HT22 cells. The present work will be able to elucidate possible interaction mechanisms between the parasites and the neuronal cells.
Keywords: Trypanosoma evansi, cell interaction, CNS

DA.45 - Search for new therapeutic targets for the treatment of tegumentary leishmaniasis through *in silico* functional prediction of *Leishmania braziliensis* proteins

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Tegumentary leishmaniasis (TL) is a neglected parasitic disease whose main agent is *Leishmania braziliensis*, it is estimated that 1 million people are diagnosed with the disease annually. Currently, the pharmacological guideline for the treatment of TL is highly toxic and with reports of acquired resistance (monotherapy with meglumine antimoniate). Through the analyzes of the *L. braziliensis* genome available at the NCBI (Genbank: GCA_900537975.1) we will suggest new targets for drug repositioning through functional prediction. Using the 3,373 proteins with unknown function present in the genome of L. braziliensis and the databases, Uniprot, Psipred, SignalP, Psort, Pfam, CDD, SMART and STRING, a functional prediction based on sequence was made. Using the BATS and FILTERS filters of the MHOLline software, 3D models for these proteins were generated and selected. The selection of 3D models was based on identity (>25%) and coverage (>70%) values and using FastSCOP, SCOPe and CATH databases reached a consensus on the prediction of function of these proteins. In the classification obtained by BATS, 8 proteins were selected: SYZ66351, SYZ69098, SYZ64647, SYZ69127, SYZ63617, SYZ66198, SYZ66915 and SYZ67025. After the analysis of metabolic pathways, we followed with the protein SYZ64647 with predicted function of UDP-Galactopyranose that is present in the parasite, but not in humans. Using the programs AutoDock Vina, AutoDock Tools and the 3D model UDP-Galactopyranose (PDBid template: 4DSG) the GRID was parameterized based on the amino acids that interact with the ligand (center x: 63.988 y: -9.335 z: -43.684 size x/y/z: 30) and exhaustive tests (8, 16, 64, 100). With the parameters validated, the virtual screening was carried out with the World database, reaching 10 promising substances. With the studies carried out with the SYZ64647 protein, we saw this as a promising target for the repositioning of drugs for the treatment of tegumentary leishmaniasis. K**eywords:** Comparative Modeling, Leishmaniasis, *Leishmania braziliensis.* **Supported by:** CNPq, CAPES and IOC

DA.46 - Characterization of Photo-induced Oxidative Modifications of Parkinson´s Disease Related Protein α-Synuclein

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A gain of toxic function of the protein alpha-Synuclein (aSyn) has been largely linked to the development and progress of Parkinson's Disease (PD) and related Synucleinopathies. However, the identity of aSyn toxic species remains elusive. In this way, several post-translational modifications (PTMs) that derived from oxidative stress could be possible modulators of the physiopathology of aSyn in neurons. In this work we investigated the susceptibility of aSyn to be modified when this protein adopts different conformations, allowing us to identify potential pathological modifications. Materials and Methods: Recombinantly expressed aSyn was modified employing optimized photochemical methods that uses Rutheniun (II) Tris(bipyridine) as photosensitizer to produce either Tyrosine (Tyr) crosslinking or, in the presence of NaNO2, nitration. The reactions were characterized and quantified by Fluorescence emission, UV-Vis absorption and Mass Spectrometry. Results and Conclusions: Three conditions were analyzed: disordered aSyn free in solution, bound to membranes (helix-rich structure) and fibril conformation (cross β-sheets). Tyrosine crosslinking of disordered aSyn mainly involved Tyr39 and C-term residues Tyr133 or Tyr136. On the other hand, Tyrosine nitration were identified in all Tyr residues, being Tyr125 the most nitrated during photo-reaction. When bound to negatively charged SUVs, modification of Tyr39 is highly restricted. Similar outcome was found in the fibrillary form. Nitration of C-term residues is only reduced when aSyn interacts with membranes with a negative net charged, not in the unstructured C-term in fibrils. In conclusion, particular modifications, such Tyr39 nitration or Tyr39-Tyr133/136 crosslinking, could only emerge in pathological conditions and their identification in complex biological samples could be employed for the development of innovative tools for the early diagnosis of Synucleopathies. **Keywords:** alpha-Synclein, Oxidative Stress, Post-translational Modifications. **Supported by:** CONICET (Argentina), ANPCyT (Argentina), UNLP (Argentina), Bunge & Born, Max Planck Society and Williams Foundations (Argentina-Germany), DFG (Germany)

DA.47 - Identification and prediction of HbAg d-chain sequence.

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The giant hemoglobins from annelids have particular biotechnological characteristics. Sequence data from *Amynthas gracilis* hemoglobin are scarce and the improvement of the knowledge about what sequences are associated to the protein structure is important to biotechnological studies. The aim of this work was the obtaining of the transcriptome of the earthworm species *A. gracilis* to identify the coding sequence of one of the Hb subunits, the d-chain. A cDNA library was constructed from mRNAs extracted from adult individuals of *A. gracilis*. The sequencing was carried out on an Illumina MiSeq device, generating 2x300bp paired-end reads. The assembly of the transcriptome was performed using the Trinity 2.0.6 software and the prediction of the open reading frame (ORF) was made with the TransDecoder. The identification of the HbAg d-chain was performed using BLAST against the sequences deposited in the UniprotKB database considering a minimum e-value of 1.0e-20. Around 68% of the obtained data showed a phred quality score ≥30, with 25,644,392 reads. The assembly of the transcriptome generated a N50 of 883 bp, the lengh average of the contigs was 626.24 bp and the total of bases assembled was 681845025. After BLAST analysis, six sequences homologous to other annelid species were found, more specifically, sequences homologous to the d-chains of *Lumbricus terrestris* and *Glossoscolex paulistus*. A potential ORF corresponding to the d-chain of HbAg was found from the transcriptome obtained.

Keywords: transcriptome, giant proteins, earthworms. **Supported by:** Fapesp, CNPq

DA.48 - Architecture and dynamics of the yeast aquaglyceroporin Fps1 in response to osmotic and oxidative stress

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Membrane proteins play key roles at the interface between the cell and its environment by mediating selective import and export of molecules via plasma membrane channels. The Fps1 protein is the principal membrane aquaglyceroporin of the yeast *Saccharomyces cerevisiae* with its primary purpose to regulate intracellular pressure via facilitating glycerol efflux. Fps1 belongs to the Major Intrinsic Proteins superfamily, which is present in the vast majority of organisms from bacteria to plants and humans, fulfilling life essential tasks such as water content regulation and cellular signalling. Despite a multitude of studies on these transmembrane channels, understanding of their dynamics directly within living systems is limited. To address this, we correlated molecular scale information from living cells with real time changes to their microenvironment. We combined traditional molecular biology approaches with the Slimfield microscopy, a powerful new technology which enables detection of biomolecules directly in living cells with millisecond sampling, to track labelled molecules of interest in real time. We use the Fps1 aquaglyceroporin as an exemplar membrane protein to dissect and correlate its stoichiometry and molecular turnover kinetics with various extracellular conditions. We show that Fps1 resides in multi-tetrameric clusters and is also present as an intracellular pool, while hyperosmotic conditions cause Fps1 reorganization. We also demonstrate that rapid exposure to oxidative stress causes Fps1 assembly into larger foci which are further subjected to hydrogen peroxide-induced degradation and may thus be a regulatory step. Hence, we provide novel insights into understanding of cellular adaptation to the microenvironment through characterisation of the plasma membrane channels. **Keywords:** single-molecule, plasma membrane channels, cell stress

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DA.49 - PROJECT: Membrane interaction of the S. cerevisiae Golgi Reassembly and Stacking Protein
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The Golgi reassembly and stacking proteins (GRASPs) were initially identified by *in vitro* experiments as one of the main factors necessary for stacking the Golgi cisternae in mammalian cells. Over the years, data about GRASPs have reported their participation in many other cellular processes and the additional involvement of members of the GRASP family in unconventional protein secretion pathways. Although we already have some knowledge on their structure and function, such as their presence in membrane of organelles and vesicles that participate in the early and late secretory pathway, no data on how this interaction occurs have been reported this far. This was the main objective of this project. To understand more about how this protein can anchor in the membrane of organelles, the yeast *Saccharomyces cerevisiae*, a model organism, was used in this work. This budding yeast has a single homolog of GRASP65, which is called Grh1, and has already been reported in the early secretory pathway. Grh1 interaction with membranes are thought to involve the acetylation of its N-terminal. We used the heterologous expression of Grh1 to produce samples destined for fluorescence and circular dichroism experiments so as to unravel the molecular determinants of Grh1-model membrane interactions.
Keywords: GRASP65/55, Membrane, protein
Supported by: CAPES

DA.50 - Exploring novel environments for effective searching of unique xylose isomerases to lignocellulosic materials fermentation
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Xylose is regarded as the second-most abundant sugar in nature. Thus, its usage is crucial for the development of a sustainable biobased economy. Xylose isomerases (XI) catalyze the first reaction of xylose metabolism, enabling downstream metabolic steps to convert xylose into a value-added bioproduct. However, XIs suffer from low activity at fermentation conditions since most XIs present optimum thermophilic and alkaliphilic range conditions. Therefore, in this work, we explored a metagenomics approach to identify novel XIs from the two unique environments, the mangrove region and Antarctica soil, searching for higher activity at milder conditions. To set our targets, we performed a sequence-similarity network (SSN) which indicated three potential XIs (ORF1, ORF3 from mangrove, and AraXI from Antarctica soil). We performed an in-depth biochemical and molecular characterization, from cloning to detailed biochemical characterization. Our results show a thermophilic behavior even at XI prospected from Antarctica. Although the targets in this study showed higher susceptibility to inhibitors, the ORF3 presented better catalytic properties at fermentation conditions than the control XI. Because XIs are metalloproteins, we studied a range of different conditions biochemically and biophysically, accessing the thermal stabilization through Differential Scanning Fluorimetry. Interestingly, the ion which maximized the activity (Mg^{2+}) generated the lowest thermal stabilization. To understand the molecular aspects underpinning these properties, we investigated their high- and low- resolution structures. Small Angle X-ray Scattering experiments revealed XIs are found as homotetramers in solution, and the crystallographic structures are in the refinement process. Our findings suggest that metagenomics approaches exploring different environments gave useful information about the thermostability of XIs; their activities and stabilities are fine-tuned by several reaction conditions; and structural information could give important insights for protein engineering.
Keywords: Biorefinery, Lignocellulose, Xylose
Supported by: FAPESP

DA.51 - Production, purification, characterization and biotechnological application of endoglucanase isolated from *Pycnosporus sanguineus*

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The increase in the production of agro-industrial residues has required alternatives to minimize the global problem. Clean alternatives have been developed, including the use of agro-industrial residues as a source for fungal growth and production of enzymes of biotechnological interest. The objective of this work was the production, isolation, characterization and biotechnologically application of the endoglucanase produced from the filamentous fungus *Pycnosporus sanguineus*. Five agro-industrial residues, from industries in Maceió-AL, were used (wheat bran, sugarcane bagasse, sawdust, coconut fiber and paper). It was isolated using ethanol fractionation and ion exchange and chromatography. It is characterized in terms of temperature, pH, halotolerance, enzymatic kinetics and biotechnological application. Wheat bran was the residue where the fungus showed the highest enzymatic production. The ethanolic fractionation fractionated the enzymatic extract into 5 fractions, the 80-100% fraction concentrated greater activity and was subjected to ion exchange chromatography (DEAE-Sepharose) in AKTA pure M1GE, the isolated enzyme was obtained in a single fraction, observed through of the SDS-PAGE. The biochemical characterization of the enzyme determined the optimum temperature of 50°C and thermal stability between 30 and 60°C with up to 50% of the enzymatic activity. The optimum pH was 5.0, with stability at pH between pH 4.0 and 8.0. The isolated enzyme maintains more than 100% of the activity in up to 5 M NaCl with Km of 3.18 ± 100 mg/mL and the Kcat of 4.53 S-1. The isolated enzyme produced 273.096 mg/mL reducing sugars in 24 hours in rice husks. The present work concluded that the fungus Pycnosporus sanguineus is a producer of an important endoglucanase, with biochemical characteristics compatible with various industrial processes, which can be produced, isolated and applied biotechnologically quickly and with low cost.
Keywords: Agro-industrial residues, saccharification, halotolerant. **Supported by:** CAPES

DA.52 - Evaluation of the interaction between p53 tumor suppressor protein and heparin

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The p53 tumor suppressor protein is known as the guardian of the genome due to its functions of maintenance and conservation of cell stability, responsible for DNA repair or blocking of gene alteration. Mutations in the central domain of the protein are present in approximately 50% of human cancers, causing loss of function of p53 functions, promoting rapid cell proliferation, associated with the formation of intracellular protein aggregates. The mechanism of propagation of these structural alterations still needs to be elucidated, and interaction with cofactors may influence this process. Among these molecules, glycosaminoglycans, negatively charged carbohydrates, such as heparan sulfate and chondroitin sulfate are found in amyloid deposits of tumors, and this presence is related to an increase in malignancy. Our goal is to observe the effects of heparin on p53 aggregation (WT and R280K mutant) and analyze the structural alterations after interaction *in vitro* and evaluate the effect in cancer cell models. We used spectroscopic techniques such as fluorescence, light scattering and circular dichroism to evaluate *in vitro* interaction, and used p53-expressing tumor cell lines (MDA-MB-231 and MCF-7) to investigate the cytotoxic effects of heparin. The interaction between p53 and heparin was analyzed through polarization, showing higher affinity for R280K p53. This interaction did not change the secondary and tertiary structures of the protein at 25°C. However, at 37°C, it induced an increase in protein aggregation, time-dependently, leading to the formation of amyloid structures. Regarding cell viability, high concentrations of heparin showed significant cell death. The data emphasize the importance of heparin in p53 aggregation, suggesting that this ligand may remodel p53 structure during its aggregation process, acting as a chaperone. If this remodeling leads to the formation of more or less toxic species it still has to be further evaluated.
Keywords: amyloid, cancer, glycosaminoglycans. **Supported by:** CAPES, CNPq e FAPERJ

DA.53 - Kinect characterization of V(H+)-ATPase activity from gill microsomal fractions of two hololimnetic populations from shrimp *Macrobrachium amazonicum*

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Macrobrachium amazonicum is endemic to South America and it can be separated into hololimnetic and coastal. In freshwater, this shrimp hyper-osmoregulates their hemolymph energized by the gill (Na^+, K^+)- and V(H^+)-ATPase. This study aimed to characterize the kinetic properties of gill V(H^+)-ATPase activity of *M. amazonicum* from Grande and Tietê Rivers' populations. The gill microsomal fractions were prepared by differential centrifugation. The ATPase activity was carried out at 25 °C using PK/LDH coupling system and monitored spectrophotometrically at 340 nm ($\varepsilon_{340nm,\ pH\ 7,5}$ = 6200 M^{-1} cm^{-1}). The V(H^+)-ATPase activity represents the difference in activity measured in the presence of orthovanadate and orthovanadate plus bafilomycin. The V(H^+)-ATPase maximum activity was 16.7 ± 1.7 nmol Pi min^{-1} mg^{-1} protein for the fresh-caught shrimps from Grande River and 27.2 ± 1.1 nmol Pi min^{-1} mg^{-1} protein for the Tietê River population. The apparent affinity by ATP and Mg^{2+} of the gill *M. amazonicum* V(H^+)-ATPase from the Grande River population was 0.16 ± 0.03 mmol L^{-1} and 0.14 ± 0.02 mmol L^{-1}, respectively. On the other hand, from Tietê river the apparent affinity was 0.27 ± 0.04 mmol L^{-1} and 0.28 ± 0.05 mmol L^{-1} for ATP and Mg^{2+}, respectively. The gill V(H)-ATPase showed a constant enzyme inhibitor for bafilomycin, 35.1 ± 1.1 nmol L^{-1} and 21.2 ± 1.3 nmol L^{-1} for Tietê and Grande rivers populations, respectively. The V(H^+)-ATPase activity shown by those *M. amazonicum* populations was close to another population inhabiting a São Paulo state reservoir, although it is twice smaller when compared to the cultivated shrimp from broodstock, which was originally collected in the coastal Amazon basin region.

Keywords: V(H+)-ATPase, *Macrobrachium amazonicum*, osmoregulation
Supported by: Capes, FAPESP, CNPq, FAPEAM, INCT-ADAPTA

DA.54 - Strategies to release protein material from alpha-lactalbumin protein microparticles: exploiting their pH-responsiveness

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In destabilizing conditions, proteins may create intermolecular interactions and self-assemble into protein aggregates with different size and morphology. A great interest is focused on a peculiar characteristic group of protein aggregates with a common structure (the cross beta-sheet) stabilized by H-bonds named Amyloids. Their assembly and disassembly processes are of special interest for the design of biomaterials. Among amyloid structures, micron-sized spherical aggregates formed at pH near protein`s isoelectric point called protein particulates, have gained relevance for their ideal size, low citotoxicity, the ability to be formed from most of globular proteins and being unrelated to neurodegenerative diseases. Combining spectroscopic and quantitative fluorescence microscopy methods, we study the assembly and disassembly of protein particulates made of alpha-lactalbumin in different conditions. I have formed protein microparticles of alpha-lactalbumin and I have characterized them using advance fluorescence microscopy and spectroscopic techniques. We show that, structure, size and compactness of protein particulates can be controlled depending on the initial protein concentration and the time of incubation. These properties have a direct effect on the stability of these protein microspheres. We prove that disassembly of protein particulates occur differently varying the pH from hard acidic (pH2) to neutral/basic pH (pH 7.4) and the addition of salt. At hard acidic conditions the disassembly is instantaneous resulting in small oligomeric and/or large fragments depending on the maturation stage of the aggregate. At neutral/basic pH in the presence of salt disassembly is characterised by a slower releasing non-toxic monomeric alpha-lactalbumin. As a conclusion, protein particulates can act as reservoir of protein material presenting different strategies for the release that control not only the speed of the process, but also the final product that is delivered.

Keywords: Protein Microparticles, Disassembly, Amyloids
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DA.55 - Exploration of decarboxylase profile of putative P450 peroxygenases

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Cytochrome P450 proteins (CYP) are a superfamily of heme-containing monooxygenase enzymes. CYPs catalyze a wide variety of chemical reactions, making them of great interest for biotechnological applications. In recent years, the P450 peroxygenase OleT (CYP152) has attracted attention due to its ability to catalyze the oxygen removal from medium to long-chain fatty acids, with hydrogen peroxide as co-substrate, and thereby producing terminal alkenes. This characteristic is of great scientific and industrial concern as it can be explored to produce drop-in biofuels. Drop-in biofuels are composed of renewable hydrocarbons, chemically identical to petroleum fuels, and fully compatible with existing petroleum infrastructure. This similarity would allow the development of fuels with less environmental impact without the need for structural changes in vehicles. However, little is known despite the importance of the peroxygenases capable of fatty acid decarboxylation. This study aimed to discover new putative P450 enzymes previously selected by amino acid sequence analysis based on a unique decarboxylase described in the literature to expand the knowledge in the field. The genes were synthesized, fused to a histidine tag, and transformed into BL21 *E. coli* cells. The purification was performed using nickel-immobilized resin columns. Out of the six chosen candidates, three of them were possible to be purified and SDS-PAGE analysis together to spectrophotometric assays (Soret-peak ~ 420nm) indicated a high likelihood for functional CYPs. Activity test using C14 was carried out demonstrating a possible activity to this fatty acid. Further experiments will be performed to evaluate peroxygenase activity and the degree of activity against other fatty acids.
Keywords: Cytochrome P450 proteins, Drop-in biofuels., Terminal alkenes

DA.56 - Structural and DNA binding characterization of the glycine-rich protein AtGRP7 RRM domain

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AtGRP7 (*Arabidopsis thaliana* glycine rich protein 7) is a glycine-rich, RNA-binding protein that plays a central role in plant growth, development, and abiotic stress response. AtGRP7 consists of an N-terminal RNA recognition motif (RRM) followed by an intrinsically disordered region enriched in glycines. Despite the role played by AtGRP7 in cold adaptation and flowering time regulation in *Arabidopsis thaliana*, the biochemical mechanisms underlying its function are largely unknown. Here, we used a plethora of biophysical techniques to characterize the structure, stability, and DNA binding affinity of AtGRP7-RRM. The gene sequence enconding the RRM domain of AtGRP7 (residues 1-90) was cloned into RP1B and expressed as a His6-tag fusion protein. AtGRP7-RRM showed partially soluble expression in *Escherichia coli* BL21 DE3 at 18oC and 0.5 mM IPTG. Recombinant AtGRP7-RRM was purified by a combination of nickel-affinity and size exclusion chromatography. Circular dichroism revealed features of a typical RRM fold, containing a mixture of α-helices and β-sheets. AtGRP7-RRM displayed a melting temperature of 38 oC, suggesting a lowly stable protein. AtGRP7-RRM interaction with a 7-mer DNA oligonucleotide consisting of a previously identified binding site was investigated by fluorescence spectroscopy. The fluorescence emission spectrum showed a maximum at ~349 nm, indicating that the sole tryptophan residue is solvent exposed. Increasing concentrations of oligonucleotide led to fluorescence quenching and AtGRP7-RRM interaction with DNA occured with an apparent K_D of 17.9 ± 4.2 μM. Using multidimensional, triple resonance NMR, we unambiguously assigned 90% of AtGRP7-RRM resonances. This is an important first step toward the sctructural determination of AtGRP7-RRM, which will shed light into its mechanism of action.
Keywords: AtGRP7, Arabidopsis, structure

DA.57 - Partial purification of the posterior gills (Na^+,K^+)-ATPase from the swimming crab *Callinectes danae* by Molecular Sieve Chromatography

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Improving enzymatic activity while keeping the conformational structure of membrane proteins after purification has been a challenge over the past 70 years. The (Na^+,K^+)-ATPase is a transmembrane protein responsible for transporting 2 K^+ in and 3 Na^+ out of cells. Although this enzyme has been the main focus of several purification procedures in mammal tissues, it has been slightly neglected for crustaceans. Therefore, the main goal of this study was to purify the gill (Na^+,K^+)-ATPase from the swimming crab *Callinectes danae* using Molecular Sieve Chromatography. Aliquots (3.0 mL) of concentrated (Na+,K+)-ATPase microsomal gill fraction was purified in a Sepharose 4B column (2.5 cm X 32.5 cm) equilibrated and eluted with 50 mM HEPES buffer, pH 6.8, containing 250 mM sucrose using the AKTA Pharmacia Protein Purifier. The Electrophoresis and Western Blot analysis, as well as the (Na^+,K^+)-ATPase activity of each eluted fraction (1.0 mL) were estimated according to Moraes et al. (2020). two well resolved protein peaks were eluted from the column and only Peak I showed (Na^+,K^+)-ATPase activity (154.9 nmol Pi min^{-1} mg^{-1}) representing a 14-fold purification compared with that of concentrated microsomal fraction. SDS-Page of Peak I revealed a clearer protein profile compared with that of microsomal fraction prior purification and the Western Blotting analysis showed a single immunoreactive band for (Na^+,K^+)-ATPase α subunit (≈110 kDa). Peak II revealed a more complex protein profile (molecular weight lower than ≈70 kDa), but no (Na^+,K^+)-ATPase activity was observed. These primary results allow great advances in the area.

Keywords: (Na^+,K^+)-ATPase, Purification, *Callinectes danae*
Supported by: FAPESP, CAPES, CNPq, FAPEAM, INCT-ADAPTA II

DA.58 - Expression, Purification and Refolding of the Receptor Binding Domain (RBD) of the Spike protein from SARS-COV-2.

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In 2019, a new outbreak of a respiratory disease was identified in Wuhan, China, caused by the new coronavirus SARS-CoV-2, which is a positive sense, single stranded RNA virus. The SARS-CoV-2 spread all over the world very fast leading to a pandemic state declared by the WHO in March 2020, responsible for more than 4 million deaths. The SARS-CoV-2 interacts with Angiotensin 2 Converting Enzyme (ACE-2) on the host cells by spike protein subunit S1 (containing RBD) to enter and initiate the virus replication. The long period of SARS-CoV-2 pandemic state has favored the emergence of mutations. A variant of concerning has been the variant P1 (gamma) identified in Manaus. Despite the development of different vaccines, none of them promoted a complete immunization to control the virus spreading, making it important to continue searching for new inhibitors. In this scene, the objectives of this work are the expression, purification, and refolding of recombinant RBD and RBD-P1. The plasmid containing RBD-wt sequence was kindly given by Dr. Lima from School of Life Sciences, Keele University, UK. The plasmid containing RBD-wt was used as template for the construction of RBD-P1 by side-direct mutagenesis using PCR, which was confirmed by sequencing. Both constructions were used to transform *E. coli* Bl21(DE3)plyS and protein expression was performed in auto induction media at 37°C for 4 h. Protein expressions were also performed in LB medium and 1 mM IPTG at 30°C for 16 h. The proteins were purified and refolded on an affinity chromatography column. Both proteins presented a 34 kDa in the SDS-PAGE. We successfully obtained purified recombinant RBD and RBD-P1. The perspectives will be the selection of peptide phage display library for both purified proteins, in attempt to find specific peptide for each protein.

Keywords: SARS-CoV-2, Receptor Binding Domain, Recombinant Protein

DA.59 - Heterologous Expression, Purification and Characterization of TRAP1 and PINK1

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Inside eukaryotic cells are located the mitochondria, organelle responsible for a lot of physiological processes, such as energy production, synthesis of biomolecules and it also has an important role in some mechanisms of cellular apoptosis. Imbalances in its operation can disturb cell homeostasis, resulting in the emergence of many diseases (*e.g.* cancer). Molecular chaperones, including Hsp90 and its mitochondrial homologue TRAP-1, are involved in the good performance of mitochondrial functions. TRAP-1 ensures the correct protein folding and it's essential for the mitochondrial integrity, protecting cells against apoptosis, besides avoiding toxic effects of oxidants and anti-cancer drugs. To perform its functions, it interacts with other proteins, such as PINK-1, a mitochondrial serine-threonine kinase: a fundamental regulator of the cytoprotective activity of TRAP-1 through the chaperone phosphorylation. Recombinant proteins production as well as their structural characterization. The proteins were produced in *E. coli* BL21(DE3) strains and protein expression were induced with isopropyl-β-D-thiogalactoside (IPTG). The pellets were lysed and purified using immobilized metal affinity (IMAC) and size-exclusion (gel filtration) chromatographies. The efficiency of the methods was verified by polyacrylamide gel electrophoresis (SDS-PAGE) and protein concentration determination by spectrophotometric measurements. To characterize TRAP-1 and PINK-1 circular dichroism and intrinsic tryptophan fluorescence assays were performed. A preliminary pull-down test was also performed to detect the interaction between the recombinant proteins. Both proteins were successfully expressed and purified, with a high degree of purity and homogeneity. Both proteins are well folded as characterized by circular dichroism spectrum and intrinsic tryptophan fluorescence assays. The interaction was detected in the pull-down assay, but control optimizations are still needed. Proteins were obtained with a high degree of purity and well folded. As future perspectives, we intend to carry out other tests focusing on the protein interaction, such as the ITC experiments. **Keywords:** structural characterization, TRAP1, PINK1. **Supported by:** CNPq e FAPESP.

DA.60 - Conformational changes of freshwater shrimp hemocyanin induced by urea

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Hemocyanins are giant extracellular proteins with molecular weight between 0.45 to 3.0 MDa and have several biotechnological applications associated with their immunogenic properties, which motivates the performance of biophysical studies on these proteins. In this work the objective was to study the effect of urea on the hemocyanin structure of freshwater shrimp *Macrobrachium acanthurus* (HcMac) by different spectroscopic techniques. The hemolymph was centrifuged at 3,000 x g by 15 min, dialyzed for 12 h in 100 mmol.L-1 Tris-HCl + 20 mmol.L-1 CaCl2 buffer at pH 7.0. Followed of ultracentrifugation at 250.000 x g by 5 h. Spectroscopies analysis of the concentration of HcMac was 1.6 mg.mL-1, prepared in 30 mmol.L-1 acetate-phosphate-borate buffer at pH 5 and 7, equilibrated for 2 h, depending on the urea concentration (0 to 8 M). Dissociation of the quaternary structure and drastic conformational modifications occur while the concentration of the denaturant increase. Optical absorption spectrum shows changes in the maximum absorption intensity at 340 nm (di-copper center) due to the increase in urea concentration. Fluorescence data show a decrease in fluorescence emission intensity between 0.5 - 4.0 mol.L-1 of urea. Above 4.0 mol.L-1 there is an increase in the emission intensity and an evident shift in the maximum emission wavelength from 330 nm to 347 nm. A reduction in LSI occurs after the addition of urea until it reaches a concentration of 4 mol.L-1, associated with protein dissociation. Concentrations below 4.0 mol.L-1 urea promoted a greater exposure of the di-copper center to the solvent, showing changes in the quaternary and tertiary structure of HcMac. The results suggest that the HcMac denaturation process starts at concentrations greater than 4.0 mol.L-1 of urea, that is, after the protein dissociates. **Keywords:** hemoprotein, biophysical characterization, urea

DA.61 - Phosphatidic acid vesicles induce the formation of prion protein PK-sensitive fibrils with stable secondary structure

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The mechanism of cellular prion protein (PrPC) conversion into prion scrapie (PrPSc) is considered the most important factor in the development of prion diseases. PrPC is anchored to the outer cell membrane into lipid rafts through a GPI anchor. PrPC conversion mechanism is not fully understood. Recent studies show phospholipids may interact with PrP leading to its conversion. In a previous work from the group Phosphatidic Acid (PA) vesicles were shown to interact with recombinant PrP (rPrP), leading to aggregation (). Our aim was to elucidate PA-induced rPrP conversion into amyloid fibrils, characterizing the aggregate structure, stability, and resistance. We used light scattering, circular dichroism, fluorescence spectroscopy, proteinase K (PK) digestion and electron microscopy over different temperatures to obtain structural and stability information. PA vesicles induced rPrP aggregation and fibrillization in a temperature-dependent manner, the lower the temperature, the greater the effect. Fibrils formed at 25 °C kept their secondary structure unchanged when submitted to high temperatures, but lost thioflavin T binding. Return to 25 °C restored fibril signal. Using acrylamide as fluorescent quencher agent, we observed that tryptophan accessibility to solvent was decreased in lower temperatures, and these residues were more hidden in the fiber core. The fibrils formed were fully digested by PK. Dynamic light scattering showed no significant changes in PA vesicle diameter submitted to high temperatures, but we observed a decrease in laurdan generalized polarization. Our results suggest that rPrP:PA interaction leads to formation of PK-sensitive fibrils. PrP dynamics and flexibility changes are important to follow a fibrillization pathway induced by PA. Vesicle bilayer rigidity is also important for rPrP interaction and conversion. The quaternary structure of these fibrils seems to be sensible to high temperatures, although its secondary structure is stable, forming an intermediate capable of reorganizing into fibers. **Keywords:** Amyloid fibrils, Prion, Phosphatidic acid. **Supported by:** CNPq, FAPERJ, INBEB, CAPES

DA.62 - Effects of chronic toxicity to ammonia from *M. amazonicum*

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The Amazon River shrimp *Macrobrachium amazonicum* is widely distributed throughout South America and shows elevated potential for aquaculture investment. The successful aquaculture activity depends, among other parameters, the water quality, particularly to the ammonia concentration, which increases considerably during cultivation. We investigate the in a homolimnetic population of the *Macrobrachium amazonicum* from Paraná/Paraguay River acclimated to “safe” ammonia concentration (0.1 x LC_{50}-96h) for 10 days. Adult shrimps were collected from the Rio Grande River, state of Minas Gerais, Brazil, near the Água Vermelha Dam. Western blotting of gill microsomal homogenates from fresh caught shrimps showed a single immunoreactive band of ≅120 kDa, corresponding to the (Na^+, K^+)-ATPase α-subunit. The ammonia-exposed shrimps also exhibited a weak diffuse band of ≅130 kDa. The ammonia-exposure of *M. amazonicum* shrimps expressed a secund α subunit isoform that is 2.5-fold increased affinity (Na^+, K^+)-ATPase for NH_4^+. Exposure of *M. amazonicum* to 4.9 mg L^{-1} total ammonia (1.8 mg L^{-1} un-ionized ammonia) increase gill (Na^+, K^+)-ATPase (1.3-fold), V(H^+)-ATPase (2.5-fold) and K^+-ATPase (2.5-fold) activities; these are key enzymes in the active ammonia excretion process. These changes suggest that the mechanism, still unknown for freshwater shrimp, is not very different from that described for marine species, where NH_4^+ would enter the gill ionocytes via substitution for K^+ on the hemolymph-facing basal (Na^+, K^+)-ATPase K^+ binding sites, and K^+ binding sites on the presumably basal K^+-ATPase, then being excreted into the subcuticular space via the apical Na^+/H^+ (NH_4^+) exchanger. Although ammonia-exposure does not cause mortality, lethargy or any indication of sulfuring for 10 days, further studies are needed to improve the determination the maximum acceptable toxic concentration in this population shrimps. **Keywords:** ammonia toxicology, gill (Na+, K+)-ATPase, freshwater shrimp aquaculture. **Supported by:** FAPEMIG, CNPQ, FAPESP

DA.63 - Structural-Functional Study of domains NBD (Nucleotide Binding Domain) and PBD (Peptide Binding Domain) of human HspA1A protein

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Among the class of molecular chaperones, there is a superfamily called Heat Shock Proteins (Hsps) that are highly expressed in cells under thermal stress conditions and act, in general, on cellular proteostasis. The Hsp70 family, 70 kDa heat shock proteins, have about 40-60% identity, ubiquitous and ATP-dependent monomeric proteins. Among the different members belonging to the human Hsp70 family, the cytosolic HspA1A isoform is an important constituent of the cellular network of molecular chaperones and providers of folding within cells. Structurally, Hsp70 are proteins composed of two domains, the nucleotide-binding domain (NBD), responsible for the weak ATPase activity, and the peptide-binding domain (PBD), responsible for the interaction with sequences of hydrophobic amino acid residues in the client protein. The investigation of the function, dynamics, structure and stability of the individualized PBD and NBD domains of HspA1A, can help in elucidating its molecular mechanism. Thus, the present work consists of evaluating some structural and functional properties of the NBD and PBD domains of the HspA1A protein, such as circular dichroism, intrinsic fluorescence of tryptophan and analytical size exclusion chromatography, showing its structural characteristics, hydrodynamic and stability. The obtained results showed that the recombinant proteins referring to the constructs HspA1A_PBD and HspA1A_NBD were produced in their folded form, with secondary structure content, rich in β-sheets and α-helices, respectively, and behave, respectively, as a mixture of oligomers and monomer in solution. The proteins showed different thermal stability, the PBD construction being partially reversible and the NBD irreversible. Chemical stability, for both, showed two transitions, so that for the PDB, was characteristic of cooperative unfolding and, for NBD, partially cooperative. In summary, the results obtained enrich the information about the individual characteristics of the domains and their influence on the canonical HspA1A. **Keywords:** Molecular chaperones, HspA1A, domains

DA.64 - The Cryo-EM-elucidated structure of an early oligomer of alpha Synuclein and its relation to Parkinson's disease toxicity

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The neurodegenerative Parkinson's disease (PD) is characterized by the aggregation of the intrinsically disordered protein alpha-Synuclein (α-Syn) into fibrils with a cross-β amyloid structure, in the Lewy body plaques within dopaminergic neurons of the mid-brain. The fibrillation pathway of α-Syn encompasses a multitude of transient oligomeric forms differing in size, structure, toxicity and prion-like seeding activity. According to a recent solid state NMR study, the core residues within a fibril of α-Syn are arranged into in-register parallel β sheets with a unique C-terminal Greek key topology (PDB 2N0A). Here, we report the formation of stable, non-toxic (to both liposomal membranes and neuroblastoma cells) 'mace'-shaped oligomers, when the physiologically-available small molecule heme (hemin chloride) is added at sub-stoichiometric ratios, to either monomeric or aggregated α-Syn. Using cryo-electron microscopy, we found that a tetrameric form of the Greek key model could be fit into the density of these mace oligomers, albeit with a distortion in the head-neck junction of the mace structure. The 'Greek key oligomer' fits well as a segment of the previously-described annular oligomers and appears to be its structural predecessor in the hierarchical pathway of fibril formation. We propose that the distortion in the heme-treated oligomer prevents further appending of the twisted units into annular oligomers. Furthermore, heme binds to a crucial histidine (His50) residue located in the inter-protofilament pre-non amyloid β component interface, thus interfering with a salt bridge formation with a Glu57 residue located in the opposite protofilament, thereby weakening the inter-protofilament steric zipper integrity. Overall, we describe a mechanism of inhibition of fibrillation and associated of α-Syn using a physiologically-available small molecule. **Keywords:** oligomer, neurodegeneration, Greek key fibril

DA.65 - Isolation and characterization of Canavalia ensiformis serine proteases: enzymes with pharmacological potential

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Canavalia ensiformis is a tropical legume with high content of active proteins, that have important biological functions, and their pharmacological activities have been extensively investigated. Previous studies from our group demonstrated important serine protease activity from *C. ensiformis* leaf aqueous extract (CE-A), suggesting a possible pharmaceutical application. The aims of the present work was to isolate and characterize serine proteases from CE-A. Fresh *C. ensiformis* leaves collected in Plataforma Agroecológica de Fitomedicamentos (PAF), of FIOCRUZ were processed with N_2 and proteins extracted with distilled water. Protein content was determined by Bradford method and protein profile evaluated by SDS-PAGE. Serine proteases were isolated by affinity chromatography using ρ-aminobenzamidine–agarose column. Serine proteases rich fraction (CE-AρBza) was isolated from CE-A, 3.00 times with total yield of 79.8%. SDS-PAGE showed major proteins molecular weight about 52, 37 and 25 kDa. The enzymatic activity was studied using L-N–α–p–tosyl–L–arginyl–methyl ester ($_L$-TAME) and N-Benzoyl-L-tyrosine ethyl ester (BTEE) as substrates. The maximum protease activity was observed in typical serine protease pH range (9.0), besides, was expressively inhibited only by serine protease inhibitor, such as benzamidine which inhibited 100% of CE-AρBza using BTEE as substrate and 77% when using $_L$-TAME. This fraction preserved 88% of activity in 24h at 70ºC using BTEE as substrate and 46% when using $_L$-TAME. CE-AρBza has similar affinity to BTEE (K_M =11,65 μM) and TAME (14,97μM). These results indicated that CE-AρBza proteases had expressive and thermostable activity, and the methodology for isolation was efficient. Thus, these proteases have potential biotechnological and pharmacological employment.

Keywords: biochemical characterization, Canavalia ensiformis, serine proteases

DA.66 – PROJECT: **Auxin as mitigating agent of salt stress in two species of forage grasses**

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The grasses Urochloa brizantha and Megathyrsus maximus have little tolerance to salinity. And Plant hormones are fundamental to the adaptation to salinity by mediating several adaptive responses. The aim of the proposed study is to evaluate the effect of auxin (indole-3-acetic acid) on salt stress adaptation methods in Urochloa brizantha and Megathyrsus maximus submitted to salinity. The seeds will be germinated in substrate moistened with auxin with three repetitions. Beginning on the 30th day, salinity will be applied with a combination of ions (Na+, Mg+2 and Ca+2). On the day of collection, one group of plants will be decapitated at the collum level to collect fluid from the xylem, estimate the concentration of ions Na+ and K+, and estimate the flow rate of xylem sap. In parallel, another group of plants will be divided into leaves, colla and roots to estimate the content of ions Na+ and K+. The quantification of ions in these samples will be performed using flame photometry. The Western blot method and polymerase chain reaction (qPCR) will be used to analyze the expression of transporters involved in the control of the transport of Na+, such as plasma membrane (SOS1) and vacuolar (NHX) antiporters in roots. Treatment with indole-3-acetic acid is expected to stimulate the Na+ homeostasis regulating mechanism. Thus, Urochloa brizantha and Megathyrsus maximus could have perform better under conditions of salinity.

Keywords: Auxin, Forage grasses, Tolerance to salinity

DA.67 - Inositol monophosphatase 2 (IMPase 2) and its inhibition with lithium(I) and hesperetin

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Individuals with bipolar disorder (BD) have a characteristic and sudden change in mood from euphoria to depression. In Brazil, there are around 6 million people diagnosed with BD. The most used drug to alleviate BD symptoms is lithium along with other drugs and therapies, but Li(I) medicinal dose is close to toxic. Besides, the benefits of lithium are observed just in 60% of patients with BD that use this drug. In addition, the use of lithium therapy provokes a drop in myo-inositol, which is a precursor of phosphatidylinositol that plays an important role in intracellular signal transduction through the production of second messengers. Thus, an important connection between bipolar disorder and the altered performance of enzymes from the family of inositol monophosphatase (IMPase), especially IMPase 2, was hypothesized. This project aimed to propose a flavone, hesperetin, as a possible phytotherapeutic agent for BD and an inhibitor of IMPase 2. To perform the *in silico* assays and preliminary experiments conducted *in vitro*, it was first necessary to obtain the protein using the recombinant DNA technique. IMPase 2 was purified using affinity chromatography and characterized using biophysical and biochemical techniques as a folded and active enzyme. Then, the interactions among hesperetin-IMPase 2, lithium-IMPase 2, and hesperetin+lithium-IMPase 2 were studied. The results obtained in circular dichroism, fluorescence, and enzymatic activity experiments pointed to an interaction between the flavone of interest and IMPase 2, and that this interaction enabled to inhibit the enzyme's action. Finally, when associated with lithium, hesperetin inhibited the IMPase 2 hydrolytic properties, especially against inositol-1-monophosphate. There are still to determine the IMPase 2 hesperetin binding site, and propose the alteration in the IMPase 2 mechanism of action in the presence of the flavone and lithium. **Keywords:** Inhibitors , Enzymes , Flavone. **Supported by:** FAPESP

DA.68 - Mechanism elucidation of not conventional peroxygenases applied on production of drop in biofuels

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Alkenes or olefins are precursors for drop-in biofuels production, such as biokerosene and green diesel. OleTJE is the only studied enzyme from the P450 family that can promote decarboxylation activity on free fatty acids, the reaction that produces alkenes as a final product. Our research intends to find new enzymes with decarboxylation activity to understand what drives this type of reaction and propose a biotechnology route to produce drop-in biofuels. Herein, we discovered a novel decarboxylase P450 (named RN) using bioinformatics tools that cluster enzymes by their activity and amino acid sequences. RN enzyme was expressed in a host cell (E.coli) and purified by chromatography methods. The purified enzyme was submitted to SEC-MALS, SAXS, crystallization, and X-ray diffraction experiments to determine its molecular properties. Moreover, the spectroscopic characterization was done by methods as UV-Visible to calculate dissociation constant (Kd) with different chain size substrates (C12, C14, C16, C18, and C20) and circular dichroism (CD) to analyze the secondary structure besides to melting temperature (Tm). Hydrodynamic studies revealed RN as a dimer, conformation never reported in this class of enzymes, and conserved amino acids in its active site, written in OleTJE as crucial to the decarboxylation route. The native enzyme presented higher affinity by myristic acid (C14, Kd: 0.59 μM) and Tm of 52.23 °C. To better understand the role of dimer conformation on RN, mutants S26F and E405F were investigated. The mutants were confirmed as monomers, presenting a similar CD profile and Tm (53.3 and 49.5°C). Still, there is a lower affinity for myristic acid (Kd: 0.91 and 2.07 μM) and alkene production compared to the wild-type enzyme. The results presented here expand the knowledge on the molecular bases for decarboxylation activity of an enigmatic hydrocarbon-producing P450 before based on a unique structure available in the literature.
Keywords: alkenes, decarboxilation, drop in biofuels. **Supported by:** FAPESP

DA.69 - Antimicrobial resistant bacteria as treasure troves of protein evolution

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The intensive use of antibiotics in hospital settings and in the management of animals for human consumption, coupled with limited success in the development of new classes of antimicrobials, has created the possibility of an imminent public health crisis: the number of infections caused by antimicrobial resistant bacteria is on the rise and there are estimates that by 2050 more people will die of infections by resistant bacteria than due to cancer. Our aim is to evaluate how substitutions resulting from antimicrobial selective pressure lead to resistance, by looking at their effect on protein function and structure. Specifically, we look at the transcriptional repressor EthR of Mycobacterium tuberculosis and the two component systems PhoPQ and PmrAB of Klebsiella pneumoniae. We employ bacterial genetics and protein biochemistry to assay how substitutions change DNA binding, protein-protein interaction, and or enzymatic properties. We find that certain substitutions in EthR and PmrAB and PhoPQ occur in key functional regions. In the case of EthR we show that two substitutions enhance transcriptional repression, likely by disrupting allosteric changes predicted to happen upon ligand binding. In the case of PmrAB and PhoPQ we show that mutations selected by polymyxin treatment fall in discrete functional regions, hinting at the mechanisms of resistance. Albeit a major health problem, antibiotic selective pressure on bacteria works as an experiment on the evolution of protein function and structure.

Keywords: AMR, bacterial two component systems, M. tuberculosis

Supported by: Fiocruz; Institute Pasteur International Network

DA.70 - P53 protein and Liquid Liquid Phase Separation (LLPS): Aggregation studies in anticancer therapy

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The p53 tumor suppressor protein is a cellular sensor for DNA damage that induces cell cycle abrogation to avoid misbehavior on cellular phenotype. In this sense, mutants of the TP53 gene have been found in more than 50% of cancer cells. The hotspot mutations are placed on the DNA-binding domain of the p53 protein and they are related to loss of function that results in tumor development. On the other hand, the mutations can also induce gain of oncogenic function by the formation of amyloid aggregates that impair DNA damage recognition. The goal of this work is to investigate whether the p53 aggregation route involves liquid-liquid phase separation (LLPS). The first step was the preparation of soluble p53, p53 WT-EGFP and p53 mutants, by heterologous expression. After, we tested whether p53 LLPS could occur, varying some conditions as temperature and molecular crowding, using Differential Interference Contrast (DIC) and fluorescence recovery after photo bleaching (FRAP). The results showed that p53 phase separates in the presence of the molecular crowding agent ficoll under low temperatures. Longer incubation times or increased temperature led to the transition of p53 to aggregates. Polyanions, such as heparin and RNA, were able to modulate the phase separation and phase transition *in vitro*. Heparin led the p53 condensates in a gel-like state, whereas RNA resulted in the conversion into a solid-like state of the protein, similar to that found in proteins involved in neurodegenerative diseases. The possibility to probe different conformational states on the misfolding pathway at an atomic level has highlighted microscopy tests as the state of the art in the structural biology field to study invisible intermediate states.

Keywords: Liquid Liquid Phase Separation, protein, p53

Supported by: FAPERJ, FINEP, CNPq and CAPES

DA.71 - A biophysical and *in vitro* characterization of an amyloidogenic and highly tolerated cell penetrating peptide

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Due to the increasing number of new therapies based in the delivery of nucleic acids or even drugs with great therapeutic appeal that are poorly absorbed by the tissue/cells, we face an increasing demand for biocompatible carriers. In the last 30 years, nearly 2000 cell penetrating peptides (CPPs) have been reported in the literature, CPPs are small amino acid sequences with the capacity of translocating through cell membranes, and since the presence of cationic residues such as arginine and lysine are reported as potential enhancers for the translocation of these sequences, most CPPs are highly hydrophilic and cationic. However, this increase in charge is generally followed by an increase in cytotoxicity, which is a major problem for the use of CPPs in living organisms. In this work we propose the formation of non-covalent complexes between the sequence PFVYLI, a hydrophobic and non-charged CPP, conjugated with nucleic acids. The self-assembly of PFVYLI was characterized by classical biophysical techniques such as small angle scattering X-ray (SAXS), Circular dichroism (CD) and Atomic force microscopy-based infrared spectroscopy (AFM-IR). the delivery of non-covalent aggregates between PFVYLI and a model fragmented DNA into HeLa cells was observed by fluorescence confocal microscopy. Cytoxicity was determined by MTT assays. It was possible to observe the self-assemble of PFVYLI into rod-like supersctructures with lengths ranging from a few nanometers to micrometers, and the the AFM-IR, fluoresnce in the presence of Thioflavin and CD assays indicates an Amyloid-rich secondary structure. We also succesfully formulated non-covalent aggregates between this peptide and nucleic acids, which were well tolerated and internalized by HeLa cells, possibly via an endocytic pathway. The peptide PFVYLI self-assembled into rod-like super structures with an intrinsic β-amyloid secondary structure, we also successfully produced conjugates between this peptide and DNA that were well tolerated and internalized for HeLa cells.

Keywords: biophysics, molecular biology, cell penetrating peptides
Supported by: FAPESP, CAPES

DA.72 - An anterior transcriptional repression mechanism in the segmentation cascade of Drosophila

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This cascade is formed by sequential transcription factors that set the polarity of the egg and establish the division of the body in segments. The cascade comprises three hierarchical levels formed by gap, pair-rule and segment polarity genes. Our goal is to understand transcription regulation during development. To that end, we investigate the segmentation cascade that specifies the antero-posterior axis in Drosophila during its syncytial blastoderm. In the laboratory we study the segmentation cascade in the anterior region of the embryo. Our approach is to combine genetics, cellular, biochemical and bioinformatics to understand molecular mechanisms underlying transcription at DNA regulatory regions. According to our results, a mechanism comprised by the combined activity of gap repressors prevents the formation of pair-rule stripes in the anterior blastoderm. So far, we identified Sloppy-paired, Tailless and Huckebein as repressors that operate in additive manner to set the borders of anterior stripes of pair-rule genes.

Keywords: Drosophila, repression, transcription

DA.73 - The conformational dynamics of the flanking polyQ regions in the membrane-bound state of huntingtin exon 1

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The pathological expansion of the polyglutamine (polyQ) repeat within the first exon of huntingtin (HTTex1) protein is a hallmark of Huntington's disease (HD). Multiple evidences support that the membrane interaction of huntingtin is critical for its misfolding and aggregation in HD. Here, we focus on obtaining a detailed understanding of the initial steps of HTTex1-lipid interaction and the conformational dynamics of the flanking polyQ regions. We initially employed single-molecule approaches based mainly on fluorescence correlation spectroscopy (FCS) to monitor the binding of HTTex1 to synthetic lipid vesicles. The dependence on membrane curvature and lipid composition was studied, considering as models anionic lipids, raft-mimicking mixtures, and total brain lipid extract. The FCS results show a preferential binding of HTTex1 towards POPS (anionic lipid)-containing vesicles. Moreover, time-resolved fluorescence intensity and anisotropy measurements of HTTex1 site-specifically single labeled at the adjacent polyQ regions reveal distinct conformational dynamics of these flanking regions in the HTTex1 membrane-bound state. Notably, the proline-rich domain remains highly flexible and solvent exposed even upon membrane binding. Instead, the N17 segment converts into a less dynamic state. Our findings provide unique insight into how membrane composition and flanking polyQ sequences modulate the early stages of HTTex1 aggregation at the membrane surface.

Keywords: Intrinsically disordered proteins, lipid-protein interaction, Neurodegeneration
Supported by: Work supported by FCT-Portugal (PTDC/BIA-BFS/30959/2017 grant and CEECIND/00884/2017 contract to AMM, and UIDB/04565/2020 funding to iBB-IST).

DA.74 - Improving inhibition potential of an ACE2-derived peptide against SARS- CoV-2 spike protein by rational design

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In the recent SARS-CoV-2 pandemic, the importance of designing neutralizing strategies for the virus is evident. During viral infection, the spike protein is responsible for the attachment to the host cell surface via binding to angiotensin converting enzyme 2 (ACE2) and for the fusion of viral and cell membranes releasing the viral genome into the cytoplasm. In this sense, some peptides are capable of competitively inhibiting the interaction between SARS-CoV-2 and ACE2. Earlier studies of the interaction between the spike receptor binding domain (RBD) of the SARS-CoV-1 and ACE2 identified characteristic regions of the receptor, from which a derived peptide, called p6, was found to have potent antiviral activity. In this work, we propose five new peptides derived from ACE2. Using atomistic MD simulations, we started analyzing p6, and then we rationally designed five new peptides using two different strategies: On the one hand, the hydrophobic and solvent-exposed residues were mutated expecting a stabilization of the secondary structure and on the other hand, we tried to improve the interaction based on per-residue energy calculations and published mutational analysis. We compare the analysis of the computer simulations to experimentally obtained Kd values, and highlight key factors that contribute to the tight binding of the p6 peptide to the SARS-CoV-2 RBD.

Keywords: SARS-CoV-2, antiviral peptides, ACE2
Supported by: CONICET

DA.75 - P53 protein and Liquid Liquid Phase Separation (LLPS): Aggregation studies in anticancer therapy

Elaine da Conceição Petronilho [1], Murilo Martins Pedrote[1], Mayra de Amorim Marques[1], Yulli Moraes Passos[1], Michelle Ferreira Mota[1], Benjamin Jakobus[3], Gileno dos Santos de Sousa[1], Filipe Pereira da Costa[1], Adriani de Lima Felix [1], Giulia Diniz da Silva Ferreti[1], Fernando Pereira de Almeida[1], Yraima Cordeiro[1], Tuane Cristine Ramos Gonçalves Vieira[1], Guilherme Augusto Piedade de Oliveira[1], Jerson Lima Silva[1]
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The p53 tumor suppressor protein is a cellular sensor for DNA damage that induces cell cycle abrogation to avoid misbehavior on cellular phenotype. In this sense, mutants of the TP53 gene have been found in more than 50% of cancer cells. The hotspot mutations are placed on the DNA-binding domain of the p53 protein and they are related to loss of function that results in tumor development. On the other hand, the mutations can also induce gain of oncogenic function by the formation of amyloid aggregates that impair DNA damage recognition. The goal of this work is to investigate whether the p53 aggregation route involves liquid-liquid phase separation (LLPS). The first step was the preparation of soluble p53, p53 WT-EGFP and p53 mutants, by heterologous expression. After, we tested whether p53 LLPS could occur, varying some conditions as temperature and molecular crowding, using Differential Interference Contrast (DIC) and fluorescence recovery after photo bleaching (FRAP). The results showed that p53 phase separates in the presence of the molecular crowding agent ficoll under low temperatures. Longer incubation times or increased temperature led to the transition of p53 to aggregates. Polyanions, such as heparin and RNA, were able to modulate the phase separation and phase transition *in vitro*. Heparin led the p53 condensates in a gel-like state, whereas RNA resulted in the conversion into a solid-like state of the protein, similar to that found in proteins involved in neurodegenerative diseases. The possibility to probe different conformational states on the misfolding pathway at an atomic level has highlighted microscopy tests as the state of the art in the structural biology field to study invisible intermediate states.
Keywords: Liquid Liquid Phase Separation, protein, p53
Supported by: FAPERJ, FINEP, CNPq and CAPES

DA.76 - From evolution to folding of repeat proteins

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The coding space of protein sequences is shaped by evolutionary constraints set by requirements of function and stability. Repeat proteins are made with tandem copies of similar amino acid stretches that fold into elongated architectures. Due to its symmetries, they are a unique system to model how evolutionary constraints at the sequence level can impact tertiary structure, folding and function. In this work we depart from pairwise identity patterns emerged from a curated Ankyrin 150000 sequence dataset. We combine an inverse Potts model scheme with an explicit mechanistic model of duplications and deletions of entire repeats for calculating the evolutionary parameters of the system, such that the observed identity patterns can be reproduced. We use the evolutionary energy obtained to input a folding toy model for repeat proteins, a coarse-grained 1D ising model where each spin corresponds to a protein sequence fragment and has to be folded or unfolded. We perform Monte Carlo simulations of the model using only sequence information and we get thermal unfolding curves that are compatible with experimental ones of several proteins. We perform a large scale analysis of the dataset and we develop a predictor of several folding mechanism features for the Ankyrin family.
Keywords: proteins, evolution, folding
Supported by: UBA, CONICET, ECOS-Sud

DB - Structural Biology

DB.01 - Structure of a Short Peptide Derived from the Transform Growth Factor beta1

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Peptide nanostructures have been studied as alternative therapeutics due to their potential selectivity for certain molecular targets. One of the promising uses is related to autoimmune diseases, which in many cases are correlated to either central or peripheral tolerance processes. The peripheral process is mediated by the transform growth factor beta 1 (TGF-β1), which is secreted by T-regulator cells, and plays anti-inflammatory function in autoimmune diseases. In this context, the structural characterization of peptide fragments derived from TGF-β1 may assist the elucidation of mechanisms implied in the action and open possibilities for developing biomimetic nanomaterials based on this growth factor [1]. We aimed to provide detailed characterization on both self-assembly and structure of a heptamer with amino acid sequence Ac-ESPLKRQ, derived from TGF- β1, and correlate structure and anti-inflammatory action. The peptide has been synthesized through standard solid-phase approaches. Characterization has been performed through a range of biophysical techniques including fluorescence, circular dichroism and infrared spectroscopy, small-angle X-ray scattering, and electron and atomic force microscopy. Fluorescence assays have shown the formation of stable aggregates above a critical concentration ~ 3 mg/mL, with secondary structure dominated by disordered conformations; however, at higher concentrations, these supramolecular assemblies present signature of α-helix conformations, the same secondary structure exhibited by the active in the native TGF-β1 protein, which may be the conformation responsible for docking to cell receptors. The aggregates present nanometer lengths, composed by subunits with globular shape and sizes ~60 nm, which may assist interaction with cell membranes. The peptide sequence mimetizing TGF-β1 investigated here is able to form nanoscopic aggregates with secondary structure that reproduces conformations of active sites found in the native protein. These characteristics make this fragment a promising candidate to produce bioactive nanoparticles able to exert similar anti-inflammatory responses to those observed for TGF-β1. **Keywords:** peptide self-assembly, TGF-beta1, biomaterials. **Supported by:** CAPES, CNPq, FAPESP

DB.02 - Structural and functional characterization of the N-terminal domain of the human coronavirus hCoV-HKU1 nucleocapsid protein

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Since 2002, three beta coronaviruses (bCoVs) have gained notoriety: SARS-CoV, MERS-CoV, and SARS-CoV-2, which is causing the Covid-19 pandemic. Besides these more aggressive CoVs, there are endemic human viruses, such as hCoV-HKU1, associated with mild respiratory tract disease, that can progress to acute respiratory failure. During infection, the transcription of the viral genome generates full-length and subgenomic RNAs, through a discontinuous process regulated by the transcriptional regulatory sequences (TRSs). The nucleocapsid (N) protein of CoVs acts as an RNA chaperone and its N-terminal domain (N-NTD) binds to TRS, forming a high-affinity complex. Comparing the structure, dynamics, and TRS interaction of HKU1-N-NTD with those of more aggressive CoVs will enable the understanding of the molecular factors leading to severe infections and the development of treatment strategies. Determining the structure of HKU1-N-NTD, mapping its interaction with different DNA/RNA fragments, including TRS, and probing its dsDNA/RNA melting activity. NMR assignments of the HKU1-N-NTD were determined using a set of classical 2D and 3D experiments. Structure calculation was performed using ARIA and CS-Rosetta softwares. HKU1-N-NTD interaction with TRS was evaluated by protein NMR titration with DNA/RNA. HKU1-N-NTD melting activity towards double-stranded DNA/RNA was assessed by fluorescence. The calculated structural model presents 4 β-strands and 4 flexible regions, including an extended loop known as the finger. In the presence of TRS, chemical shift perturbation (CSP) was observed for several residues, including Asp57, Gln59, and Val105, which presented high CSP for all evaluated protein-fragment interactions. N-NTD melting activity was greater for dsRNA. HKU1-N-NTD interacts with DNA and RNA fragments and promotes double-strand melting. CSP data indicate that the main binding region is located between the finger and the central β-sheet. These results may help better understand the role of N protein in the discontinuous transcription, enabling new approaches to fight CoVs. **Keywords:** hCoV-HKU1, nucleocapsid protein, TRS

DB.03 - Determination of the three-dimensional Structure of Schistosoma mansoni Universal Stress G4LZI3 Protein by X-ray Crystallography

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Schistosomiasis is the most potent water-based disease in tropical and subtropical regions of the world from a global health perspective, infecting more than 252million people with 90% resident in SSA. Despite its existence from antiquity, efforts to eradicate and control the disease have failed and unfortunately current treatment now displays drug resistance and reduced efficacy. Schistosomes have over time developed mechanisms to cope with the myriad of stresses they encounter throughout their developmental cycle through the up-regulation of Universal Stress Proteins (Usp). The G4LZI3 USP has since been identified as a potential 'lead' molecule in schistosomal treatment hence, the aim of this study was to determine the 3D structure of this protein and its biological function. The G4LZI3 protein was over-expressed in M15 cells and purified using Ni-NTA affinity chromatography and gel filtration. Characterization of the protein was done by Differential Scanning Calorimetry, Fluorescence Spectroscopy, Fourier-transform Infrared Spectroscopy, Mass Spectrometry and 1D Nuclear Magnetic Resonance. These showed the protein was folded, consisted of various secondary structure elements and is thermodynamically-stable. Pooled purified fractions were concentrated to yield samples for crystallization trials. Various conditions yielded small crystals that were seeded and thereafter used to generate considerably-sized 3-dimensionally-shaped crystals. Diffraction data was remotely collected at Diamond Light Source (United Kingdom) and processed at the University of Cape Town using MR Rosetta Software tools. Coot was thereafter used for model building and refinement of the structure. Lastly, bioinformatics tools were used to identify small molecule inhibitors which were docked onto the protein. Those with the best scores were used to determine parameters such as molecular dynamic simulations, post dynamic analysis and binding free energy calculations. These results provide basis for further investigation of the G4LZI3 protein towards the design of new anti-schistosomals.

Keywords: Characterization, Crystallography, G4LZI3
Supported by: Diamond Light Source (UK), National Research Foundation (NRF).

DB.04 - SAXS e DLS: a theoretical review

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In order to determine nanoparticles structure and size, SAXS and DLS techniques are the best to be used. DLS is a simple technique and can return parameters as particle size and polidispersity, which mean how is the size distribution of the sample. SAXS technique can determine particle structure and supply information about lattice parameter and water channel disposition. This technique can also be used to determine protein and other molecules structures. The objective of this study is to go through an overview around parameters and the theory behind DLS and SAXS techniques. It is focused on its use to analyse cubosomes, an important lipid nanoparticle with cubic phase. As an theoretical study about the techniques mentioned above, this work followed theory placed in articles and books. The SAXS and DLS techniques are reliable to determine the parameters discussed above. In one hand, DLS is a straightfoward technique and provides the results right after the experiment is performed. On the other hand, SAXS needs some analyzing, in which the positions of peaks are used to determine particle structure. Through this analysis, it can be seen coexistence of cubic phases and phase transitions. Therefore, these techniques are the finest to use to determine particle structure and particle size. The analysis made is rather simple and the results obtained are accurate. In addition, SAXS and DLS techniques are often used by researches to characterize nanoparticles samples.

Keywords: characterization, DLS, SAXS
Supported by: FAPESP

DB.06 - Exfoliative protein C (ExhC) of Staphylococcus sciuri: inactivation and structural determination of necrotic activity

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Staphylococcus sciuri is pathogenic bacterium of significant clinical and veterinary relevance. Recently, a strain of *S. sciuri* has been described as the etiologic agent of exudative epidermitis in pigs in China and the main virulence factor involved in this clinical manifestation was the Exfoliative protein C (ExhC). ExhC of *S. sciuri*, in addition to causing epidermal exfoliation in pigs and neonatal rats, it was able to induce cell necrosis *in vitro*, specifically in the cell line of renal fibroblasts of neonatal hamsters (BHK-21) a property hitherto fore unobserved in exfoliative toxins (ETs). The production of ExhC recombinant fragments allowed us to conclude that the domain containing residues 79-128 is essential for the observed necrotic activity. The aim of this research was to verify whether the mutations of specific amino acid residues inactivate necrotic activity and to determine the structural parameters of this region and correlate it with necrosis. The amino acids of the necrotic domain were aligned with the corresponding regions of other ETs which indicated the presence of conserved amino acid residues or with similar biochemical properties in most of the ETs except in ExhC of *S. sciuri*. This *in silico* evaluation formed the basis for the design, expression and purification of ExhC protein with mutation in four amino acid residues and wild type ExhC for *in vitro* tests with the cell line BHK-21. The mutant ExhC loss of necrotic activity was observed in the *in vitro* tests with the cell line BHK-21, different results from those observed for wild type ExhC which causes a significant decrease in cellular viability. The crystal structure of mutant ExhC was determined with 1,57 Å and the stereochemical structural parameters have been correlated with the results of the *in vitro* tests and provide details of the steric requirements for the observed activity.

Keywords: exfoliative protein C, Staphylococcus sciuri, necrotic activity
Supported by: FAPESP

DB.07 - Human fumarase cocrystallization: revealing metabolite-fumarase interactions

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Fumarases are enzymes that catalyze the estereospecific and reversible hydratation of fumarate to L-malate. Human fumarase (HsFH) belongs to class II and in mitochondria participates in the tricarboxylic acid (TCA) cycle, and in the cytosol can act in the metabolism of amino acids and also play a key role in DNA damage response to DNA double strand breaks. HsFH gene mutations have been mainly associated with heritable diseases: fumarate hydratase deficiency (FHD), multiple cutaneous and uterine leiomyomatosis (MCUL) and hereditary leiomyomatosis and renal cell cancer (HLRCC). There are few inhibitors and activators reported for fumarases, and its regulation mechanisms remains unclear. This present work aimed the evaluation of different metabolites interactions in the human fumarase structure. A mass spectrometry integrated with equilibrium dialysis for the discovery of allostery systematically (MIDAS) was performed to evaluate protein-metabolite interactions. The cocrystallization experiments were performed with the recombinant HsFH and the metabolites, by sitting drop method and using PEG 10 K as precipitant agent. The preliminary analysis of MIDAS allowed the identification of maleic acid and D-2-amino-3-phosphono-propionic acid as potential ligands. Cocrystallization experiments were carried out in order to obtain the complex structure, and datasets (at 1.8 Å and 2.15 Å resolution for maleic acid and D-2-amino-3-phosphono-propionic acid, respectively) were collected. The solved structures revealed the presence of maleic acid in a pocket formed by two different chains, far away from the active site, while the D-2-amino-3-phosphono-propionic acid was found bound at the active site. MIDAS was proved to be a reliable method to identify enzyme-metabolite interactions and the results obtained so far are promising and revealed a new site of interaction in the HsFH structure.

Keywords: fumarase, enzyme-metabolite interaction, cocrystallization
Supported by: FAPESP and CNPq

DB.08 - Structural studies of a J-domain co-chaperone by NMR

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HSP40 co-chaperones cooperate with HSP70 in aiding proteins to fold in the cell. HSP40 binds a partially folded protein and delivers it to be folded by HSP70. Several lines of investigation have been used to understand the interaction between the J-domain and HSP70, however, a detailed mechanism of interaction is still missing. This work presents a high-resolution structure of the J-domain of Sis1, an HSP40 from *Saccharomyces cerevisiae*, solved by NMR. Chemical shift protection approaches were used to identify the residues that change conformation in the full-length protein and are affected by the presence of HSP70. All NMR samples were prepared in 25 mM Tris-HCl pH 7.5, 200 mM NaCl and 10% D2O. Sis1 was at 200 uM, Sis1 J-domain at 100 to 250 uM, and HSP70 peptide at 10 mM. NMR spectra were acquired at 30°C on Bruker 900, 800 MHz, or 600 MHz spectrometers at CNRMN-UFRJ. Six residues were the most affected in both cases: V2, D9, R27, T39, F52, and R73 in the full-length protein and A29, H34, T39, F45, F52, and Y67 in the presence of DnaK/HSP70. Since two of the residues, T39 and F52, as well as two regions 27-39 and 67-73, appear to be equally affected, this work suggests that the J-domain in the full-length HSP40 may be in a conformation favorable to bind HSP70, facilitating the interaction between the two proteins. The structure of the isolated J-domain and J-domain in Sis1 are similar, but there are remarkable small differences, which may account for the Sis1 function.

Keywords: NMR, co-chaperone, J-domain
Supported by: Fapesp

DB.09 - Potential Targets for Leishmaniasis Disease: New Found Binding Sites and Biophysical Characterizations

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Peptides can participate of in several physiological processes, from biological responses as well as modulation of biochemical responses. Their biological activity is determined by the sequences of specific amino acids which enables them to design short synthetic peptides with high specificity, stability and to ease of synthesis. *In silico* experiments are widely used in pharmacokinetic experiments reducing the time it takes for a drug to reach the market. However, the selection of targets and definition of the region where the drug will interact is charge for the design of peptide to act as an inhibitor. We propose a pipeline to identify sites that can be good target for these peptides inhibitors. Until now, we tried this protocol with 6 potential target proteins for Leishmaniasis described in the literature. Binding sites were detected and characterized with *PeptiMap* and *FTMap* during a first step. In a second moment, we investigated the volume and area of these sites with CHIMERA, analyzing several aspects as: i) electrostatic map for the target (using the server BLUUES), ii) protein surface accessibility and electrostatic contribution of polar residues (using the server TKSA-MC) were considered. Finally, the collective motions and structural dynamics was analyzed using I elastic network modes models with the server DYNOMICS. We found 6 new potential binding sites for each target protein, considering peptides as inhibitors. We present the results for each protein, describing their characteristics studied. The initial results showed good results for these targets. This pipeline will be improved and tried with another systems in order to be used in the drug design process with the purpose to obtain a better specificity of these compounds.

Keywords: Biophysical Characterization, Rational Drug Design, Computational Biology
Supported by: FAPERO, FAPESP, CNPq and CAPES

DB.10 - Evaluation of bovine lactoferrin as an antiprion drug

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The cellular prion protein (PrPC) is found in various tissues but abundantly in the central nervous system. A structural modification can occur on its endogenous rich α-helix form to a pathogenic isoform, PrP scrapie (PrPSc), turning into a β-sheet rich structure. This conversion triggers protein aggregation, which accumulates in the nervous tissue and progressively causes the loss of neuronal cells. Bovine lactoferrin (bLf) is known by its multiple functions, such as antiviral, antimicrobial and others. Lf is found in brain cells that were damaged by various neurodegenerative diseases, such as Parkinson's and Alzheimer's disease. It is important to investigate the possible antiprion activity of bLf because little is known about the role of this protein in prion disease. Our goal was to evaluate the interaction between recombinant PrP and bLf, characterizing the molecular details involved in this interaction. The interaction of the complex PrP-bLf was monitored by techniques such as polarization, dinamic and static light scattering, circular dichroism and isotermal titration calorimetry. The RT-QuIC assay was performed to induce the *in vitro* formation of fibrillar aggregates. The dot-blot assay was used to assess whether apo and holo-bLf were able to decrease the presence of proteinase k resistant PrP in ScN2a cells. We showed that apo and holo bLf were able to interact with similar affinity, but holo bLf induces the formation of an oligomeric complex. PrP N-terminal domain modulated this interaction. We observed that bLf was able to decrease the presence cellular proteinase K resistant PrPSc. Both apo and holo-bLf were able to totally inhibit *in vitro* fiber formation even at very low concentrations. This effect was observed using infected brain homogenates, liquor from CJD patients or *in vitro* produced fibrils as seeds. These studies are important to understand the possible application of bLf as an antiprion agent.
Keywords: bovine lactoferrin, prion protein, interaction
Supported by: FAPERJ, CNPq, INBEB and CAPES

DB.11 - Structural insights into SARS-CoV-2 Main Protease maturation process

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SARS-CoV-2 is the causative agent of COVID-19 and responsible for the global pandemic. The viral genome contains the ORF1ab, that codifies two polyproteins containing the non-structural proteins, essential for viral replication. The Main Protease (Mpro) is responsible for cleavage of the viral polyproteins in 11 sites, including its own N and C-terminus. Due to its importance in the viral cycle, it represents an important target for antiviral development. Yet, the details of its self-maturation process remain still poorly understood. In here, we used the MANACA beamline at Sirius to characterize the mechanism of self-maturation of the SARS-CoV-2 Mpro. For that, three different constructs were obtained: the immature form (IMT Mpro), the native form (Mpro) and an inactive mutant (C145S Mpro). All constructs were biochemically and structurally characterized. The IMT Mpro crystal structure revealed several structural changes compared with native protein, including differences in the substrate binding pocket. Also, this form of the enzyme exhibited reduced activity when compared with mature form, as well as to form monomers in solution. The crystal structure of C145S Mpro revealed a complex with the endogenous N and C-terminal peptides substrates during the formation of the tetrameric complex. Further biochemical characterization revealed how oligomeric state shifts overtime in this sample. Based on all evidences, we propose a mechanism for Mpro maturation process that start with the formation of an intermediate immature dimer for N-terminal cleavage, followed by the association of two dimers and C-terminal cleavage resulting in the mature form of the enzyme. The elucidation of this process sheds light in our understanding of viral cycle, and can be used to propose specific inhibitors targeting intermediate states of the SARS-CoV-2 Mpro.
Keywords: Main-Protease, maturation, SARS-CoV-2

DB.12 - Structural characterization of the type VI secretion system of *Klebsiella pneumoniae:* the inner tube Hcp protein

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Klebsiella pneumoniae is the causative agent of acute infections of the respiratory and urinary tracts. These infections represent a major challenge to public health, as strains resistant to multiple antibiotics are circulating throughout the world. The comprehension of the mechanisms associated with virulence is important for understanding and fighting these infections. Comparative analysis of the genomes of K. pneumoniae revealed that all strains analyzed contain genes encoding type VI secretion system (T6SS) proteins. T6SS is a needle-like complex made up of about 13 families of proteins. It's used by the bacteria to deliver toxins into target eukaryotic and prokaryotic cells. However, the molecular mechanisms involved in the secretion of effector proteins have not yet been fully elucidated. In this work, we intend to characterize the structure of the Hemolysin Co-regulated Protein (Hcp), responsible for the formation of the inner tube of the T6SS and for the transport of effector proteins to the interior of a target cell. Recombinant expression of Hcp2; Nickel affinity purification; Negative staining transmission microscopy; Thermal Shift. We successfully overexpressed and purified the K. pneumoniae Hcp2 in Escherichia coli strains, but it has low stability *in vitro*, forming aggregates and precipitating. Aggregates were processed for negative staining and small structures of different sizes similar to hexameric Hcp were observed by transmission electron microscopy. Also, using Thermal Shift assay, we were able to optimize the ideal pH for the protein buffer, increasing its stability. Aiming to optimize *in vitro* stability and stabilize a single conformational state of the protein, site-directed mutations were performed. We are currently overexpressing the mutants and doing *in vitro* stability tests. Our data will contribute to the better understanding of the internal tube assembly and transport of effector proteins through the T6SS of K. pneumoniae, promoting the basis for the developing of new drugs. **Keywords:** Klebsiella pneumoniae, HCP, T6SS.
Supported by: FAPERJ

DB.13 - Structure and biophysical characterization of the exfoliative E toxin mutant of *Staphylococcus aureus*

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Staphylococcus aureus is a commensal and opportunistic bacterium that can infect a variety of hosts, including humans and different species of cattle, represents a global public health problem, and causes enormous damage to the livestock industry. Exfoliative toxin E (ETE) is a virulence factor that facilitates the spread of the pathogen by inducing the generalized detachment of the granular layer of the epidermis in sheep. This serine protease is inactive in its native state. Occasional secondary interactions, which are still unknown in this system, and uncommon between serine proteinases induce specific hydrolysis of the peptide bond between Glu381-Gly382 of desmoglein 1 (Dsg1). A better understanding of the mechanism of action of ETs is necessary to develop strategies to inhibit the pathogen. This work seeks to elucidate the mechanisms and modes of action at the atomic level, in interactions with the substrate Dsg1. For this, the native and mutant ETE protein (Ser219Ala) were expressed in *E. coli* BL21 (DE3) -T1R. Subsequently, they were purified by gel filtration, on a Superdex® 75 10/300 GL column in 20 mM MES buffer pH 7.03, and pH 5.5. We analyzed its secondary structure by circular dichroism, showing that both proteins have a predominance of random coil, in dynamic light scattering the particle size for the two proteins was 2.093 ± 0.3 (r.nm). Thermal unfolding experiments using circular dichroism and differential scanning calorimetry allowed us to establish the values of the melting temperature (49.0 ± 1.4), the calorimetric enthalpy (220 ± 50), and the Van't Hoff enthalpy (270 ± 70). Crystals obtained from the proteins were diffracted in the MANACÁ line of SIRIUS from Campinas-SP, with an initial resolution of 2.4 Å. Data collected from ETEmut diffraction are in the process of refinement and will be used to analyze the functioning of the protein.
Keywords: Staphylococcus aureus, exfoliative toxin E (ETE), X-ray crystallography
Supported by: CAPES

DB.14 - Druggable hot spots in the dimerization interface of trypanothione reductase enzyme from trypanosomatids.

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Identification and characterization of druggable hot spots of trypanothione reductase enzymes (TRs) from trypanosomatids and comparison with the homologous human enzyme glutathione reductase (GR). In the PDB (Protein Data Bank) 36 TRs of trypanosomatids and 26 human GRs are available. The structures were selected exploiting different species, different oxidation states and different ligands. The prediction of druggable hot spots in the selected structures were performed using the FTMap server , excluding the regions where the FAD and / or NADPH interact. Based on the criteria predefined by Kozazov, the DrugPy Plugin classifies and characterizes the identified hot spots. Complementary analysis for the characterization of these hot spots were carried out with the aid of the pocket match.RESULTS:Prediction studies of druggable hot spots suggest the dimeric interface of TRs as a potential site for the development of inhibitors. Out of the different hot spots identified, the one with the highest frequency and strength (average concentration of 27 probes, 16 in Cs0) was analysed for their chemical environment characteristics and the Pmin results of TRs compared with the human enzyme GR range from 0.3 to 0.58, which suggests important differences in the dimeric interface between them. It is worth mentioning that the region of the active site, highly explored to date and also identified in this study, has greater structural similarity than the interface. The results open the opportunity to explore a new region for drug design, capable of modulation by potent and selective molecules. S: The characterization of a potential allosteric site in TRs marks a paradigm shift in the search for inhibitors for TR target and it enables the search of ligands with different chemical diversity of known inhibitors. The next step of this project is the chemical / biological validation of the potential allosteric site.

Keywords: Trypanothione reductase, Druggable hot spots, DrugPy Plugin
Supported by: FAPESP and CNPq

EA - Bioenergetics and Metabolism

EA.01 - Effects of the exposure of different cell lines to the steroidal hormone gestrinone

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Gestrinone is a steroidal hormone with anti-estrogen and anti-gonadotropic actions, used for the treatment of endometriosis, contraception, and other estrogen related conditions. However, there are no studies analyzing the systemic effects of this hormone, what would be important to prove its efficacy and safety. Thus, we proposed an analysis of its effects on different cell lines: mammary, uterine, endothelial, and hepatic, at different concentrations and exposure times. Cell viability in the presence of the hormone was assessed by MTT. So far, we analyzed HuH-7 (hepatocyte) and HUVEC cells (from umbilical cord endothelium), in the absence and presence of different concentrations of gestrinone: 1, 5, 10, 50 and 100 µmol/L. Both cell lines showed a concentration-dependent reduction in viability when compared to the control after 24h. After 48h, cell viability decrease was observed at the lowest tested hormone concentration (1 µmol/L), suggesting that, in addition to the concentration, the exposure time has also an important impact on cell viability. Gestrinone will be tested in the other mentioned cell lines and the metabolites secreted by these cells will be identified using nuclear magnetic resonance, followed by metabolomic analysis, which may provide us a broad profile of the cellular pathways affected by gestrinone.
Acknowledgements: FAPERJ, Formédica Rio
Keywords: gestrinone, endometriosis, implant

EA.02 - Artichoke extract reduces DNA damage in the blood of mice submitted to animal model of obesity

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Obesity results from excess fat stored in adipose tissue, developing a low-grade chronic inflammatory disease. Some plants admit substances that help to treat the effects of obesity, artichoke, for example, has beneficial properties to health. Evaluate the effect of artichoke on the levels of damage to deoxyribonucleic acid (DNA) in mice submitted to an animal model of obesity. 24 male Swiss mice [Mus musculus] with 30 days of age, weighing from 25 to 35 grams, were used. The mice were divided into two groups, one classified as obese and the other non-obese. 12 mice belonging to the obese group were fed with a high-fat diet and the other 12 were fed with a normolipid diet. Water and feed were provided and maintained in light/dark cycles of 12 hours each and a temperature of 23°C. After six weeks of obesity induction, orally and gavage, the animals received daily treatment with artichoke extract at a concentration of 370 g/ml for four weeks. The mice were killed by decapitation, followed by blood collection and cerebral cortex. Afterwards, the structures were sent to perform the comet assay and check for DNA damage. Greater damage was observed in the blood and cerebral cortex of animals in the obese group compared to the control group. A significant reduction in DNA damage in the blood was seen with the artichoke-based treatment, but in the cerebral cortex the reduction was not significant. The active ingredient of the artichoke leaf extract was carried out by chromatography. The chromatographs showed peaks corresponding to chlorogenic acid (4.2 per minute), on the other hand, caffeic acid was not expressive. Although the results were positive for blood, further biochemical investigations are important to elucidate the different mechanisms of action of artichoke extracts.
Keywords: Obesity, artichoke, DNA damage

EA.03 - Sugarcane arabinoxylan composition profiling using combinations of hemicellulolytic enzymes
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Lignocellulosic biomass is an abundant source for biofuels and food supplements production, but its recalcitrant and heterogeneous structure is a major factor limiting industrial use. The main plant cell walls components are cellulose, a homopolymer of β-1,4 linked glucoses organized in microfibrils, and hemicellulose, an heterogenous polymer containing pentoses, hexoses and sugar acids. Xylans, the most common hemicellulose, consist of a heteropolysaccharide with a backbone chain of β-1,4 linked xyloses that can be branched with arabinose, glucuronic or 4-O-methyl glucuronic, ferulic and p-coumaric acids and acetyl groups. Use of diverse hemicellulolytic enzymes is an alternative for xylan deconstruction since enzymes break specific linkages, producing less subproducts. In this work we propose to use combinations of 5 hemicellulolytic enzymes: Arabinofuranosidase (GH43), Endoxylanases (GH10 and GH11), Acetyl Xylan Esterase (CE4) and α-Glucuronidase (GH67) to obtain a composition profile and a better enzyme combination for a most complete degradation of sugarcane extracted arabinoxylan. For this, enzymes with sequences cloned in pET-28 vectors were expressed in *Escherichia coli* STAR strain, purified with nickel affinity chromatography, and reacted against sugarcane DMSO extracted arabinoxylan powder in a 2% suspension in buffer, in different combinations, to access the effect of the enzymes both alone and in groups. Reaction products were analysed by hydrophobic interaction liquid chromatography coupled with mass spectrometry (HILIC-MS) and reducing sugar quantification. Mass spectrometry results analysis indicated the arabinoxylan has acetyl (Ac) and methyl glucuronic (MG) modifications in the main xylose backbone and arabinofuranosidase activity indicated the presence of arabinoses. A recalcitrant structure, that no enzyme combination was able to hydrolyse, was also observed and the best combination observed for hydrolysis was GH10, CE4 and GH67. This method can be extended to more complex polymers to access its structure and look for a better enzymatic treatment for complete hydrolysis.
Keywords: Hemicellulolytic Enzymes, Arabinoxylan, Mass Spectroscopy. **Supported by:** FAPESP

EA.04 - Effect of atorvastatin on blood pressure and its autonomic modulation in humans and rodents: a systematic review with meta-analysis
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Atorvastatin is a drug commonly used to reduce the cholesterol synthesis in hypercholesterolemia by inhibiting HMG-CoA reductase. Studies have shown that statins, in addition to their lipid-lowering action, potentially affect the autonomic nervous system (ANS), prevent cardiac dysfunction by inhibiting oxidative stress and improving endothelial function. We evaluated the atorvastatin effect on blood pressure (BP) and its autonomic modulation and clarified the relationship between these effects and cholesterol changes. Using Pubmed database, we selected primary studies that analyzed the atorvastatin effect on BP, heart rate, baroreflex and/or heart rate variability (HRV) in randomized clinical and preclinical studies performed in mammals. Included articles were stratified according to baseline characteristics of the population and their methodological quality was assessed. Independent statistical analyzes were performed for each parameter in clinical or preclinical studies considering all included articles and/or each stratification. In clinical trials, our meta-analysis showed that atorvastatin reduces systolic (SBP) and/or diastolic (DBP) blood pressure when compared to baseline and/or placebo in hyperlipidemic individuals with or without hypertension. Atorvastatin did not affect the HRV and the baroreflex. Although the meta-regression of all primary articles demonstrates that the atorvastatin effect in BP is associated with LDL reduction compared to baseline, this association is not observed when compared to placebo. In preclinical studies, atorvastatin does not affect SBP in rats fed a control diet and spontaneously hypertensive-stroke prone, but it reduces SBP in rats fed a high salt diet, hyperlipidemic, diabetics, hypertensive by renal surgery or spontaneously hypertensive (SHR) rats. The effect of atorvastatin in reducing the SBP in SHR is not associated with a reduction of serum cholesterol, showing that SBP reduction is independent of lipid changes. Our systematic review demonstrated that the cardiovascular effect of atorvastatin occurs independently of the reduction in total and/or LDL cholesterol and of the ANS modulation.
Keywords: atorvastatin, blood pressure, heart rate variability. **Supported by:** UFSJ

EA.05 - Ahp1 is an important peroxiredoxin under stress by organic peroxide under conditions of peroxisome biogenesis in yeast

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The β-oxidation of fatty acids occurs in peroxisomes, generating H_2O_2, that may cause oxidative insults. Catalase protect cells against H_2O_2 and is located in peroxisomes. However, deletion of the catalase gene does not provoke drastic phenotypes, suggesting that other enzymes may also participate in the protection of cells against oxidants derived from the peroxisomes. Peroxiredoxin (Prx) enzymes are Cys-based peroxidases that are highly abundant and efficient in hydroperoxide reduction. Ahp1 (alkyl hydroperoxide reductase 1) is a Prx from *Saccharomyces cerevisiae* with the ability to efficiently reduce alkylhydroperoxides and other peroxides, such as H_2O_2. The amino acid sequence (AHL) of this protein presents elements that suggest its localization in peroxisome. We analyzed the functions of Ahp1 under distinct physiological conditions, employing a ΔAhp1 (cells with the Ahp1 gene deleted) strain. Cells treated with 1mM tert-butyl hydroperoxyde (tBHP) underwent a growth arrest, in medium containing glucose or glycerol. By the ferrous oxidation-xylenol orange (FOX) assay, we also observed that the duration of the growth arrest correlated with the amounts of tBHP remaining in culture medium. In cells treated with 1mM tBHP, the lag time to recover cell growth was longer for ΔAhp1 (about 72 hours) than for wild type cells (about 48 hours) in Synthetic Defined medium (SD medium). By the ferrous oxidation-xylenol orange (FOX) assay, we also observed that the duration of the growth arrest correlated with the amounts of tBHP remaining in culture medium. When tBHP levels dropped, yeast growth was restored in both WT and ΔAhp1 strains. Cell fractionation-western blot assays are ongoing in an attempt to determine the cellular localization of Ahp1 under distinct physiological conditions. Ahp1 protects yeast from exogenously added organic hydroperoxides.
Keywords: Metabolism, Oxidants, Alkylhydroperoxide reductase 1

EA.06 - The p-chloro-diphenyl diselenide (p-ClPhSe)2, an organoselenium compounds, modulates the glycolytic pathway through an insulin-mimetic action in different experimental models

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Organoselenium compounds have been described by modulating rodents metabolism by stimulating glucose uptake in their liver and muscles. Notably, p-chloro-diphenyl diselenide (p-ClPhSe)$_2$ restores the metabolic defects induced by obesity by regulating the activities of enzymes involved in glucose metabolism, thus suggesting an insulin- like effect. However, to verify this putative mechanism, a genetically tractable animal model was required. We investigated the effect of (p-ClPhSe)$_2$ in modulating the insulin-like pathway in *Caenorhabditis elegans* (*C. elegans*). Knockout mutants of insulin/IGF-1 like signaling were used in this study, TJ1052 (age-1(hx546) II), VC204 (akt-2(ok393) X), DCR3791 (pfk-1.1(ola72) X) and CF1038 [daf-16 (mu86)], besides Bristol wild-type strain (WT-N2). First, we have confirmed that glucose and triacylglycerides basal levels were reduced by (p-ClPhSe)$_2$ 30 minutes treatment (from 1μM). Furthermore, we established that (p-ClPhSe)$_2$ reduced the glucose levels in *C. elegans* in an age-1, akt-1 and 2 and daf-16 dependent-manner. Considering that DAF- 16 can be activated by other pathways and to confirm that (p-ClPhSe)$_2$ was acting by this pathway, we submitted age-1 mutants to RNAi feeding to knockdown daf-16 gene. Our results demonstrate that the reduction in glucose and triacylglycerides levels caused by (p-ClPhSe)$_2$ was lost in age-1/daf-16 worms. In conclusion, (p-ClPhSe)$_2$ can reduce glucose and triglycerides levels by modulating insulin/IGF-1 like signaling in an AGE-1/DAF-16 dependent-manner in *C. elegans*.
Keywords: *Organoselenium compounds, metabolism, C.elegans*

EA.07 - Glucocorticoids decrease the thermogenic capacity and increase the triacylglycerol synthesis by glycerokinase activation in brown adipose tissue of rats

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The maintenance of adequate triacylglycerol (TAG) stores is essential for normal brown adipose tissue (BAT) functioning and requires a continuous supply of glycerol-3-phosphate (G3P). This study aimed to investigate the effects of glucocorticoids on thermogenic capacity and in G3P generation pathways for TAG synthesis in interscapular brown adipose tissue (IBAT) of rats. Male Hannover rats received a single daily injection of dexamethasone (DEXA) (1 mg/Kg) or saline 0,9% during 7 days (CEUA protocol 195/2018). The mitochondrial proteins content, the temperature after noradrenaline stimulation, and noradrenaline content were measured in IBAT. The generation of G3P was evaluated by glycolysis, glyceroneogenesis, and direct phosphorylation of glycerol, respectively, by 2-deoxyglucose uptake, phosphoenolpyruvate carboxykinase (PEPCK) activity and pyruvate incorporation into TAG-glycerol, and glycerokinase (Gyk) activity and glycerol incorporation into TAG in IBAT. DEXA treatment increases the IBAT mass and lipid content probably by increasing the de novo fatty acid (FA) synthesis, evaluated by increased glucose-6-phosphate dehydrogenase and ATP citrate lyase activities (79% and 48% respectively), compared to control. DEXA increases the content (~55%) and activity (~41%) of Gyk, without affecting the glucose uptake and glyceroneogenesis. DEXA reduces the glycerol incorporation into TAG (~54%), the AQP7 content (~50%), and the rate of basal glycerol release (~54%) in IBAT. In addition, DEXA decreases the thermogenic capacity of IBAT, evidenced by a reduction in the content of mitochondrial proteins, including UCP-1, and the respiratory complexes, reduction in the noradrenaline content (53%), and the capacity of IBAT to increase the temperature after noradrenaline stimulation. Our data suggest that direct phosphorylation of glycerol by Gyk may be responsible for maintaining the supply of G3P for the increased esterification of FA and TAG synthesis in IBAT from DEXA-treated rats. The reduction of IBAT thermogenic capacity in these animals could be probably due to reduced sympathetic stimulation of IBAT. **Keywords:** Brown adipose tissue, Glucocorticoids, Glycerokinase

EA.08 - High-density lipoprotein remodeling associates with COVID-19 severity: a quantitative proteomic study

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Coronavirus disease 2019 (COVID-19) has caused more than 4 million deaths worldwide. Alterations in lipid profile, including lower levels of high-density lipoprotein (HDL)-cholesterol and high levels of triglycerides have been linked to disease severity. Although important for lipid metabolism, HDL may also play a role in immune response in infectious diseases. Due to its very complex protein composition, HDL proteome is altered in several diseases, including metabolic, inflammatory and infectious diseases. We used quantitative proteomics to test whether alterations in HDL proteome associate with COVID-19 severity. COVID-19 patients (n=41) were divided into two groups according to disease severity (hospitalized and non-hospitalized subjects). Levels of 29 HDL proteins were quantified by high resolution mass spectrometry. We showed levels of five proteins were increased by more than 50% in hospitalized patients when compared to non-hospitalized ones. Those proteins were serum amyloid A 1 and 2 (SAA1 and SAA2), pulmonary surfactant-associated protein B (SFTPB), apolipoprotein F (APOF) and inter-alpha-trypsin inhibitor heavy chain H4 (ITIH4). On the other hand, phospholipid transfer protein (PLTP) and apolipoproteins A2 (APOA2) and L1 (APOL1) were reduced by more than 30% in those same hospitalized patients. Apolipoprotein M (APOM) levels within HDL negatively associated with odds of death due to COVID-19. Furthermore, HDL proteins were able to classify COVID-19 subjects into those two groups (error rate of 5%). Our results indicate an inflammatory remodeling of HDL proteome which reflects the severity of COVID-19 infection and contribute to the putative role of HDL in infectious diseases.
Keywords: high-density lipoprotein, proteomics, COVID-19. **Supported by:** FAPESP

EA.09 - Metabolic characterization of whole, parotid and submandibular/sublingual saliva: a valuable tool for diagnostics

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Whole saliva is a mixture of different fluids secreted by various glands such as parotids and submandibular/sublingual. Since saliva is collectible by non-invasive procedures, a growing number of studies have identified specific salivary biomarkers associated with pathological and physiological alterations, promoting the development of non-invasive tools for diagnostics. (1,2) We collected three different types of saliva (whole; parotid and submandibular – sublingual), from 20 healthy volunteers and mapped every gland-specific metabolic composition. Saliva metabolites profiles have been obtained by 1H-NMR spectroscopy, adding a freeze-drying step in sample preparation. Metabolites quantification was performed using Chenomx and MestReNova software. Statistical analysis revealed a distinct profile for the whole saliva and an overlap for the parotid and the submandibular/sublingual saliva. We identified metabolites originated both from endogenous metabolism and from oral bacterial flora, whose concentration was higher in the whole saliva. By correlating the profile of the whole saliva metabolome with the periodontal health status scores, we identified a panel of 5 metabolites that can distinguish the initial phase of bleeding disease in the healthy population. The identified metabolites are related to oral bacterial metabolism, which could potentially induce a significant host response, including oral inflammation and gingival bleeding. (3) This tool could be used for the development of an early diagnostic tool in oral inflammation. References: [1]García-Villaescusa G., et al., Plos One, 1-12 (2018). [2]Lohavanichbutr P., et al., Plos One, 1-18 (2018). [3]Liebsch C, et al., J Dent Res, 642-651 (2019). **Keywords:** Saliva, 1H-NMR, diagnostics

EA.10 - Systemic investigation of TOR kinase: a strategic regulatory hub for biomass accumulation in sugarcane

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Sugarcane (*Saccharum* spp) is an important crop in the Brazilian sucroenergetic scenario. In the past two decades, there was an expressively advance in the knowledge behind the physiology and molecular biology of sugarcane motivated by the challenge of exploring the potential of cellulosic ethanol. Thus, since some molecular targets function as regulators of biomass accumulation, one can achieve efficient crop yield improvements through a systemic integration of biological sugar signaling and sensing machinery. A strategic regulatory hub in plants is the Target of Rapamycin (TOR) kinase that synchronizes growth according to the nutritional/energetic status and environmental inputs, triggering important changes in carbon flux through the central and energetic cellular metabolism. This work aimed at investigating the role of TOR in sugarcane by using an ATP-competitive inhibitor to track how does TOR inhibition alters the primary metabolism. There were performed a TOR inhibition experiment with *in vitro* seedlings obtained through axillary meristem of sugarcane SP80-33280 variety, which were individualized and treated with the drug AZD-8055 (25, 50, and 100 μM). Another strategy adopted to investigate this signaling pathway was through bioinformatic analysis to search and filter high-quality orthologous sequences in seven publicly available genomic/transcriptome sugarcane databases. TOR suppression in sugarcane did not impact the starch content and only a few amino acids had their levels altered after 12h of treatment, diverging from classical responses of model species. These retrieved 208 sequences from the TOR kinase complex, of which 69 showed their respective predicted protein domains. This project encompasses the guidelines of the National Institute of Science and Technology (INCT) of Bioethanol, which proposes to unveil the theoretical bases for carbon participation in the metabolic status of sugarcane, contributing to set new strategies allowing higher biomass and/or sucrose content.
Keywords: Biomass accumulation, Sugarcane, Target of Rapamycin
Supported by: CNPq (115313/2019-2; 142090/2018-2), CAPES PNPD (20131621-33002010156P0), and FAPESP (2014/50884-5; 2018/03764-5).

EA.11 - Project: Induction of the enzymatic and non-enzymatic antioxidant system by *Trichoderma* sp. in *Urochloa brizantha* cv. Piatã under salt stress

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Urochloa brizantha is a tropical grass species widely used as pasture for animals. Despite its adaptability, cultivation in soils with high levels of salts reduces its growth and productivity. Salinity contributes to oxidative stress, causing physiological changes and toxicity. The use of symbiotic microorganisms proved to be promising to mitigate effects generated by abiotic stresses, highlighting fungi of the *Trichoderma* genus. There is evidence that these fungi are producers of auxins, hormones that help tissues development, root and stem growth. In view of this, the work aims to evaluate the induction potential of the antioxidant system, germination and development of *U. brizantha* inoculated with *Trichoderma sp.* under conditions of salt stress. Seeds of *U. brizantha* will be sown and the inoculation of *Trichoderma sp.* will be simultaneously performed. The seedlings will be subjected to concentrations of 0 (control) and 75 mmol/L of NaCl and collected for analysis, 1, 7 and 14 days after the onset of salt treatment. The fungal root colonization will be determined by microscopy and plant growth will be estimated. Membrane damage will be evaluated by lipid peroxidation and percentage of electrolyte leakage. To evaluate the antioxidant system, the enzymes catalase, guaiacol peroxidase, superoxide dismutase and ascorbate peroxidase will be quantified by spectrophotometry, as well as non-enzymatic compounds ascorbic acid, tocopherols, glutathione. The content of auxins, proline, amino acids and soluble proteins will also be obtained by spectrophotometry. The activity of antioxidant enzymes will be analyzed by zymography with electrophoresis following specific staining for the gels of each enzyme. With the completion of the project, it is expected to evaluate the action of colonization of *Trichoderma sp.* in *U. brizantha* seedlings under conditions of salt stress on antioxidant responses, as well as investigating the production of auxins and determining their influence on the activity of antioxidant enzymes.

Keywords: Urochloa brizantha, Trichoderma, oxidative stress
Supported by: Fundação Cearense de Apoio ao Desenvolvimento Científico e Tecnológico

EA.12 - Fatty acid based *in vitro* model of Metabolism-Associated Fatty Liver Disease and its effect on oxidative stress biomarkers

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Metabolism-Associated Fatty Liver Disease (MAFLD) refers to a multisystem metabolic disorder, whose first stage is the accumulation of triacylglycerols within hepatocytes, known as hepatic steatosis. The progression of the disease is strictly associated with oxidative stress, which is defined by the imbalance between reactive species and the neutralizing capacity of defense systems. Among the antioxidant mechanisms, the one involving glutathione system is the main endogenous response and therefore a potential target for MAFLD. Thus, this study aimed to evaluate the efficiency and oxidative stress biomarkers of an *in vitro* model of MAFLD. To induce lipid accumulation, HepG2 cells were maintained in DMEM medium in absence of fetal bovine serum at 37ºC with 5% CO_2 for 24 hours, and then incubated with a solution of palmitic acid (0.7mM); or a mixture of oleic and palmitic acids (1.0 and 2.0 mM) in a 2:1 ratio, respectively, in the presence of albumin, for 24 hours. Cells were fixed with 4% paraformaldehyde, stained with Oil Red O and lipid content determined spectrophotometrically. The cytotoxicity was evaluated through the lactate dehydrogenase release method and the oxidative stress through the quantification of lipid peroxidation biomarkers by thiobarbituric acid reactive substances assay (TBARS) and total glutathione levels. All tested concentrations shown difference ($p < 0.001$) in intracellular lipid content compared to control, not resulting in cytotoxic effect. Also, no increase in lipid peroxidation was detected. However, reduced glutathione levels were observed in cells exposed to 1.0 ($p<0.05$) and 2.0mM ($p<0.01$) concentrations. The results indicate that the treatment with fatty acids is efficient in inducing steatosis *in vitro*, especially at 1.0 and 2.0mM. Despite not observing changes in TBARS levels, reduced GSH levels, indicate a depletion of the intracellular antioxidant system thus, this model is a valuable tool for preclinical study of MAFLD and treatment targets related to oxidative stress. **Keywords:** MAFLD, glutathione, hepatic steatosis.
Supported by: FAPEMIG, CAPES, UFU

EA.13 - Project Analyzes of MC4R variants in patients undergoing bariatric surgery

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Obesity is a public health problem that becomes a risk factor for numerous metabolic diseases, greatly impacting healthcare costs worldwide. It is influenced by diet, exercise, and a susceptible genotype in 50-75% of early-onset severe cases. Among the most prevalent monogenic forms of obesity are variants of the MC4R gene (Melanocortin-4 receptor), responsible for generating the transmembrane receptor, acting in the central pathway of appetite regulation. The present study aims to evaluate the coding region of the MC4R gene of 56 patients who underwent bariatric surgery at Hospital Tereza Ramos in the city of Lages-SC, from 2016 to 2018, investigating the genotype-phenotype relationship of carriers of gene variants. Amplifications of the genomic region of the MC4R gene from 56 individuals were carried out using PCR and gel electrophoresis, followed by Sanger sequencing. So far, 18% of the material has been sequenced. A male patient, who had hypertension and altered blood glucose, has the missense alteration V103I. Although bariatric surgery is the most efficient treatment of choice, there are studies in individuals with MC4R variants highlighting post-surgical weight loss failure. There are few data related to these variants in the Brazilian population, making it necessary to provide additional clinical follow-up to these patients, as well as to reduce the costs of ineffective treatments.

Keywords: MC4R, Obesity, Variant. **Supported by:** FAPESC, PAP 2020

EA.14 - Project: Biochemical and physiological characterization of the pequizeiro (Caryocar coriaceum Wittm.) submitted to saline stress.

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The pequi tree (Caryocar coriaceum) is an important plant in the Brazilian Northeast. Soil salinity is responsible for losses in agriculture and wild environments. Check if the pequi tree is resistant to salinity. The seedlings in this work will be produced by cuttings with different concentrations of auxin (50, 150, 250, 350, 450 µg/mL). The substrate for the cutting will be sand: earth: organic compost (1:2:1). Rooted, the plants will be cultivated for two months and submitted to three levels of salinity: 0 mM (control); 50 mM (NaCl) and 100 mM salt (NaCl). For responses to salt stress, gas exchanges are verified with the Li-Cor 6400 Equipment; chlorophyll fluorescence with the Li-Cor Equipment 6400-40 (Li-Cor Inc., Lincoln, NE, USA); the chlorophyll content in acetone (80%) will be measured in DMSO extracts; potassium and sodium ions will be measured by flame photometry; chlorine will be measured by titration with silver nitrate. Proline will be quantified by a protocol using 3% aqueous sulfosalicylic acid and absorption measured by a chromophore containing toluene; the soluble sugars in ethanol extracts will be determined by the reaction with phenol/H2SO4. The leaf anatomy will be by light microscopy with the help of software. For proteomic analysis and enzyme zymogram, proteins will be extracted in phosphate buffer (pH 7.0). The mass spectrometry will be with the Gel-free/label-free protocol that analyzes the total proteins without using a 2D gel and the zymogram will use the leaf zymography technique. The activities of catalase enzymes are through the destruction of hydrogen peroxide; of superoxide dismutase by the photoreduction of nitro blue tetrazolium; aspartate peroxidase will be measured by ascorbate consumption; the guaiacol peroxidase will be determined through the oxidation of guaiacol. The perspective is to create an orchard and check if the pequi tree is resistant to salinity.

Keywords: pequi, salinity, enzymes

EA.15 - PROJECT: Environmental contaminants with disruptor endocrine action - effects of neonatal phthalate exposure on metabolism

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According to the World Health Organization, the number of obese people has tripled since 1975 (WHO, 2020). Exposure to different endocrine disruptors during critical periods of development, such as pregnancy, lactation and adolescence, can influence this condition. There is a class of endocrine disruptors known as obesogenic, due to its ability to modulate the metabolism, contributing to the development not only of obesity, but also other metabolic disorders such as cardiovascular disease, thyroid dysfunction, diabetes and metabolic syndrome. Phthalates, derivatives of phthalic acid and alcohols, are present in polyvinyl chloride (PVC) film, hospital materials, cosmetics and consumer products. Phthalates is classified as obesogenic endocrine disruptors and its exposure is associated with hormonal, hepatic, renal and pulmonary alterations, as well as with the appearance of some tumors. Exposure to endocrine disruptors has been well described in adult animals and humans as harmful however in critical stages with great plasticity and susceptibility to external factors as lactation and phthalate exposure and its consequences is not elucidated. tt research the phthalates exposure (bis-(2-ethylhexyl) phthalate) during lactation by breast milk and its endocrine-metabolic consequences at short-term (weaning) and long-term (adulthood). Nutritional assessment, glycemic homeostasis, central adiposity, serum adipokines and hormone levels, hypothalamic regulation of energy homeostasis and lipid profile. Expected results: We hope to find an association between exposure during the critical phase of lactation and a possible initially altered metabolic profile, which would lead to obese profile of the adult life. Conclusion: We suggest that phthalate is a potential agent that would be associated with the onset of late obesity.

Keywords: phthalates, metabolism, obesity
Supported by: Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro - FAPERJ

EA.16 - Quantitative Metabolic Analysis of Alcoholic Fermentation in Brewing Process by Nuclear Magnetic Resonance

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Wort fermentation by yeast is the initial step at beer production and has a high impact on its flavor. Elucidate how the fermentation can be influenced by the source and the sugar concentration is interesting for the academy and industry. A complete map yeasts' synthesis and consumption can help in the fine adjustment of fermentation and be a tool to improve beer production yield. This project aims the detailed analysis of alcoholic fermentation of high sugar content to accurately identify the metabolites and analyze the correlations between synthesis and consumption of compounds with sensory interest in beer. *Saccharomyces cerevisiae* strains were inoculated in malt extract medium (15% sugar) at 20 ° C. The 1D ^{1}H NOESY and ^{1}H ^{13}C HSQC spectra were obtained on a Bruker Avance 500 MHz spectrometer for 3 days with intervals of approximately 2 hours between the series, and at intervals of 1, 7 and 14 days with variation of 3 initial inoculation conditions. The spectra were processed using the Topspin 3.5 software and the compounds were identified on the Colmar NMR platform and in the literature. The 1H NOESY and 13C 1H HSQC spectra were used using online metabolomic databases, the Chenomx program and previous work. We quantify the consumption of glucose and maltose as a function of time and relate it to the production of ethanol. Other molecules related to the fermentation processes were mapped and, in the case of acetate esters, their production was increased in the condition of greater yeast inoculum. The results indicate that NMR can be a strong allied for the efficient identification of substances in beer. Further investigations are underway to expand the identification and the relationship between the compounds produced during fermentation and the common variables of modern beer production, such as sugars, adjuvants and different yeast strains. **Keywords:** Alcoholic Fermentation, Yeast Metabolomic, Nuclear Magnetic Resonance

Supported by: Faperj

EA.17 - Modulations of respiratory parameters in glucose metabolism of C2C12 myoblasts and myotubes induced by capsaicin

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Capsaicin (CAP) is a selective agonist for the transient receptor potential vanilloid subtype 1 and gives pungent characteristics in the genus *Capsicum* plants. Previous studies show that CAP can modulate muscle energy metabolism. However, the molecular mechanisms of action of the CAP are not elucidated. Thus, the objective of this study is to investigate the effects of CAP in glucose metabolism of skeletal muscle. C2C12 myoblasts and myotubes were grown in DMEM-high glucose supplemented with fetal bovine serum and antibiotics. 24 hours after growth, the culture medium was changed for DMEM- high (HG, 25 mM), medium (MG, 15 mM), or low glucose (LG, 5.5 mM) and treated with CAP concentrations for 24 h or 48 h for the experiments. Cell viability was determined by MTT assay. Oxygen consumption rate was measured by high-resolution respirometry. After 24 h of treatment with CAP (25-400 µM), compared with a control (DMSO), only the 400 µM concentration was able to induce cytotoxicity in myoblasts, while in myotubes the reduction was already observed in 150 µM in HG. The treatment with CAP 25 µM for 24 h in myoblasts stimulated in the HG condition a significant increase in basal (65,70 ± 9,91 nmol O2/ml HG X 29,24 ± 8,54 nmol O2/ml LG), maximum (94.74 ± 15.16 nmol O2/ml HG X 43.74 ± 11.33 nmol O2/ml LG) and non-mitochondrial respiration (33.80 ± 9.93 nmol O2/ml HG X 7.95 ± 2.37 nmol O2/ml LG) of the cells compared with LG. In myotubes, different concentrations of glucose and treatment with CAP seem to alter only the maximum respiration, but now, this increase was observed in LG compared with HG treatment. Our results show that glucose has a key role in muscle energy metabolism and treatment with capsaicin can modulate cell respiration and may improve glucose signaling.
Keywords: capsaicin, skeletal muscle, glucose metabolism. **Supported by:** FAPERJ, CNPq

EA.18 - Overexpression of the argonaute ALG-1 induces miRNA biogenesis and promotes oxidative stress resistance in *C. elegans*

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Down-regulation of components of the miRNA biogenesis pathway has been associated with chronic noncommunicable diseases and aging. Argonautes are key proteins involved in miRNA synthesis/function and have been shown to play a role in metabolism and longevity. ALG-1 is one of the argonautes required for miRNA maturation and function in *C. elegans*. We sought to investigate the mechanisms controlling the expression and function of ALG-1 in *C. elegans* and test whether ALG-1 overexpression is sufficient to promote miRNA biogenesis, increase stress resistance and extend lifespan. Worms overexpressing ALG-1 (ALG-1/OE) were subjected to lifespan and oxidative stress resistance assays (i.e., paraquat or arsenite). To identify how alg-1 is transcriptionally regulated, we used modENCODE data to search for proteins that bind to alg-1 promoter. We also performed quantitative proteomic analysis and miRNA sequencing in ALG-1/OE worms. Moreover, using knock-out mutants of miRNAs overexpressed by ALG-1/OE, we evaluated the influence of those miRNAs in the oxidative stress response of *C. elegans*. ALG-1/OE worms showed more resistance to oxidative stress, despite a normal lifespan under unstressed conditions. Among the transcription regulators that bind to alg-1 promoter, we experimentally confirmed that GEI-2, NHR-28, NHR-77, R02D3.7 and SKN-1 suppress ALG-1 expression. Interestingly, silencing of nhr-28 and R02D3.7 promotes stress resistance in an ALG-1 dependent manner. Global miRNA expression is increased upon ALG-1 O/E, and among the upregulated miRNAs, 8 were also upregulated in long-lived, stress resistant glp-1 mutants, where ALG-1 is also increased. The proteomic analysis revealed differences in proteins related to nucleotide binding, reproduction and DNA mismatch repair – all processes commonly related to aging. Together, these results support the notion that ALG-1 expression can be dynamically modified to confer protection against oxidative stress, contributing to the general healthspan of *C. elegans*.
Keywords: miRNAs, Oxidative Stress, Metabolism; **Supported by:** FAPESP

EA.19 - Mechanical properties of mitochondria depend on cytoskeletal integrity

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Mitochondria are organelles involved in many cellular processes, such as ATP generation and the maintenance of cell homeostasis. The functional requirements of a cell correlate with changes in mitochondrial dynamics, form and positioning. Recent research demonstrated that the cytoskeleton and associated proteins play a crucial role in regulating mitochondrial dynamics, organisation and function. For instance, actin filaments facilitate mitochondrial fission, while microtubules act as tracks for mitochondrial transport mediated by molecular motors. Although the interactions between mitochondria and the cytoskeleton have been found to alter mitochondrial function, the mechanisms underlying this phenomenon are largely unknown. In this work we explore how the integrity of the cytoskeleton and the forces exerted by molecular motors associated to these networks affect the morphology and the mechanical properties of mitochondria. We use an experimental approach in which we analyze confocal microscopy images of mitochondria labeled with mitoTracker Deep Red in Xenopus laevis melanophore cells, both in control conditions or treated with latrunculin, a drug that depolymerizes the actin filaments. Using a filament tracking routine, we recovered the longitudinal coordinate of individual mitochondria from the confocal images and computed the tangent angle along the organelle shape. We calculated the ensemble average of the tangent angle persistence and found an exponential behaviour. We estimated the persistence length as the characteristic length of an exponential fitting of the data, this parameter is associated with the organelle's flexural rigidity and its capacity to deform. Our results show that mitochondria are less deformed in the absence of filamentous actin suggesting that contacts with this network are a key factor in the regulation of mitochondrial morphology and probably affect the organelle dynamics.

Keywords: cytoskeleton, mechanical properties, mitochondria
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EB - Photobiology, Optogenetics and Neural Systems

EB.01 - Melanoma cell migration in response to red and near-infrared low-level light

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Cell migration plays an important role in tissue formation and cancer progression. *In vitro* scratch assay has been used for many years to study cell migration to mimic the migration of in vivo cells, and, thus, to evaluate cancer growth. Low-level red and near-infrared light (LLL) can increase normal cell migration. However, the impact of LLL on tumor cells remains unclear. In this work, we aimed to evaluate the effects of a single LLL dose on melanoma cell migration. B16F10 (murine melanoma) cells were cultivated in RPMI medium with 10% of fetal bovine serum until they reached 80% confluency. The cell line was seeded in a 6-well plate at a density of $2x10^5$ cells/well in triplicate at two different moments. A wound scratch was performed to disrupt the confluent cell monolayer with a 10 µL pipette tip. Immediately after the injury, the cells were submitted to the LLL at two distinct wavelengths (660 and 780 nm) provided by a LED and a laser, respectively, delivering 3 different energies (1.3, 3.6, and 6 J) at an irradiance of 4.2 mW/cm^2. The control group was not irradiated. Cells were photographed immediately and at 3, 12, 24, and 36 h after the scratch. The wound closure was measured using ImageJ software. To evaluate the overall migration, we calculated the areas under the curve for each group. Cells exposed to the red laser at 6 J migrated slower than control. In contrast, LLL at 780 nm promoted faster cell migration when irradiated with 3.6 J. These results suggest that low-level LEDs at 660 nm could prevent melanoma progression in higher energies. However, 780 nm should be avoided at middle energies.

Keywords: Melanoma, Photobiomodulation therapy, Scratch-wound assay
Supported by: CNPq and CAPES

EB.02 - Mechanisms of membrane protection by deuterated PUFA

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Polyunsaturated fatty acids (PUFAs) constitute one of the most abundant and important components found in membrane bilayers. PUFAs stabilize protein complexes and modulate membrane properties, ensuring homeostasis of organelles and cells. However, PUFAs are highly susceptible to oxidative damage through lipid peroxidation (LPO) chain reaction which triggers atherosclerosis, cancer and neurodegenerative diseases. Selective hydrogen replacement of bis-allylic PUFA hydrogens by deuterium offers protection against LPO, but the protection mechanism is not fully understood. To understand the protection mechanism by deuterium substitution, Giant Unilamellar Vesicles (GUV) were prepared with H-Lin-PC, in the presence of small amounts of D-PUFAs. We analyzed photo-induced oxidation in the presence of 1µM of Al(III) Phthalocyanine tetrasulfonic acid chloride. The initial steps of the membrane oxidation, which consists of lipid hydroperoxidation by singlet oxygen, are characterized by fluctuations and area expansion of the GUVs. Membrane permeabilization results from further oxidation steps, forming lipid truncates aldehyds. We show that the presence of 20% of D-PUFA in the 80%of H-Lin-PC matrix of vesicles, prevents substantially the fluctuation/area increase, and the loss of contrast. The presence of tocopherol, following the same proportion of D2-PUFA-PC in H-Lin-PC, is effective in preventing the formation of pores/membrane permeabilization, however it does not inhibit the formation of hydroperoxides, resulting in area fluctuation and increase. These findings demonstrate that a small proportion of D-PUFAs is sufficient for the protection of both contact-dependent and contact-independent oxidation processes. Deuterium reinforced lipids offer membrane protection and the relief of the oxidative stress, mitigating several diseases.

Keywords: Lipid peroxidation, Giant Unilamellar vesicles, Deuterium reinforced lipids
Supported by: FUSP - RETROTOPE

EB.03 - Effects of photodynamic inactivation mediated by Zn(II) porphyrin on promastigote and amastigote forms of *Leishmania amazonensis*

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Photodynamic inactivation (PDI) has been attracting attention as an innovative technology to treat topical diseases, such as cutaneous leishmaniasis (CL) and infections caused by multidrug-resistant microorganisms. Zn(II) *meso*-tetrakis(*N*-n-hexylpyridinium-2-yl)porphyrin (ZnTnHex-2-PyP^{4+}) is a lipophilic water-soluble Zn(II) porphyrin with improved photophysical properties, high chemical stability, and cationic/amphiphilic character that can enhance its interaction with cells. Thus, this study aimed to investigate the PDI effects mediated by ZnTnHex-2-PyP^{4+} on *Leishmania amazonensis*. Confocal fluorescence microscopy was explored to study the interaction of ZnTnHex-2-PyP^{4+} with promastigotes. The PDI action was analyzed by cell membrane integrity, mitochondrial membrane potential (ΔΨm), and cell morphology. Promastigotes were incubated with ZnTnHex-2-PyP^{4+} for 5 min at 0.62 and 1.25 μM and irradiated by a LED (410 nm) for 1 or 3 min (2.3 and 3.4 J/cm^2, respectively). PDI on amastigotes and the cytotoxicity on macrophages were also analyzed (3.4 J/cm^2). Fluorescence microscopy revealed that parasites efficiently uptake ZnTnHex-2-PyP^{4+} and displayed a punctate labeling pattern along with the cytoplasm. An intense ΔΨm depolarization was also observed, which in association with microscopy results, suggests that ZnTnHex-2-PyP^{4+} may accumulate in the mitochondrion, or other well-defined structures close to it. Moreover, ZnTnHex-2-PyP^{4+} at concentration as low as 0.62 μM led to the immediate inactivation of >95% of promastigotes, regardless of the light dose used. Loss of the fusiform shape and plasma membrane wrinkling were also observed. After a single treatment session in amastigotes, PDI led to a reduction of 70% in the infection index. No considerable toxicity was observed on mammalian cells. Thus, PDI of *Leishmania* parasites showed *in vitro* efficiency at a submicromolar concentration of ZnTnHex-2-PyP^{4+}, with short pre-incubation and irradiation times. The results encourage further studies in CL pre-clinical assays and PDI of other microorganisms.

Keywords: Cutaneous leishmaniasis, photodynamic therapy, ZnTnHex-2-PyP4+
Supported by: CAPES, CNPq, FACEPE, FAPESP, IAM/FIOCRUZ, FINEP and INCT-INFo.

EB.04 - Blue light supports the aging of the skin of Swiss mice

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Solar irradiation causes skin aging by generating biomolecular damage and oxidized structure accumulations. Although the effects of ultraviolet (UV) light are well characterized, little is known about the effects of visible light. *In vitro* studies showed that UV-A and blue light stimulate redox processes with the generation of reactive oxygen species (ROS) and nitrogen (RNS) damaging skin cells. However, there are no in vivo studies that show the consequences of blue light on the skin and its association with UV-A. In addition, there is no evidence of factors that block or even minimize damage. The objective here was to verify the effects of blue light (465nm) and in synergy with UV-A (365nm) in the skin of Swiss mice. Animal experimentation was approved by the Animal Use Ethics Committee (CEUA – 613296), with seven groups (G) (8 animals/group) divided into G0 (control group), G1 (UV-A), G2 (blue light) and G3 (UV-A+blue light). Groups G4, G5 and G6 followed the same order, but were added sunscreen (PP Photo Ultra ISDIN active unify 99) before each irradiation cycle in BlackBox Smart equipment (BioLambda – SP) with UV-A and blue doses, 20J/cm² and 100J/cm² respectively. All animals underwent dorsal trichotomy before irradiation, with subsequent photographic recording and biopsy in 3 times: zero, fifth and tenth irradiation. Statistics was performed by Origin 8.0. Through macro and microscopic analysis (HE technique at 40x magnification) it was noted that groups irradiated without PP, showed greater hyperpigmentation of the skin, indicative of melanogenesis. G3 stands out, in which 50% of the animals showed hyperkeratosis and 12% elastosis. Furthermore, accumulations were observed in G2 (blue light), suggestive of lipofuscin or hemosiderin. Therefore, blue light was more harmful and accelerated the aging process, especially when associated with UV-A.
Keywords: Blue light, photoaging, Swiss mice
Supported by: Fundação de Amparo a Pesquisa e Inovação do Espírito Santo - FAPES

EB.05 - Sun-induced modifications on collagen molecular structure explored by fluorescence spectroscopy and advanced microscopy techniques

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UV light is known to induce dangerous modifications on various biological molecule, which are linked to onset of pathological conditions. Since collagen is a structural protein ubiquitous in all human body, exploring the modifications induced by sun radiation and particularly focusing on UV range, is of crucial interest. We here present an experimental study based on steady state fluorescence spectroscopy and time resolved non-linear optical imaging aimed at characterizing collagen modifications at molecular level. Collagen was exposed to single wavelength UV lamp radiation (270 nm) and to solar simulator which emits a radiation with similar characteristic to the one Sun produces in term of spectrum and power. The results were a reduction of auto-fluorescence signal, attributed to the aromatic residues and to cross-links, and a decrease of the turbidity of samples, this highlighting an overall loss of supramolecular structure, associated to a solubility increase. CD spectra also reveal structural modifications which we investigate more in depth using 8-Anilino-1-naphthalenesulfonic acid (ANS). ANS is a fluorescent dye known to bind to collagen fibers and whose fluorescence is affected by its molecular environment, and in particular its hydrophobicity. We carried out fluorescence lifetime imaging (FLIM) measurements of ANS-stained samples, analyzed by phasor approach to observe alterations in the molecular structure of collagen triple helix. As already hypothesized from ANS steady state fluorescence measurement, FLIM analysis confirm a reduction of binding sites affinity, showing a high grade of lifetime distributions heterogeneity and an overall reduction of the ANS fluorescence lifetime. Moreover, since we observe that irradiated collagen isn't able to self-organize in fibers, we conclude that solar radiation degrades the hydrophobic terminals of collagen triple helix, vital for its fibrillogenesis, and that ANS provide a useful and simple tool to investigate them.
Keywords: Collagen, UV-light, FLIM

EB.06 - Evaluating Re(I) complexes as anticancer agent

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In recent decades, many organometallic compounds (ie, compounds containing at least one metal-carbon bond) have been extremely promising as candidates for anticancer drugs. Rhenium compounds have several intrinsic advantageous properties for the development of new anticancer drug candidates. Considering that some Re complexes have shown to be promising as candidates for anticancer agents, but have been little explored. We selected these 3 chemical structures of Re complexes, whose ligands are based on phenanthroline and lipophilicity increases as a ring is added to the ligand, to study their therapeutic potential and evaluate the interaction of these compounds with biomolecules. In addition, we also propose the use of nanocarriers to improve the therapeutic effect of the compounds. Study the properties of Rhenium (I) and its ability to interact with biomolecules, as well as evaluate its toxicity before and after encapsulated in polymeric nanospheres. Fluorescence spectroscopy was used to investigate the interaction of the Rhenium(I) complex with the biomolecules. For encapsulation of compounds, the polymeric system chosen was carboxymethylcellulose and apple pectin. The technique used consists in coacervation, which occurs the separation of phases in a colloidal solution under certain conditions, i.e. temperature, pH and solubility of the dissolution medium. The interaction studies between Rhenium complexes and BSA analyzed by Stern-Volmer treatment, indicated that as more hydrophobic is the compound stronger is the interaction with protein. Also, nanocarriers composed of carboxymethylcellulose or apple pectin has been successfully prepared by coacervation proposed method, but some characterizations are still ongoing. Our tested compounds can be useful as anticancer agents, since preliminar data suggest stronger interaction with biomolecule. However, further studies are still ongoing for solid conclusion.
Keywords: Rhenium (I) complexes, biomolecule interaction, nanocarrier systems

EB.07 - Effect of membrane deformation on electrical firing in rat cortical neurons during electrophysiological measurements

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From patch-clamp to 3D nanoelectrodes, tight mechanical coupling with the neuronal membrane is essential to secure the high amplitude electrical recording. As a byproduct, both approaches induce membrane deformation. In the former, the membrane is acutely deformed by the glass pipette, while in the latter, the membrane spontaneously engulfs the 3D vertical nanostructure. In line with the discoveries pointing to the existence of mechanosensitive ion channels in neurons, we combined electrophysiology with functional imaging to test the effect of acute and chronic membrane deformation on rat cortical neurons' electrical properties and firing dynamics. To estimate the effect of patch-clamp induced acute deformation, we combined semi-blind patch-clamp with calcium-imaging. Additionally, we utilized patch-clamp to investigate whether the long-term exposure to vertical topology on 3D nanoelectrodes influences the neurons' electrical properties. All measurements were performed on rat cortical neurons starting from 2 weeks in culture. Calcium-imaging measurements during the formation of giga-seal have demonstrated that patch-clamp targeted neurons respond to the mechanical perturbation with plateau-shaped calcium signals (N = 29). Moreover, up to 100% of neurons in 0.185 mm^2 area responded in a similar trend. This finding suggests that acute deformation affects not only the targeted neuron, but also the immediate network. Furthermore, the comparison of neurons on flat surface and neurons on 3D nanoelectrodes showed no statistically significant difference in excitability and action potential firing. Overall, these results recognize the effects of acute, patch-clamp mediated mechanical perturbation on the targeted neuron, as well as the immediate network. On the other hand, no changes were present with chronic membrane deformations during the spontaneous engulfment of vertical nanostructures.
Keywords: electrophysiology, membrane, neurons

EB.08 - Modulators of dopaminergic neurotransmission: effects on the behavior and expression of receptors in an experimental model of Epilepsy

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Epilepsies are Central Nervous System disorders characterized by irregular and recurrent electrical discharges and, frequently, epileptic patients are also affected by neuropsychiatric comorbidities, such as depression and anxiety. Both Epilepsy and comorbidities may be related to changes in dopaminergic neurotransmission. In the context of drug repositioning, we investigated behavioral and biochemical alterations induced by modulators of dopaminergic pathways in an experimental model of Epilepsy – Wistar Audiogenic Rats (WARs). Male WAR and Wistar rats were divided in four groups: Levodopa/Carbidopa (LC, 30 and 15 mg/kg), Levodopa/Benserazide (LB, 30 and 2 mg/kg), Haloperidol (H, 0.3 mg/kg) and control group (S, 0.9% saline). Doses were administered for 14 days, once a day, according to the protocol approved by Ethics Committee – 233/2019. Rotarod and open field tests were used to evaluate motor and exploratory behaviors. A significant decrease in the first fall latency in rotarod was observed in S/WAR group when compared to the S/Wistar group. Treatments with LC, LB and H in WARs attenuated the first fall latency when compared to S/WAR group. In the open field test, a significant decrease in the mobility rate, locomotion speed and total distance was observed in S/WAR group when compared to the S/Wistar group. No changes were observed in these parameters in all WAR treatment group when compared to the S/WAR groups. Interestingly, a significant increase in the type 1 dopamine receptor (DR1) expression was observed in S/WAR group when compared to the S/Wistar group. In addition, a decrease in DR1 expression was also observed in all WAR groups treated with the modulators when compared to the S/WAR groups. In summary, our treatments were able to change behavioral aspects in WAR animals when compared to Wistar animals, also modifying the expression of receptors related to dopaminergic neurotransmission in the WAR model.
Keywords: dopamine, behavior, drug repositioning; **Supported by:** FAPEMIG, CNPq and CAPES

EB.09 - Characterization of the effects of Photodynamic therapy on membrane mimetics composed by 1,2-Dioleoyl-sn-glycero-3-phospho-rac-(1-glycerol) sodium salt

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Photodynamic therapy (PDT) is an important technique that uses chemical compounds called photosensitizers to oxidize unsaturated bonds in organic molecules in the presence of light.To better understand the response of bacteria membranes to induced photodamage, we used GUVs (Giant Unilamellar Vesicles) as a simple model of its membranes, where DOPG (1,2-Dioleoyl-sn-glycero-3-phospho-rac-(1-glycerol) sodium salt) is one of the main lipids on bacteria membrane. In order to understand the effects of oxidative stress induced in the membrane of bacterias, GUVs composed by DOPG are made and subjected to light irradiation with wavelength of the order of 655 nm in the presence of two different isomer photosensitizers (PS): Methylene Blue (MB) and Dimethyl Methylene Blue (DMMB). The objective is to compare the effect of these two photosensitizers and analyze qualitatively its effects on the liposome. Phase-contrast microscopy was the principal technique used in this work. It guarantees time resolution images about de GUVs. With the images, we extract information about the contrast between internal and external parts, being able to observe fluctuations of the liposome area, loss of contrast, poration, etc. Irradiation of DOPG GUVs in the presence of DMMB resulted in the contrast loss and changes in liposome area, getting smaller than before. In general, this effect was associated with a bud emission. MB had a similar effect, but the time demanded for this was greater than for its isomer. To conclude, we consider that a better understanding of the action mechanisms of the different PS can improve the use of PDT to treat a variety of diseases. DMMB seems to be more efficient than MB in allowing the lipid bilayer to be permeated and then broken down, on the other hand, there are still many other factors to be evaluated when talking about effectiveness in implementing this for the treatment of diseases.
Keywords: DOPG, membrane mimetics, Photodynamic therapy
Supported by: conselho nacional de desenvolvimento científico e tecnológico (CNPq)

EB.10 - Highly fluorescent nanoprobe based on quantum dots and *Enterolobium contortisiliquum* trypsin inhibitor (EcTI)

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Serine proteases play crucial roles in life maintenance, and their activities are controlled through their inhibitors. The dysregulation in this activation/deactivation mechanism can trigger diseases. EcTI is a serine protease inhibitor purified from *Enterolobium contortisiliquum* seeds. EcTI has high biotechnological potential, showing anti-cancer, anti-inflammatory, larvicide, and insecticide activities. Currently, fluorescent nanoprobes, able to providing information at molecular levels, are being increasingly requested. In this regard, the quantum dots (QDs) stand out due to their unique optical properties. Thus, a nanotool consisted of between QDs and EcTI can provide valuable information on the location and interaction of specific serine proteases in biological systems by fluorescence techniques. To develop a highly fluorescent nanoprobe based on the conjugation of QDs and EcTI for studies of the interaction with serine proteases in biological processes. Carboxyl QDs were synthesized and optically characterized, then covalently conjugated to EcTI. Conjugates were characterized by absorption and emission spectroscopy. The conjugation efficiency was evaluated through fluorescence correlation spectroscopy (FCS) and fluorescent microplate assay (FMA). The QDs-EcTI conjugate presented high fluorescence, and its absorption spectra did not present noteworthy changes compared to bare QDs. Using FCS, a shorter diffusion time (135.87 ± 13.77 μs) was obtained for bare QDs compared with the QDs-EcTI conjugate (529.14 ± 46.90 μs). From diffusion times, hydrodynamic sizes were estimated as 3.4 ± 0.3 nm and 13.1 ± 1.2 nm for bare QDs and QD-EcTI conjugates, respectively. Moreover, from FMA a relative fluorescence of 3,814.8% ± 533.8% was obtained compared to controls (only QDs or EcTI). EcTI was effectively conjugated to QDs and the proposed conjugate can be a potential nanotool for studies involving serine proteases in biological systems through fluorescence-based techniques. **Keywords:** quantum dots, protease inhibitor, fluorescence. **Supported by:** CAPES, CNPq, FACEPE, INCT-INFo, and LARNANO-UFPE

EB.11 - Molecular dynamics simulations and *in vitro* assay of POPC membrane models in the presence of phenothiazinium photosensitizers

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Photodynamic therapy (PDT) treatment have been widely investigated in order to understand the photophysical mechanisms. PDT has been used for skin diseases, inactivation of virus and cancer treatment. In general PDT involves a photosensitizer (PS) molecule that it can be activate after light irradiation and generate radical species that it causes several effects in the target. In this way cell membranes composed by proteins and lipids are the major targets for photoinduced cell inactivation. After light irradiation, the free radicals and contact dependent reactions by PSs can produces oxidized lipids through lipid peroxidation process. The formation of these species can lead to membrane damage and causes cell death. To perform *in vitro* and theorical studies to analyzed PSs photochemical properties and membrane damage effects. Preparation of SUVs and GUVs vesicles with POPC lipid and molecular dynamics (MD) simulation using Gromacs software. Experiments performed in small unillamelar vesicles (SUVs) and giant unilamellar vesicles (GUVs), with a POPC (unsaturated lipid), showed that MB, DO15, DO16 and DO37 cause membrane damage in a small concentration, (1 μM), after light irradiation (630 nm). To better understand the results found in the experimental tests, we performed the molecular dynamics (MD) simulation of the PSs described above in a POPC membrane model. MD studies reinforce the importance of PS interaction with the cell membrane in PDT efficiency. As with the experimental results, the simulation suggests that DO15 is more efficient in generating damage to the cell membrane. Due to its proximity to the double bond of lipids, by a contact-dependent mechanism, followed by DO16, DO37, and finally MB. All compounds entered the membrane, and those that came closer to the double bond, internalized perpendicularly to the membrane with the piperidine ring coming close to the lipid double bond, suggesting that the site of action is in this region. **Keywords:** Photodynamic therapy, Photosensitizers, Membrane damage. **Supported by:** Fapesp

EB.12 - Reconstitution of Leishmania plasma membrane to understand the photodynamic effect

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Leishmaniasis is an important neglected disease. Photodynamic therapy (PDT) has been used to fight cutaneous leishmaniasis showing good results. However, PDT mechanisms in Leishmania parasites are not yet completely clarified. In this work, our objective was to develop a protocol to produce giant plasma membrane vesicles (GPMVs) from *Leishmania amazonensis* promastigotes to understand the mechanisms of action of methylene blue (MB)-mediated PDT on the cell membrane of parasites. For membrane extraction, several techniques were tested. The osmotic shock was the technique that presented the best yield and effectiveness. Phosphate and protein measurements were performed to confirm membrane extraction. For the growth of GPMVs, the best technique was electroforming using different frequencies and voltages in 4 cycles. Reconstituted GPMVs were observed by phase-contrast light microscopy. Subsequently, PDT was applied to GPMVs dispersed in an aqueous solution containing 50 μM MB and we verified the changes in permeability before and after exposure to light. The same process was applied to giant unilamellar vesicles (GUVs) with lipid compositions similar to the parasite membrane. The electroforming technique with the protocol developed in this work made it possible to obtain GPMVs from a promastigote membrane isolate of *L. amazonensis*. The membrane isolation technique was effective to extract the parasite's membrane while preserving lipids and proteins. In GUVs we observe an increase in the area during PDT in different compositions and loss of contrast. The GPMVs showed a loss of contrast as well as the GUVs but did not show an increase in area. This factor could be explained by the high degree of complexity of the membrane, which contains membrane proteins in addition to containing lipids.
Keywords: *Leishmania amazonensis*, GPMVs (Giant Plasma Membrane Vesicles), PDT (Photodynamic Therapy)

EB.13 - Enhanced action of nanoencapsulated herbicide on photosynthesis and antioxidant activity in spinach leaves: toward a sustained weed control?

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Despite a wide range of possible applications of nano-enabled pesticides, the mechanisms involved in their enhanced action remain largely unknown. Understanding the interaction between nanopesticides and plants is crucial for evaluating their potential safety application. Using an experimental and theoretical approach, this study aimed to investigate the target effect of paraquat-loaded chitosan/tripolyphosphate nanoparticles on the photosystem I (PSI). Chitosan/tripolyphosphate nanoparticles carrying paraquat was prepared by ionic gelation method. Nanoformulation was characterized, and the amount of lipid peroxidation, photooxidizable P700 reaction center content, NADPH/NADP+ ratio levels, and antioxidant enzymes were evaluated in spinach leaf tissue exposed to the nanoherbicide compared to the non-encapsulated herbicide. Biochemical traits of PSI were significantly decreased in spinach leaf tissue exposed to the nanoherbicide. Our data also revealed that nanoformulation might act promoting oxidative stress by changes observed on antioxidant enzymes. Also, the molecular docking results showed a preferential disposition of the herbicide paraquat and paraquat-tripolyphosphate complex (TPP:PQ) into the ligand domain close to FAD and Glu312. Due to the inhibitor's strategic position into the catalytic pocket, a model of electron-capture is proposed, where the herbicide disturbs the redox process NADP+ ⇌ NADPH by capturing electrons to reduce itself. Our findings provide important insights into changes induced on targeted action mechanisms may play a key role in its increased herbicidal efficiency. Thus, our findings contribute to a better understanding of the mode of action of herbicides encapsulated in polymeric nanoparticles.
Keywords: Nano-enabled agrochemicals, Enzymes, Photosynthetic electron transport
Supported by: CAPES

EB.14 - Red LED irradiation impacts the cytotoxic response of murine breast cancer cells to ionizing radiation

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Breast cancer is a disease of worldwide importance since it is considered the 5th leading cause of cancer deaths. Triple-negative breast cancer (TNBC) is a molecular subtype that presents resistance to conventional radiotherapy, demanding high doses of ionizing radiation (IR) for a prolonged period of treatment. On the other hand, low-level light irradiation (LLLI) has been studied to sensitize cells before IR exposure. However, the literature is poor regarding the association of both techniques in TNBC cells. Thus, we aimed to assess the effect of LLLI before IR exposure on two TNBC cell lineages. MDA-MB-231 (human TNBC) and 4T1 (murine TNBC) were cultivated, seeded at a density of 2.5 x 10^5 cells/cm², and maintained in an incubator (37ºC, 5% of CO_2) overnight. LLLI was performed with a red LED (λ= 660 ± 11 nm, 38.2 mW/cm²) delivering energies of 1.2 J and 6.0 J. One-h after LLLI, the cells were submitted to both 2.5 and 5.0 Gy doses from a ^{60}CO source. After 24-h, mitochondrial activity (MA) was quantified by MTT assay with n= 9/group. Our data showed that 4T1 cells exposed to LLLI at 1.2 J exhibited higher MA than cells exposed to IR2.5. In contrast, cells exposed to 6 J of LLLI showed lower MA than IR5. Concerning MDA-MB231 cells, no statistically significant differences were noticed among groups regardless of IR and LLLI doses. These findings indicate that LLLI before IR could sensitize only murine breast cancer. Besides, an appropriate combination of IR and LLLI doses seems to play a role to kill TNBC cells.

Keywords: Photobiology, radiotherapy, radiomodifier
Supported by: CAPES, CNPq, CNEN

EB.15 - Fetuin detection by a promising optical-magnetic multimodal nanoprobe functionalized with Cramoll lectin

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Many researchers are seeking to develop smart nanomaterials due to the diversity of potential applications. Bimodal nanoprobes (BNPs) have gained attention, especially those consisted of quantum dots (QDs) and iron oxide nanoparticles (SPIONs), due to the possibility of combining the advantageous superparamagnetic response of SPIONs and the singular optical properties of QDs. Furthermore, BNPs can be conjugated with biomolecules, such as Cramoll lectin, a glucose/mannose-binding protein purified from *Cratylia mollis* seeds, to become site-specific. To obtain a multimodal system (BNPs-Cramoll) to detect the fetuin glycoprotein whose levels may be altered in pathologies. Carboxyl-coated QDs were covalently combined to aminated SPIONs, and then BNPs were conjugated with Cramoll. The optical properties and the zeta potential of nanosystems were determined. *Candida albicans* yeasts were incubated with BNPs-Cramoll and analyzed through fluorescence microscopy and flow cytometry to evaluate the specificity/efficiency of the nanoprobe. Fetuin detection was performed through fluorimetry. The QD absorption band was absent in the supernatant of BNPs, indicating effective conjugation with SPIONs. There was a redshift in the maximum emission of BNPs compared to bare QDs; lectin conjugation did not cause a spectral shift. BNPs-Cramoll had a less negative surface charge than BNPs. Approximately 90% of yeast cells were homogeneously labeled by BNPs-Cramoll and after inhibition with methyl-α-D-mannopyranoside, a labeling reduction of *ca.* 3x was observed. When incubated with different concentrations of fetuin (0.675-10.8 mg/mL), a linear decay in the BNPs-Cramoll fluorescence was identified. Incubation with bovine serum albumin (control) did not significantly decrease the fluorescence. BNPs-Cramoll showed to be a specific fluorescent-magnetic nanoprobe able to detect fetuin, promising for the biosensing of this glycoprotein.

Keywords: quantum dot, magnetic nanoparticle, lectin
Supported by: CAPES, CNPq, FACEPE, INCT-INFo, and LARNANO-UFPE.

EB.16 - Interaction of ruthenium complex with biomolecules and its outcomes of photodynamic efficiency

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Photodynamic therapy (PDT) is related to two non-toxic actors, which are combined to induce cellular and tissue effects in an oxygen-dependent manner. The main actors are the photosensitizer (PS), light absorption and oxygen, which together generate reactive oxygen species (ROS) to inactivate undesirable cells. The PSs approved by FDA involve compounds from the classes of porphyrin, chlorins and phthalocyanines. Recently, metal complexes and especially polypyridine Ru(II) complexes have received increasing attention due to their intriguing photophysical and biological properties. TLD-1433 Ru(II) complex is the first inorganic complex (not belonging to the classes of porphyrins, chlorins and phthalocyanines) to enter phase II clinical trials as an PS for PDT against bladder cancer. Study properties of Rubpy and Rubpe and their ability to bind and photooxidize biomolecules in order to correlate the PS-biomolecule interaction and photodynamic efficiency. UV-Vis spectrophotometer was used to study properties of PS such as absorption spectra in organic solvent and aqueous solution as well as determination of pka values. Fluorescence spectroscopy was used to evaluate interaction of Rubpe and Rubpy with lipids (liposomes made of DMPC) and proteins (BSA). Rubpe exhibited binding constant (Kb) value 2.5 times more than Rubpy in liposomes made of DMPC. Also, the interaction of Rubpe with BSA (protein) were larger compared to Rubpy. Among the 2 PS studied, one of them presents an ethylene group separating two pyridine rings, providing more hydrophobicity to the compound which probably increase PS interaction to biomolecules. Studies of photosensitization eukariotic cells with both compounds are ongoing in order to correlate the stronger interaction of Rubpe and the phototoxicity. The ethylene group added between the pyridine rings increases the ability of PS to interact and photo-oxidize biomolecules such as lipid and protein. It may improve photodynamic effects.

Keywords: Photodynamic Therapy, Ruthenium complex, biomolecule interaction

EB.17 - How to make protoporphyrin IX more efficient?

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Protoporphyrin IX (PpIX) is a derivative of ALA (5-aminolevulic acid) an intrinsic photosensitizer (PS) of the human body. PpIX is highly fluorescent, however it photobleaches rapidly under production of singlet oxygen[a] which makes PpIX an efficient FS used in photochemotherapy. The affinity of PpIX for diseased tissues can be improved by the use of carriers[b]. To study the interaction between PpIX and Bovine Serum Albumin (BSA) to be used as a carrier justified by its similarity with Human Serum Albumin (HSA) that has affinity to cancer cells[b]. Optical absorption and fluorescence emission spectroscopy, particle size and zeta potential were performed to characterize PpIX and its complexes with BSA. Langmuir monolayers containing Dipalmitoylphosphatidylcholine (DPPC) were used as a model to study the interaction between both PpIX and PpIX-BSA complex with biological membranes. All experiments were carried out in acidic (4.5) and physiological (7.3) pH buffer solutions. Changes in the profile of the optical absorption spectra and fluorescence emission show that BSA binds to PpIX. These photophysical changes do not impair PpIX photochemical efficiency. Particle size measures showed the formation PpIX-BSA complexes depending on the BSA concentration with sizes ranging from 5-1000 nm at pH 4.5 and from 3-400 nm at pH 7.3. The formation of the complex was favored at pH 4.5, close to the BSA isoelectric point. Surface pressure (π–A) curves measured through the Langmuir monolayers show that both porphyrin and its albumin complex interact with the DPPC monolayer. PpIX binds to BSA with enhanced photophysical properties. Both PpIX and PpIX-BSA interact with the cell membrane model suggesting enhanced performance of PpIX as PS. a LEE, H.-S.; LEE, J.-B.; YUN, S. J.; et al. Spectrofluorometric Determination of Protoporphyrin IX in Cells Using Acridine as Internal Standard. Bull. Korean Chem. Soc., v. 27, n. 7, p. 1067–1070, 2007. b ELSADEK, B.; KRATZ, F. Impact of albumin on drug delivery — New applications on the horizon. Journal of Controlled Release, v. 157, n. 1, p. 4–28, jan. 2012.

Keywords: Protoporphyrin XI, BSA, Langmuir Monolayer.
Supported by: FAPESP, CNPq and CAPES

EB.18 - Photodynamic therapy associated with ionizing radiation in the treatment of triple-negative breast cancer cells

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Breast cancer is the most common cancer for women worldwide. According to the World Health Organization, it is considered the 5 th leading cause of death from cancer. Triple-negative breast cancer (TNBC) is a subtype of this disease that represents around 20% of all invasive breast cancer, whose main characteristics are resistance to conventional treatments, such as exposure to ionizing radiation (IR). On the order hand, the photodynamic therapy (PDT) using porphyrins and their derivatives has been described in the literature as a potential therapy against cancer. Thus, our goal in this work was to associate PDT and IR in the treatment of TNBC. MDA-MB-231 cells at a concentration of 2x104 cells were submitted to PDT using TMPyP porphyrin (30 μM) and a red light (660 ±11 nm) with fluences of the 23 and 57.5 J/cm 2 (57.3 mW/cm 2). Immediately post-PDT, cells were divided into groups: non-treated (control), only IR and PDT associated with IR (PDT57+IR and PDT23+IR) and then, exposed to IR with a dose of 2.5 Gy. Past 24-h of the PDT-session, the cell viability, clonogenicity and total glutathione were verified. Cells exposed to IR not presented statistically significance difference compared to the control group. However, treated groups showed around 38% lower cell viability in relation to the control and IR groups. For the clonogenic assay a reduction of the approximately 65% was observed between IR and treated groups. Regarding to the total glutathione, all groups showed an increase when compared to control group. Nonetheless, no were identified differences between IR and treated groups. Taken together, our results indicate that PDT associate with IR may be an ally in TNBC treatment.

Keywords: radiotherapy, combined therapy, cancer
Supported by: CNPQ

EB.19 - Combination Therapy of Antimicrobial Photodynamic Inactivation: Potential of Nanoparticles and Plant Based Compounds

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The multidrug resistance of pathogenic bacteria has become a serious problem to public health, finding novel approaches to combat multidrug resistant bacteria have therefore become increasingly important. Microbes in biofilm form can tolerate higher levels of antibiotics than their planktonic form. An “ideal” anti-biofilm agent should be able to penetrate the matrix and/or to inhibit/interfere with its accumulation; combined with the ability to recruit immune cells and/or modulate the host immune response would be an added value. One promising approach is antimicrobial photodynamic therapy (APDT) which involves the use of photosensitizer (nontoxic dyes) that are excited by visible light and produce oxygen free radicals in the presence of oxygen. APDT can be combined with other agents or drugs, improving the overall result while reducing individual concentrations and avoiding host tissue damaging. Among these options nano formulations of photosensitizer or using nano vehicle for drug delivery got enormous interest and advance in the recent. Also polyphenol compounds from plants showed antibacterial activity against different pathogen which could be consider as an adjuvant in APDT. In our work, after performing MTT assay (to evaluate photosensitizer toxicity on human fibroblast cells), the effect of APDT on bacteria in the planktonic and biofilm forms were investigated. We combined APDT based methylene blue as photosensitizer with polyphenols or used curcumin nano particle as photosensitizer. Our result showed that there was a reduction in the number of bacteria in planktonic condition, bacterial biofilm production and also enhance in destruction of the biofilm in combination mode compared to single mode. Therefore, combination therapy with APDT could be suggested as novel approach in the treatment of multi drug resistant bacteria in chronic ulcer condition.

Keywords: Antimicrobial photodynamic inactivation, Combination therapy, Nano particles

EC - Redox Processes

EC.01 - Plasmalogens pro-oxidant action by generation of excited singlet molecular $O_2(^1\Delta g)$ in the dark

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Plasmalogens are glycerophospholipid with a vinyl–ether linkage at the sn-1 position of the glycerol backbone. Even though they are found in all human tissues, being especially abundant in the brain and heart, the biological role of plasmalogens remains unclear. It has been suggested that plasmalogens are antioxidants because of the high reactivity of their vinyl ether groups with reactive oxygen species (ROS). However, plasmalogen reaction with singlet molecular oxygen (O2 (1Δg) can produce two primary unstable oxidation products, a hydroperoxide and a short-lived dioxetane intermediate whose decomposition can produce O2 (1Δg) by transferring energy to triplet molecular oxygen. Herein, we describe evidences of the generation of O2 (1Δg) by chemical trapping and monomol IR light emission at 1,270 nm. We have also characterized the main O2 (1Δg)-oxidation products of phosphatidylethanolamine plasmalogen (pPE) by high resolution tandem mass spectrometry (ESI-MS/MS). The results from mass spectrometry confirms the formation of pPE hydroperoxides and unstable dioxetanes, which were degraded to formyl phosphoethanolamine (formyl-PE), lyso-PE and fatty aldehydes with 15 and 17 carbons. These findings demonstrate that although plasmalogens are considered antioxidants, they can act as a pro-oxidant by promoting singlet molecular oxygen generation in the dark.
Keywords: photochemistry in the dark, Plamalogen, singlet molecular oxygen
Supported by: FAPESP

EC.02 - The contrast agent 2,3,5-triiodobenzoic acid (TIBA) induces cell death in tumor cells through the generation of reactive oxygen species

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TIBA is an iodine contrast agents used for diagnosis of tissue structures in X-ray techniques. However, its ionic form increases blood osmolarity, generating physiological complications such as contrast-induced nephropathies (CIN). The CIN leads to an increase in the generation of reactive oxygen species (ROS) in tubules and renal epithelium, causing the death of these cells. An antitumor activity of TIBA has been described in the non-small cell lung cancer (H460). In this model, TIBA induced cell death for mitochondrial intrinsic pathway, in considerably lower concentrations than iodinated contrast agents used in the clinic. But the subcellular mechanisms involved in TIBA-induced cell death are still unknown. Thus, the objective of this work was to evaluate whether the anti-tumor activity of TIBA involves ROS increase, in cell lines of non-small cell lung cancer (H460), chronic myeloid leukemia (K562), and its cytotoxicity in normal renal epithelial (VERO). The MTT assay was used for evaluation of cell viability, the fluorescent probe H2DCFDA to evaluate ROS induction, cell cycle analysis using flow cytometry to measure cell death, and immunofluorescence with annexin/7-AAD, to assess the association of cell death with the ROS generation. TIBA decreases cell viability in a dose-dependent manner for the H460 and K562. However, VERO cells showed less response to the drug, with 70% viable cells after 72 hours of treatment in the highest concentration of the drug. And the tumor cells with only 20% viable cells. Thus, tumor cells exhibited higher DNA fragmentation, compared to the renal line (VERO with 5% of fragmented DNA, H460 with 26%, and 56% in K562). Finally, TIBA-induced ROS and apoptosis in all lines, which is significantly decreased after treatment with the antioxidant N-acetyl-cysteine (NAC). These data demonstrate the relationship between the increased cellular oxidative stress and the anti-tumor action of the TIBA.
Keywords: TIBA, Cancer, Contrast Agent
Supported by: FAPERJ

EC.03 - Antioxidant capacity of Petroselinum crispum (parsley) in yeast cells

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Oxidative stress is caused by an imbalance that occurs between oxidizing species such as reactive oxygen species (ROS), and the insufficiency of intracellular defense mechanisms. These species cause damage to cells and tissues and can be precursors of a series of pathologies. For this reason, several studies investigate the prevention of the generation of this oxidative stress through substances that have an antioxidant action capable of maintaining intracellular redox homeostasis. In our previous studies was demonstrated the antioxidant activity of apiin, a glycosylated flavonoid present in 6% in the aqueous extract of *Petroselinum crispum* (parsley) in *Saccharomyces cerevisiae* cells. The aim of this study was to verify whether non-cytotoxic concentrations of the aqueous extract of parsley would present in *S. cerevisiae cells* a protective antioxidant effect upon oxidative stress induced by hydrogen peroxide. Toxicity and cell viability assays by colony forming units as well as damage to membrane lipids (TBARS) were performed in yeast cells. Both concentrations of 0.113 and 0.226g.L-1 of the aqueous extract did not show toxicity to the cells, obtaining survival percentages of 81.67 ± 4.16% and 105.33 ± 6.81% respectively. In relation to the extract, both concentrations showed a similar protection to oxidative stress. Analysis of cells under stress revealed 26.50 ± 8.88% cell viability while pre-incubation with extracts it increased to 54.25 ± 20.67% and 46.33 ± 5.51% respectively. Pretreatment with the extract also showed a reduction in lipid peroxidation, so with significant results when evaluated at the concentration of 0.565g.L-1 of parsley extract. In conclusion, it is likely that this results with previous antioxidant analyzes using the flavonoids apiin and apigenin are intrinsically related to the antioxidant potential of *P. crispum* extract, mainly to the glycosylated flavonoid apiin.
Keywords: apiin, Petroselinum crispum, *Saccharomyces cerevisiae*
Supported by: FAPERJ, CNPq and CAPES

EC.04 – Project: Oxidative and membrane parameters in Wistar rat hippocampus with intrauterine development in the absence of maternal melatonin or in the presence of valproic acid

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According to the World Health Organization in 2021, it is estimated that 1 in 270 people in the world have autism spectrum disorders (ASD) with an increasing incidence in the last five decades. The development of ASD can be associated with genetic components and environmental factors, such as exposure to toxins, drugs and infections that lead to a possible induction in epigenetic changes. Prenatal exposure to valproic acid (VPA) may lead to the development of ASD and, therefore, it is used as a model to study these disorders of the neurological system. People with ASD may suffer from recurrent changes in sleep and this may be a consequence of insufficient levels of melatonin, a hormone produced by the pineal gland that has a fundamental role in regulating the circadian rhythm, in addition to its antioxidant properties. Elevations in oxidative stress parameters are also reported in individuals with ASD. In search for the relationships between oxidative stress, melatonin and ASD, in the present study we will evaluate the possible changes in oxidative and membrane parameters in hippocampi of rats prenatally exposed to VPA, comparing to hippocampi from offsprings of pinealectomized Wistar rats, that is, with prenatal development in the absence of the pineal gland.
Keywords: Autism, Oxidative stress, Melatonin
Funding: FAPEMIG

EC.05 - Piperine in association with N-acetylcysteine protects against acetaminophen-induced hepatotoxicity

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Acetaminophen (APAP) is widely used in the world for presenting analgesic properties and antipyretic however, its use in high doses can result irreversible liver damage. Preliminary studies suggest that piperine acts by inhibiting cytochrome P450, whom is involved in the metabolism of different xenobiotics, including paracetamol. With that our hypothesis that piperine could be a possible therapeutic target to minimize APAP-induced hepatotoxicity. The aim of this study was to evaluate the hepatoprotective effect of piperine in association or not with N-acetylcysteine (NAC) in a model of paracetamol-induced hepatotoxicity in C57BL/6 mice. The groups (n=7) were distributed into: control, APAP, APAP+P20, APAP+P40, APAP+NAC, APAP+P20+NAC, APAP+P40+NAC. Paracetamol was administered (500mg/Kg) and after 2 hours the treatments were performed with piperine (20 and 40 mg/Kg) in association or not with NAC (300 mg/kg). All treatments were performed orally through gavage. The animals were euthanized 12 hours after APAP administration. We evaluate hepatic and renal function, in addition to histological analysis and redox status in the liver. All procedures were approved the Ethics Committee on Animal Use (CEUA) from the Federal University of Ouro Preto, Brazil. The results showed that treatment with piperine 20 mg/kg associated with NAC reduced the activity of the ALT, reduced MMP-9 and increased the sulfhydryl group (-SH) compared to the APAP group. AST, urea and TBARS decreased in NAC, NAC+P20 and NAC+P40 groups when compared to APAP. The area of necrosis, TNF and carbonyl protein decreased in groups P40, NAC, NAC+P20 and NAC+P40 compared to APAP. The cytokine IL-6 reduced in all treated groups compared to APAP. Based on these results, piperine has been shown to be a possible ally with NAC in the treatment of hepatotoxicity.
Keywords: Acetaminophen, Piperine, hepatotoxicity. **Supported by:** UFOP, FAPEMIG e CAPES

EC.06 - Bioactive compounds and in vivo and *in vitro* antioxidant capacity of biquinho pepper (Capsicum chinense)

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Biquinho pepper (*Capsicum chinense*) is one of the most produced and consumed domesticated peppers in Brazil. Besides its commercial and economic value, this pepper has a significant health-promoting potential, since it contains bioactive compounds, which are responsible for nutritional and antioxidant potential. The objective of this work was to identify bioactive compounds present in *C. chinense*, and to evaluate the antioxidant capacity by in vivo and *in vitro* assays. Bioactive compounds were identified by UHPLC-MS analysis. For the antioxidant capacity were used DPPH, ORAC and β-carotene/linoleic acid assays and extracts toxicity and cell viability assays using *Saccharomyces cerevisiae* cells. Thirteen compounds were detected, such as carotenoids (β-carotene (m/z 537), lutein (*m/z* 551), zeaxanthin (*m/z* 569), antheraxanthin (*m/z* 585), cryptoxanthin (*m/z* 735), capsanthin (*m/z* 767), and capsorubin (*m/z* 811)), capsaicionoids (nordihydrocapsaicin (*m/z* 294), capsaicin (*m/z* 306), dihydrocapsaicin (*m/z* 308) and homocapsaicin-I (*m/z* 320)), and capsinoids (nornorcapsiate (*m/z* 301) and nordihydrocapsiate (*m/z* 317)). Capsaicionoids and capsinoids are recognized due to their anti-inflammatory, antioxidant, and antitumoral properties. Carotenoids have a series of conjugated C=C bonds that are responsible for their ability of quenching molecular oxygen and scavenging free radicals. *C. chinense* showed an antioxidant potential in DPPH radical scavenging activity (33.17±0.08%). The oxygen radical absorbance capacity (ORAC) was measured by fluorescein method and it was 32.35±0.02μM TE/g and result of β-carotene/linoleic acid assay was 55.80±1.30%. In vivo antioxidant analyses demonstrated that the pepper extract (170μg/mL) decreased the damage promoted by H_2O_2 in *Saccharomyces cerevisiae* cells. The antioxidant potential was demonstrated by both in vivo and *in vitro* assays. Additionally, *C. chinense* extract showed low toxicity to *Saccharomyces cerevisiae* cells under the evaluated conditions. Therefore, the *biquinho* pepper becomes a potential target for more advanced studies, both in the health and food areas, which may be related to disease prevention and potential food preservatives. **Keywords:** antioxidant, Capsicum chinense, *Saccharomyces cerevisiae*

EC.07 - Modified protocol for redox proteomics in melanoma cells

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In the development and progression of cancer, chemical modification of proteins is as important as the amount of protein synthesized. Although the involvement of reactive oxygen species (ROS) in signaling processes is well established, sensors and targets of these events are still not well defined. In this context, it becomes necessary studies that can clarify the modifications generated in the proteins, that act in the malignancy or even in the resistance of these tumors to the treatment. These modifications are intimately related with post-translational protein modifications, e.g., highly reactive thiol moiety of cysteines enables structural rearrangements resulting in redox biological switches. In this context, redox proteomics emerge as a fundamental tool to identify and quantify redox-sensitive proteins and to understand redox mechanisms behind thiol modifications. Given the great variability in redox proteomics protocols, problems including decreased resolution of peptides and low protein amounts even after the enrichment steps may occur. Based on this, our objective was to determined a protocol to analyse redox proteomics in melanoma. Protocols for redox proteomics were revised and tested. Considering the biological importance of thiol's oxidation in melanoma and based on previous protocols mainly described by Zaccarin e colleagues (2014), and our own insights, we adapted a protocol of Biotin switch assay technique with Biotin-HPDP and NEM for a cell line. This adaption surpassed limitations on the traditional method, improving a protocol of redox proteomics focused on thiol-protein studies in melanoma cells, whose biological importance of study concerning thiol´s oxidation were extensively reviewed.

Keywords: redox, proteomics, melanoma

Supported by: CNPQ and CAPES

EC.08 - Effects of Potential Antioxidants on Insulin-Producing Cells

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Oxidative stress may be a common link in pancreatic beta cell dysfunction. The use of chemical compounds mimicking the situations encountered in diabetes, such as sodium nitroprusside (SNP), menadione and hydrogen peroxide, has made it possible to clarify the various deleterious or protective molecular mechanisms that act in the beta cell. In contrast, bioactive compounds such as glycyrrhizin and rutin, which may act to prevent or mitigate the consequences of this process, are of great interest. To evaluate the toxicity of oxidizing agents and nitric oxide donors and to test the possible cytoprotective effects of natural compounds on RINm5F insulin-producing cells. RINm5F insulin producing cells were grown in an appropriate medium and exposed to cytotoxic compounds (hydrogen peroxide, menadione, and sodium nitroprusside) and antioxidants of interest (glycyrrhizin and rutin). The MTT spectrophotometric reduction method used to cell viability assays. The status of redox (nitroxy) cells, anionic production of intracellular mitochondrial superoxide and detection of phosphatidylserine in the cytoplasmic membrane were determined by the fluorogenic probes in the cytoplasmic membrane were determined by the fluorogenic probes DCFH-DA, MitoSOX and Annexin V - FITC, respectively, by fluorescence microscopy. There was a significant decrease in cell viability and an increase in the production of intracellular reactive oxygen species against oxidative challenge. However, co-incubation of RINm5F cells with glycyrrhizin had three different types of effects: partial protection against the use of hydrogen peroxide; other deleterious exposure to menadione exposure and no effect on donor sodium nitroprusside (SNP) use. It is possible that cell type and sub-localization of reactive oxygen species production will determine the ultimate effect of glycyrrhizin and cell fate. Although rutin had no protection against oxidative damage induced by hydrogen peroxide, menadione and SNP, no cytotoxic effect on RINm5F cells was observed under the conditions tested.

Keywords: antioxidants, beta-cells, reactive species

Supported by: FAPERJ

EC.09 - Antioxidant effect of phenolic compounds fermented by probiotics on *Saccharomyces cerevisiae* strains deficient in antioxidant systems

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Studies show that a diet rich in foods containing phenolic substances helps in the body's redox balance acting against free radicals, which in high concentrations are associated with a series of diseases, such as degenerative and cardiovascular diseases, tumors, diabetes and others. Most bioactive compounds are metabolized by microorganisms present in the intestinal lumen before being absorbed, which directly impacts their biological activity. The objective these study was to evaluate the influence of microbial biotransformations on the antioxidant activity of the phenolic substances phenethyl ester of caffeic acid (CAPE) and mangiferin in *Saccharomyces cerevisiae* cells. Cells were treated with CAPE and mangifetin (0.1mM) isolated and after fermentation of these phenolics with a probiotic blend, followed by exposure to hydrogen peroxide (2.0 mM) for 1h. Antioxidant analysis was determined by cell viability, membrane lipid peroxidation (TBARS) and redox environment by dichlorofluorescein. Our results revealed that pure CAPE and mangiferin (0.1 mM) were able to decrease the oxidative damage induced by hydrogen peroxide in the control strain (BY4741) and in the mutant strains Δsod1, Δgsh1 and Δctt1, deficient in superoxide dismutase, glutathione and catalase, respectively. After fermentation, the phenolics maintained their antioxidant capacity, but this activity related only to mangiferin was not relevant in the intracellular viability and oxidation tests. These studies suggest that the antioxidant activity was maintained in the presence of microbial biotransformations, showing that these compounds can be potential antioxidants even after their fermentation by microorganisms present in the intestinal flora using a eukaryotic study model.
Keywords: *Saccharomyces cerevisiae*, probiotics, phenolics substances

EC.10 - A novel diselenide attenuates the carrageenan-induced inflammation in mice by inhibiting neutrophil chemotaxis

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Myeloperoxidase (MPO) is a neutrophil haem peroxidase that catalyzes the formation of free radicals and HOCl. Despite their role in immune defense, these species may oxidize host biomolecules and lead to human inflammatory diseases. Therefore, there is interest in the development of MPO inhibitors for clinical application. Here we investigated the *in vitro* and in vivo anti-inflammatory effect of a new selenium compound, dibenzyl[diselanediylbis(propane-3,1-diyl)]dicarbamate. We first evaluated the reaction of diselenide and HOCl by the taurine-chloramine method. The diselenide effect on oxidative burst of PMA-stimulated *d* HL-60 was also investigated. Carrageenan-induced peritoneal and paw inflammation models were employed to monitor its anti-inflammatory activity by measuring some redox and inflammation biomarkers. The toxicity was assessed by single dose and brine shrimp assays. *In vitro*, the compound rapidly reacted with HOCl ($k=9.2\times10^7$ M^{-1} s^{-1}) similarly to glutathione ($k=1.2\times10^8$ M^{-1} s^{-1}). It also reduced the HOCl formation by human neutrophils, but tyrosine did not restore it. Interestingly, the diselenide inhibited the oxidative burst of *d*HL-60 cells (O_2 consumption, $O_2^{\bullet-}$ and H_2O_2 production) in a concentration-dependent manner with no cell toxicity (≤25 μM). *In vivo*, carrageenan increased leukocyte migration into mouse peritoneal cavity, and the compound (25, 50 and 75 mg/kg, i.p.) reduced the total leukocyte and neutrophil numbers, MPO activity, lipid peroxidation, albumin exudation, nitrite, TNF-α and IL-1β levels in peritoneal fluid. Likewise, the diselenide (50 mg/kg, i.p.) decreased the paw edema after carrageenan-subplantar injection in mice over 4 hours. Histological and immunohistological analysis from the inflamed paws showed a reduction in neutrophil counting, edema area, and marking for MPO and protein carbonyl. No toxicity was observed by the single dose toxicity study (50 mg/kg, i.p.) over 15 days and the brine shrimp bioassay. Collectively, these data indicate that the new diselenide attenuates carrageenan-induced inflammation mainly by suppressing neutrophil migration and the resulting oxidative burst-mediated damage. **Keywords:** selenium, inflammation, oxidative burst.
Supported by: FAPESB

EC.11 - Air pollution in Recife (PE): evaluation of cell death, inflammatory responses and oxidative stress in A549 cells

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The World Health Organization (WHO) estimates that air pollution is responsible for seven million deaths annually, triggering or aggravating cardiorespiratory disorders and some types of cancer. Recife, with more than 1.6 million inhabitants and a fleet of almost 700.000 vehicles, showed a considerable increase in the number of hospitalizations and deaths from diseases associated with air pollution in the last decade, which may be related to the increase in the fleet and greater formation of particulate matter (PM). PM generates reactive oxygen species (ROS) and causes inflammation when inhaled. This study determined the concentration of $PM_{2.5}$ in Recife atmosphere (PM-Recife) and evaluated its effects on human alveolar type II cells (A549), comparing with the diesel-derived PM (PM-Diesel). We analysed cytotoxicity, cell death, levels of inflammatory cytokines, ROS production, and transcriptional activation of antioxidant enzymes. Our results show that the concentration of PM2.5 in Recife is according to the current Brazil standard but it is close to WHO standards. PM-Diesel reduced cell viability after 48 h ($p<0.0002$) and 72h ($p<0.0001$) of exposure, affecting more than 60% of the cells while PM-Recife reduced cell viability by almost 50% after 72h ($p<0.03$). PM-Diesel increased the levels of IL-6 after 48 h (< 0.03) and 72h ($p<0.005$) of exposure and both PMs increased IL-8 after 72h ($p<0.02$). Both PMs induced apoptosis and intracellular oxidative stress in A549 cells after 72h ($p<0.02$), in addition to transcriptionally activating the enzymes superoxide dismutase, catalase, glutathione peroxidase, thioredoxin and ferredoxin reductase ($p<0.02$). We conclude that PM-Recife, like PM-Diesel, is toxic to lung cells, capable of inducing apoptosis, inflammatory responses, oxidative stress and altering the cellular system of antioxidants. Such findings point to the need for further studies to understand the mechanisms involved in the pathogenesis of PM-Recife.

Keywords: Air pollution, Apoptosis, Oxidative stress
Supported by: CNPq, CAPES and FIOCRUZ

EC.12 - Influence of ferulic and p-cumaric acids on the catalase enzyme in oxidative stress condition

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Oxidative stress is a condition generated by the inability of the antioxidant defense system to control the action of oxidants in cells, which can react with lipids, proteins and DNA, damaging the properties of cells structures. Oxidative stress has been associated with several pathologies, such as neurodegenerative diseases, diabetes and cancer. Ferulic and *p*-coumaric acids are exogenous antioxidants related to positive effects against neurodegenerative diseases. Study the influence of ferulic and *p*-coumaric acids on catalase in *Saccharomyces cerevisiae* cells.Cells viability, catalase activity, antioxidant activity and molecular modelling experiments were carried out. Both acids did not show toxicity at the concentration of 10 µg.m/L for cells. The effect of these acids on catalase activity under different concentrations of hydrogen peroxide (0.5mM, and 2.5mM) revealed 77.76% and 53.63%; 127.52% and 104.39%; and 83.67% and 70.61% for ferulic and *p*-coumaric acids in relation to the negative control, respectively. Regarding the antioxidant potential, pre-treatment with acids showed an increase in cell viability after oxidative stress with hydrogen peroxide (2.0mM). These results were observed in the control BY4741 (increase greater than 20%) and mutant strain Δctt1 (increase greater than 25%) in catalase. Analyzing by molecular docking (GOLD) and *in silico* analysis it was found that both acids can establish possible interactions with the enzyme, such as hydrogen bonding with the amino acid Gln163, in addition to hydrophobic interactions with Val111, Pro124, Phe148, Phe149 and Phe159 in catalase, amino acids outside the enzyme catalytic site. In conclusion, ferulic acid and *p*-coumaric acid provide cellular protection against oxidative stress and this protection is apparently unrelated to their effect on catalase.

Keywords: hydroxycinnamic acids, catalase, *Saccharomyces cerevisiae*
Supported by: FAPERJ, CNPq and CAPES

EC.13 - Time-course of redox status, redox activity-related and mitochondrial dynamics-related gene expression after acute bout of different physical exercise protocols

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The magnitude of exercise-induced adaptations depends on exercise parameters, such as intensity, duration and execution mode (continuous or with intervals). In this context, the present study aimed to investigate the expression of redox activity-related and mitochondrial dynamics-related genes in mice skeletal muscle along 24 hours after an exercise session carried out with different protocols. Sixty-five male Swiss mice were allocated into a control group (5 animals) and others 3 groups (20 animals/group), which were submitted to a forced swimming bout with the follow protocols: low-intensity continuous (LIC), high-intensity continuous (HIC) and high-intensity interval (HII). Five animals from each group were euthanized immediately (0h) and at 6h, 12h and 24h after the exercise session. Gastrocnemius muscle was removed for analysis of expression of mitochondrial dynamics-related genes: Ppargc1a (mitochondrial biogenesis), Mfn2 (fusion), Dnm1L (fission), and Park2 (mitophagy); and redox activity-related genes: Nos2 Nfe2l2 and GPx1. Within-group and between-group comparisons were performed with ANOVA, with significance level set as $p \leq 0.05$. Despite opposites in exercise intensity and duration, similar temporal behavior was observed in the expression of the Ppargc1a in LIC and HII, with greater expression at 6 and 24h, while HIC showed significant increases only at 0h and 6h after exercise. Mfn2 was significantly higher at 0h only in HIC and HII, remaining high at 6h only in HII. Only HII exhibited repression of Dnm1L and Park2 genes along 24h after exercise, while HIC was the unique with significant increase. Nos2 was significantly higher only in HIC (0h) and HII (6h). Nfe2l2 increased along the 24h in LIC and HII. GPx1 was significantly higher only in HIC (0h) and HII (24h). The use of intervals during high-intensity exercise could suppress the expression of fission and mitophagy-related genes, and enhances the molecular profile related to mitochondrial biogenesis and fusion, as well as antioxidant defense.
Keywords: activity redox, exercise, mitochondrial dynamics. **Supported by:** FAPESB

EC.14 - Neutrophil granules isolation: a new miniaturized method

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Neutrophils are the most abundant leukocytes in the bloodstream and play a key role in the immune system. The activation and functionality of neutrophils depends on the exocytosis of storage particles, that are divided in 2 groups: granules and secretory vesicles. The granules are subdivided in 3 subtypes: azurophil, specific and gelatinase. These particles are differentiated mainly through their protein content; therefore, abundant specific proteins can be used as markers for each storage particle. The markers are myeloperoxidase (MPO) for azurophil, lipocalin-2 (NGAL) and lactoferrin for specific, gelatinase for gelatinase granules and latent alkaline phosphatase (latent AP) for secretory vesicles. Characterization of granule's content is essential to comprehend the different functions of these cells, hence the importance of the granule's isolation. Currently, the protocols of isolation are performed in large density gradients, leading to the necessity of major cell quantities, about 3x108, that makes unfeasible to compare stimuli and biological replicates in the same experiment. Thus, the main goal of our work was to create a miniaturized isolation method based on a discontinuous percoll density gradient. In order to achieve this goal, 9 x 106 neutrophils were isolated, lysed, put on top of a 3-layer percoll density gradient and centrifuged. The resulting gradient (~940µL) was collected from the bottom to the top of the tube in 19 fractions. The protein markers were then analyzed for each fraction using western blot (MPO, lactoferrin and NGAL), gelatin zymography (gelatinase) and enzyme assay (latent AP). Our results showed we successfully isolated the granules and secretory vesicles using a gradient with less than 1 mL of total volume. The miniaturized method allows new experiments to be conducted, including a comparative study of neutrophill's response to diverse stimuli.
Keywords: Fractionation, Granules, Neutrophil. **Supported by:** FAPESP, CAPES e CNPq

EC.15 - Effects of redox modulation on quiescin/sulfhydryl oxidase activity of melanoma cells with stimulated melanogenesis

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Secreted quiescin/sulfhydryl oxidase (QSOX) is overexpressed in many tumor cell lines, including melanoma and it is usually associated with a pro-invasive phenotype. Glutathione (GSH) is one of the main responsible to control redox homeostasis in cells. Our previous work described that B16-F10 cells enter in a quiescent state as a protective mechanism against ROS-damage generated by melanogenesis stimulation. This work aimed to investigate how modulation of GSH levels in B16-F10 murine melanoma cell line under condition of stimulation of melanogenesis affects cell surviving and QSOX activity. Melanogenesis was stimulated by culturing B16-F10 cells with RPMI 1640 medium in the presence of L-tyrosine and NH4Cl during 48h. After 48h, cells were treated with GSH or BSO for 24h. Cell viability was evaluated by MTT assay and Crystal violet dye, which stains nucleic acids of adhered and fixed cells. QSOX activity in supernatant was measured using TBR 4100/1025 Free Radical Analyzer. Quantification of total glutathione, GSSG and GSH was performed by enzymatic/colorimetric assay. The redox homeostasis was impaired by treating cells with GSH in excess or depleting its intracellular levels through BSO treatment. Cells under melanogenesis stimulation showed lower GSH/GSSG ratio (8:1) in comparison with control (non-stimulated) cells (20:1), indicating a pro-oxidative state after stimulation. This was accompanied by a decrease in cell viability after GSH-depletion, and no alterations in QSOX activity. We suggest that melanogenesis stimulation together with redox impairment caused by GSH-depletion enhanced the oxidative stress in these cells, leading to the effects we observed and changing the phenotype of proliferation to quiescence. Another important observation that remains to be elucidated is that GSH-depleted control cells kept high levels of viability, suggesting a possible adaptative mechanism of survival even under low GSH levels. **Keywords:** QSOX, melanogenesis, glutathione. **Supported by:** CAPES and CNPq

EC.16 - The antioxidant peroxiredoxin AhpC1 is a key protein in *Pseudomonas aeruginosa virulence and in protection against oxidative response*

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Pseudomonas aeruginosa is an opportunistic pathogen with a plethora of virulence factors and antioxidant enzymes that help to subvert the immune system. Here we investigated the role of a 2-Cys peroxiredoxin, alkyl-hydroperoxide reductase C1 (AhpC1), in P. aeruginosa (PA14) survival and virulence. *In vitro*, wild-type and ΔahpC1 PA14 were incubated with HOCl, H2O2, urate hydroperoxide, and human neutrophils before CFU counting. The rate constant for the reaction of urate hydroperoxide and AhpC was determined by stopped flow. Mice were intranasally instilled with wild-type and ΔahpC1 PA14 strains 24-hours before CFU counting in lung, liver, and spleen, and evaluating some lung inflammatory biomarkers. Mice were monitored for survival after intranasal instillation with wild-type, ΔahpC1 and ΔahpC1 complemented with ahpC1 gene in normouricemic and hyperuricemic animals. Deletion of AhpC1 led to a higher sensitivity to all oxidants. ΔahpC1 was more sensitive to the killing by neutrophils, and less virulent in a mice model of infection. All mice instilled with ΔahpC1 survived for 15 days, whereas 100% died within 3 days with wild-type and complemented strain. A significantly lower number of colonies was detected in lung and spleen of ΔahpC1-infected mice. Total leukocytes, neutrophils, myeloperoxidase activity, pro-inflammatory cytokines, nitrite and lipid peroxidation were much lower in lungs or bronchoalveolar liquid of ΔahpC1-infected mice. Purified AhpC reacted with urate hydroperoxide at 2.3±0.1×106 M−1s−1, and only the ΔahpC1 was sensitive to this oxidant. Uric acid, the urate hydroperoxide precursor, impaired the killing of wild-type by neutrophils but improved the killing of ΔahpC1. Hyperuricemic mice presented higher levels of serum cytokines and succumbed much faster to PA14 infection when compared to normouricemic mice. Collectively, ΔahpC1 PA14 presented a lower virulence due to a poorer ability to neutralize the oxidants generated by inflammatory oxidative burst, especially urate hydroperoxide, leading to a more efficient killing by the host. **Keywords**: inflammation, oxidants, uric acid **Funding**: FAPESB, FAPESP, UESB, CNPq and CAPES

FA - Modeling, Big Data and Collective Behaviour

FA.01 - Genomic characterization of HIV-1 BC recombinant viruses

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The HIV-1 genetic classification presents 4 groups, subdivided into 10 subtypes which may present subsubtypes and circulating or unique recombinant forms. Currently, 11 CRF BC (07, 08, 31, 57, 60, 61, 62, 64, 85, 86 and 88) are described in the Los Alamos database and the CRF108, CFR110 and CRF118 were identified in Spain and China (2020 and 2021). According to the authors, the recombinant forms represent 22.9% of infections worldwide and may represent a challenge in the development of prophylactic agents. The aim of this study is the characterize the genetic diversity of HIV-1 BC recombinants, mapping the preferential points of recombination (R), exploring the genetic and biological aspects of their evolution. We collected 223 BC sequences in Los Alamos database and all sequences were evaluated in the jpHMM tool. To characterize the sequences, where there is recombination, the generated mosaics were used to estimate the frequency between the B and C subtypes along the genes. To estimate the phylogenetic relationship the alignment was performed using MAFFT online and manually edited using BioEdit software and submitted in IQtree. The dataset contains BC sequences collected between 1992 to 2018 from 15 countries and 220 sequences were used, 134 (60.9%) of them correspond to 14 CRFs. The mosaics revealed a low frequency of B with the maintence of C. Concerning the structural genes, the R occurred more frequently in pol (69%) and env (63%) whereas gag presented only 34% of intrasubtype recombination. This result was not observed in the accessory and regulatory genes of the virus, only the exception of nef gene, which is shown a high rate of recombination (53%). Based on this preliminary results, posteriorly the genetic and biological importance of recombination events will be investigated, in addition to the phylogenetic relationship between the samples and the worldwide distribution.
Keywords: HIV-1, Recombination, BC subtype
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FA.02 - Quantitative analysis and simulation of a liquid crystal organizational model in human liver tissue

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Cell polarity coordination is a key phenomenon underlying epithelial tissue structure and function. For complex three-dimensional tissues, such as liver tissue, a proper mathematical characterization of cell polarity has proven to be complicated to develop. The first structural model describing the organization of hepatic tissue was developed by Hans Elias in 1949, but could not describe the complex tridimensional organization of the liver. Nowadays, the current development of imaging techniques and digital reconstruction methods have allowed scientists to revisit the principles of liver organization with unprecedented accuracy. Previous studies have quantified the spatial patterns of apico-basal hepatic cell polarity in mouse liver tissue. Through a conceptual and algorithmic framework, it was found that the structure of liver tissue is in an intermediate state between an amorphous structure and a perfect crystal, best described as a liquid crystal. In this study, we quantify the nematic cell polarity in three-dimensional tissues to the case of human liver tissue under two physiologically relevant conditions: healthy tissue and tissue with the pathology of Non-Alcoholic Fatty Liver Disease (NAFLD). Then, we elucidate the mechanisms underlying the liquid-crystalline order in human liver tissue. We apply the methodology developed for the quantification of nematic cell polarity in three-dimensional tissues for the mentioned study cases. Then, we use Monte-Carlo simulations to characterize the mechanisms (i.e. apico-basal repulsion, local cell-cell interactions, external fields interactions, boundary conditions) underlying liquid-crystalline order in human liver tissue. Our preliminary results show that human liver tissue follows a liquid-crystalline type of organization as the one found in the mouse liver. The use of quantitative tools combined with simulations has helped to identify the physical properties of complex biological systems such as the liver, and it will continue to be useful in understanding the mechanisms underlying the organization and function of tissues.
Keywords: active matter, liquid crystals, tissue biophysics

FA.03 - Calcium transport by the Plasma Membrane Ca^{2+} pump (PMCA)

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Calcium (Ca^{2+}) is an important second messenger that participates in many cellular activities. Cells display different mechanisms to maintain low levels of cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$) (100-200 nM) needed to regulate its targets with optimal effectiveness. These mechanisms include transport systems at the plasma membrane level (e.g. Ca^{2+}-ATPases -PMCAs-, Na^{+}/Ca^{2+} exchanger) and at certain intracellular organelle membranes (e.g. the sarcoplasmic reticulum Ca^{2+} ATPase -SERCA-). The aim of this study was to investigate the Ca^{2+} transport by the plasma membrane Ca^{2+} pump (PMCA) in living cells. HEK-293T cell line was transfected transiently with PMCA4 and $[Ca^{2+}]_{cyt}$ was measured in real-time by loading cells with the fluorescent probe Fluo-4. $[Ca^{2+}]_{cyt}$ kinetics were examined by studying the alterations in $[Ca^{2+}]_{cyt}$ generated by Ca^{2+} released from the sarcoplasmic reticulum (ER), and by extracellular Ca^{2+} entry through store-operated Ca^{2+} channels (SOCs). Finally, the results were interpreted in terms of a mathematical model of $[Ca^{2+}]_{cyt}$ kinetics and the parameters were obtained. The typical experiment of $[Ca^{2+}]_{cyt}$ kinetics showed two phases: (a) transient elevation in $[Ca^{2+}]_{cyt}$ generated by the addition of 1 μM thapsigargin, a SERCA inhibitor that causes Ca^{2+} released from ER and (b) $[Ca^{2+}]_{cyt}$ increase generated by the addition of 4 mM Ca^{2+} to the external medium which that induced the SOC activity. In both phases, $[Ca^{2+}]_{cyt}$ reached a maximum and then its stationary level was reestablished. Overexpression of hPMCA4 led to a significant decrease in the global $[Ca^{2+}]_{cyt}$ at all times after the stimulus. On the other hand, the results were interpreted in terms of a mathematical model of $[Ca^{2+}]_{cyt}$ kinetics suggesting that Ca^{2+} transport by PMCA must increase slowly after $[Ca^{2+}]_{cyt}$ increase to explain the $[Ca^{2+}]_{cyt}$ kinetics. In conclusion, the model built in this study is a useful tool to analyze the PMCA Ca^{2+} transport activity in living cells.

Keywords: Ca^{2+}-signaling, Ca^{2+} homeostasis, Mathematical model, PMCA, SERCA, SOCS

FA.04 - Digital holographic microscopy of diatomite embedded in transparent resins

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According to the improvement of digital holographic microscopy (DHM), numbers of academic papers that reported various micron size objects are increasing in the past several years. On the other hand, sample preparation techniques for DHM is still in the primitive stage. In many papers, micron size objects including living cells were just deposited on a glass surface, and then, observed in air or in liquids. In this work, we propose a new procedure of sample preparation using several types of resins for digital holographic microscopy (DHM). Diatomaceous earth (61790-53-2, MP Biomedicals, LLC) was used as a test sample. Diatomite is fossils of diatoms that are photosynthetic planktons. 0.5 mg of diatomite powder was deposited in a Tomodish (Tomocube Inc.), and then, embedded the diatomite in SYLGARD 184 (761036, Sigma-Aldrich), mount media (139-06682, FUJIFILM Wako Pure Chemical Corp.), or 1.5% agar (016-15812, FUJIFILM Wako Pure Chemical Corp.). Finally, the Tomodish was sealed with a coverslip. The prepared samples were observed by a commercially available DHM (HT-2, Tomocube Inc.). For comparison, the diatomite was also observed in water. Although the diatomite includes aggregates of diatom fossils, isolated individual diatomite (a fossil of one diatom cell) was observed. As a result, the embedding method was effective to stabilize DHM imaging. In the control sample (water), sometimes position of individual diatomite fluctuated. Furthermore, when diatomite was embedded in SYLGARD 184, higher resolution was obtained. With SYLGARD 184, mesoporous structures of diatomite were well resolved in all the 10 independent individuals. With mount media and 1.5% agar, the mesoporous structures were resolved in 1 and 1 sample among 10 samples, respectively. In water, the structures were observed in 4 samples among 10 samples. Our results suggested that the embedding method drastically affects quality of DHM observation.

Keywords: Diatomaceous earth, Digital holographic microscopy, Embedding resin

FA.05 - Acceleration of Evolutionary Processes by Learning and Extended Fisher's Fundamental Theorem

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Natural selection is a universal and powerful concept not only to explain biological evolution but also to design engineering algorithms like genetic algorithms. Although conventional natural selection is passive in that the mutation is directionless and random, there is an increasing interest in the systems where natural selection and learning interplay. Precisely, we are interested in the natural selection of the population of agents that can learn and replicate. In biological systems, we have increasing pieces of evidence that an organism can transmit information to its descendants via epigenetic states and cultures. By learning from such information, an agent might increase the population fitness without relying on natural selection and thereby accelerate the evolutionary process. In engineering systems, the interplay between natural selection and learning is important to extend and to improve the genetic algorithms. Although these systems are individually discussed in many fields, we lack theoretical foundations. We do not have a proof that learning can actually accelerate natural selection other than numerical simulations. We also do not know what information or communication is sufficient to optimize the population fitness, which is a trait of the population not of individual agents. In addition, we do not have a methodology to quantify the acceleration. In this work, we give a unified theoretical framework and solve these problems. To solve the problem, we propose a learning rule called an ancestral learning. Also, we extend the Fisher's fundamental theorem of natural selection to quantify the acceleration. We showed that the ancestral learning can accelerate the evolutionary process. Additionally, we successfully extended the Fisher's fundamental theorem of natural selection. Since ancestral learning uses the information transmitted from ancestors, such ancestral information is sufficient to optimize the population fitness. The extended theorem relates the acceleration to the variance of the individual fitness of the agents.

Keywords: evolution, evolutionary algorithms, learning
Supported by: JSPS and JST

FB - Systems Biology

FB.01 - Different oxidative response between sexes in *Drosophila melanogaster* exposed to Biphenol S

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The intensification of regulation and prohibition of the use of Bisphenol A (BPA) in some countries, led the industry to use other types of bisphenols in the production of utensils and plastic packaging. Even without in-depth studies, Bisphenol S (BPS) is one of the most used substitutes in the production of products called “BPA-free”. Therefore, evaluating the toxicological action of BPS, and establishing whether there is a difference in the action of this chemical between the sexes, is pertinent information to be considered by scientists, legislators and society. Thus, our aim was to evaluate the effect of BPS on the oxidative stress biomarkers of *Drosophila melanogaster*, male and female, separately. Flies were separated by sex, divided into groups: Control (standard diet only), BPS 0.25 mM, BPS 0.5 mM and BPS 1mM (BPS mixed with standard diet). After 7 days of exposure, analyzes were performed on whole body samples to quantify reactive species (RS), lipid peroxidation (LPO), mitochondrial and cell viability. Female flies exposed to BPS 0.25, 0.5 and 1mM obtained increased levels of RS. In addition, they showed increased lipid peroxidation, decreased mitochondrial and cell viability at concentrations of BPS 0.5 and 1 mM, when compared to the control group. Men exposed to BPS (0.25, 0.5 and 1mM) also showed increased RS levels and decreased mitochondrial viability at BPS 0.5 and 1mM, when compared to the control group. However, male flies did not show changes in lipid peroxidation and cell viability. Changes in RS levels and decreased mitochondrial viability did not alter the cell viability of male flies. Thus, we conclude that BPS triggered different changes between genders, showing greater damage to female flies, and encouraging us to carry out future studies.
Keywords: Bisphenol S, Bisphenol A, *Drosophila melanogaster*
Supported by: CNPq and CAPES

FB.02 - Inference and analysis of a gene regulatory network of angiogenesis in an oxygen-induced retinopathy mouse model

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Angiogenesis, the formation of new blood vessels from pre-existing ones, is an important therapeutic target. Nevertheless, not all patients benefit from current drugs available for angiogenesis-dependent diseases (i.e. cancer and retinopathies). To address this problem, we sought to achieve a better understanding of gene regulation in angiogenesis, which may help the identification of new targets for drug development. Previous work by our group has identified by RNA-seq several differentially expressed (DE) protein-coding genes in a mouse model of angiogenesis (OIR, oxygen-induced retinopathy). Here, we expanded on these studies to analyze the mRNA isoforms and regulatory RNAs. These data will then be integrated to generate a comprehensive gene network of the OIR model. Seven-day-old mice were placed in a hyperoxic environment (75% O_2) for five days and then returned to room air. This causes a sudden hypoxic condition leading to VEGFA overexpression, resulting in abnormal vascular growth and pathological angiogenesis. Retinas were dissected for RNA extraction and sequencing right after the return to room air, but also 3 and 5 days later. The RNA-Seq data were analyzed using bioinformatics pipelines. We identified 60 DE mRNA isoforms, of which 26 were inferred to be homologous to human isoforms. We also identified 99 DE miRNAs, of which 5 are new candidates. Most of the predicted targets for these miRNAs are related to angiogenesis. Among lncRNAs, we identified 218 DE, of which 57 are already described and 161 are new candidates. We predicted 5421 circRNAs, half of which are novel. The next step is to build the co-expression network using WGCNA to identify the interactions between mRNAs and these regulatory RNAs. Most of the RNAs that were identified as DE are promising candidates for future studies and validation as potential targets for drug therapies, or as diagnostics/prognosis biomarkers for angiogenesis-dependent diseases.
Keywords: angiogenesis, network, RNA
Supported by: FAPESP and CAPES

FB.03 - Trans fatty acid during the developmental period induces cognitive deficit in *Drosophila melanogaster*

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Trans fatty acid (FA) is highly consumed by the population, this FA can be incorporated into neural membranes, causing changes in its structure, fluidity, and permeability, and consequently, impairing cognitive functions. These changes occur more intensely during the development, so the type of FA consumed during this period is important. In this work, we use the model of *Drosophila melanogaster*, which has demonstrated great homology with human systems. The objective of the work was to evaluate parameters related to the cognition of *Drosophila melanogaster* exposed to hydrogenated vegetable fat (HVF) during the developmental period and associated with the incorporation of these fatty acids in the nervous tissue of the fly. Progenitors were divided into 4 groups, containing 50 flies of both sexes each: (1) RD (corn flour, sugar, wheat germ, salt, powdered milk, and agar), (2) SHVF (RD fat values were replaced by HVF in the same proportion), (3) HVF 10% (RD with 10% HVF) and (4) HVF 20% (RD with 20% HVF). After 7 days of exposure, the progenitors were removed from the medium, leaving the larvae and eggs. Flies born on this medium (1-3 days) were used to assess short and long-term memory and FA composition in the head. There was a reduction in memory at 6h and 24h in flies exposed to HVF in all concentrations compared to the RD. An increase in the presence of trans FA was observed in the heads of flies exposed to HVF in all concentrations, compared to the RD. In addition, there was a reduction in monounsaturated FA and an increase in polyunsaturated FA in the group exposed to SHVF when compared to the HVF 10% and HVF 20% groups. It was concluded that trans FA can be incorporated in the flies' heads and thus causing changes related to cognition deficit.
Keywords: memory, membranes, diet
Supported by: CAPES and CNPq

FB.04 - Biochemical implementation of optimal control for run-and-tumble chemotaxis

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Various organisms search for food, good environments, and other targets with remarkable efficiency. One representative search strategy is run-and-tumble chemotaxis. Organisms can climb a spatial gradient of ligand concentration by controlling the frequency of random directional changes (tumble) based on sensed temporal changes of the concentration during ballistic swimming (run). It has been a fundamental question how efficient the run-and-tumble chemotaxis can be in principle and whether organisms have a biochemical mechanism that achieves that efficiency limit or not. This question has been addressed by deriving optimal control of run-and-tumble motions theoretically and comparing it with measured motor responses of organisms. Due to theoretical difficulties, the previous derivations of the optimal control ignore sensory noise and nonlinearity of response, hindering the detailed relation between the optimal control and biochemical systems. Here, we overcome such difficulty by deriving the nonlinear optimal control of run-and-tumble motion under sensory noise. We combine the nonlinear filtering theory and Kullback-Leibler control of partially observed Markov decision process (POMDP). The derived optimal control consists of two parts: the filtering dynamics, which extracts necessary information from the noisy signal, and the optimal control function, which converts the information to the motor control. Furthermore, the optimal control reproduces a standard biochemical model of the sensory system of Escherichia coli and a nonlinear response relation observed experimentally, indicating that the *E. coli*'s biochemical pathway can implement the optimal structure for achieving efficient run-and-tumble chemotaxis. We derived the nonlinear optimal control of run-and-tumble chemotaxis under sensory noise and show its implementation in the biochemical pathway of *E. coli*. Our derivation can work as a theoretical basis for investigating the efficiency of more complex sensory-motor integrations in run-and-tumble chemotaxis.
Keywords: Chemotaxis, Optimal control, Nonlinear filtering. **Supported by:** JSPS, JST

FB.05 – PROJECT: Investigation of abortion cases at almeida castro maternity in Mossoró/RN

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Abortion is defined as termination of pregnancy in which the fetus is unable to survive outside the uterus. Statistics show that, of diagnosed pregnancies, the incidence of this complication occurs in 10 to 15% of cases and 80% of cases occur in early pregnancy. Given the above and associated with the scarcity of information on the etiology of these losses, the objective of the research is to analyze the possible causes and effects of abortions that occur in the Almeida Castro maternity hospital, as well as to identify the predisposing factors and factors of pregnancy loss, seeking to characterize the profile of parents and fetus; investigate possible teratological changes, describing the possible macroscopic and microscopic changes associated with each case of abortion. This is a quantitative experimental research, carried out in collaboration with the Maternity Almeida Castro in the Municipality of Mossoró-RN. The study population will be composed according to the cases of abortion documented by the Hospital Almeida Castro of the Municipality of Mossoró-RN and the samples will be based on the casuistry. Data collection will be done following a pre-established script. First, the mother's history will be investigated, based on the data described in her medical record. Once selected, in the second moment, the macroscopic analysis of the fetus will be carried out in the maternity ward, if any abnormality or suspicion of alteration is found, in the third subsequent moment, samples of at most 1 cm² from specific regions will be collected. and these punctual biopsies will be forwarded for microscopic analysis at UFERSA's Histopathological Processing Laboratory. It is expected, therefore, to be able to understand the causes and risk factors for abortion cases in this population, as well as to arrive at a diagnosis to understand its causes, which is a much neglected area of research.
Keywords: Abortion, Birth Defects, Teratology

FB.06 - Blood serum lipidomics of patinete with severe respiratory syndrome, positive and negative for SARS-CoV-2 by NMR techniques

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COVID-19 disease, which affects patients infected with the SARS-CoV-2 virus, has a different fatality and behavior from respiratory infections caused by other viruses. In this context, understanding metabolic variations is fundamental to understand how the disease may affect us. Important components of blood serum, lipids are related to several metabolic functions and their variations in quantity and variety related to the disease can guide the understanding of the pathogen's action or the search for therapeutic targets. Therefore, this study aims to identify the lipids responsible for distinguishing between two classes of patients with severe acute respiratory syndrome (one group of individuals with a positive RT-qPCR test for SARS-CoV-2), and a group of age-matched healthy volunteers. Serum samples were obtained from patients and volunteers at the University of Campinas Hospital and stored at -80 °C until the moment of analysis. Lipid extractions were performed by diluting 150 µL of blood serum in 350 µL of MiliQ water, mixed with 500 µL of methanol and chloroform. The samples were shaken for 2 min, then centrifuged at 2200 rpm for 20 min at 25 °C and transferred to a freezer at -20 °C for 30 min. Then the polar and non-polar phases were separated, and both were analyzed by NMR. High resolution 1H-NMR spectra were obtained on the Bruker AVANCE III 600 MHz instrument at 25 °C. Sera from positive to SARS-CoV-2 patients showed lower levels of cholesterol, polar lipids, and lysophosphocolines. But, higher VLDL and phosphocholine levels were measured in the same cohort. On the other side, serum samples from negative to SARS-CoV-2 patients showed similar trends in levels of lipids, although in lesser extent. The data show that NMR spectroscopy can be applied to patient identification, in addition to demonstrating differences in patient lipid levels and types in blood serum samples.
Keywords: COVID-19 , Respiratory infections , 1H-NMR
Supported by: CAPES

FB.07 - Peripheral blood as a tool to determine gene expression patterns in patients with psychiatric and neurological disorders: a systematic review and meta-analysis

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Psychiatric disorders have been investigated on several biological levels. One that reflects the interaction of genes, proteins, and transcription factors is the transcriptome. Thus, we aim at summarizing the evidence regarding gene expression levels of peripheral blood samples between subjects with psychiatric and neurological disorders to healthy controls and later comparing those results to genome-wide association studies (GWAS) through systems biology. The Gene Expression Omnibus and the Array Express databases were searched with specific strategies to find studies that included blood samples and investigated the following disorders: major depressive disorder (MDD), schizophrenia (SCZ), autism spectrum disorder (ASD), Parkinson's disease (PD), or Alzheimer's disease (AD). The duplicates were removed and the records were screened independently by two reviewers. Studies were meta-analyzed using the R environment package metaMA. The genes found were also compared to the GWAS mapped genes. Moreover, analyses concerning protein-protein interaction (PPI) networks were conducted. Meta-analysis procedures revealed that the expression seems to be consistently altered for 5 genes in the MDD group, 1105 genes in SCZ, 8 genes in ASD, 22 genes in PD, and 717 genes in AD. From the genes found in SCZ, seven are also mapped genes in Schizophrenia GWAS studies. Additionally, from the ones found in ALZ, 10 genes are also mapped genes in Alzheimer's disease GWAS studies. Furthermore, when PPI networks are investigated based on the differential expressed genes found, they show that their proteins are connected. Enrichment of PPI networks found an association with biological processes related to the synapse, cellular signaling, circadian rhythm and inflammatory response. It is certainly interesting to show that some genes already associated in GWAS were found with a consistently differentially expressed pattern across transcriptome studies. This approach can be an ally to transcriptome-wide association studies in the investigation of genomic variants and their impact on gene expression.
Keywords: biochemistry, neuropsychiatry, transcriptomics. **Supported by:** CNPq, CAPES, and FAPERGS/PPSUS

FB.08 - Lutein Nanoparticles Protect Anxious-Like Behavioral Changes and Oxidative Damage in Rat Brain in a Prenatal Model of Autism

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Autism Spectrum Disorder (ASD) has increased in prevalence in recent years, but there are still many diagnostic criteria and therapeutic resources to be established. Bioactive compounds such as lutein, an important carotenoid, have antioxidant and neuroprotective potential, as it crosses the blood-brain barrier, demonstrating a neuroprotective effect in diseases such as ASD. Thus, this study aims to evaluate the action of lutein-loaded nanoparticles on anxiety-like behavior and oxidative stress in the experimental model of valproic acid-induced ASD (VPA) in rats. Fifteen adult Wistar rats (90 - 120 days old) were used, constituting the F0. On the 12.5th day, the pregnant females were separated and received an intraperitoneal injection of VPA (600 mg/kg) or saline (0.9% NaCl, 1 mL/kg). On the 21st day, the female offspring (F1) were subdivided into two groups and received nanoparticles loaded with lutein in the form of nanoparticles (5 mg/kg) or saline (1 mL/kg), orally for 14 days. On the 15th day, the open field behavioral test (OFT) was performed and after euthanization, the brain structures were used to quantify reactive species (RS). To measure the animals' anxiety, the length of stay in the center of the OFT was determined, a characteristic associated with reduced cognitive control in the ASD. Concomitantly, changes in RS cause oxidative stress and neurological dysfunction supporting the pathogenesis and/or severity of ASD. Our results showed that lutein was able to protect changes in the anxious and apathetic-like behavior characteristic of ASD, as well as VPA-induced cerebral oxidative damage in this animal model of autism. These findings suggest that lutein may be a natural alternative to protect the anxiety phenotype and oxidative alterations associated with autism. **Keywords:** neurodevelopmental disorder, carotenoid, valproic acid
Supported by: CNPq; CAPES; Unipampa - PPG biochemistry

FB.09 - Bisphenol A alters the development of *Drosophila melanogaster* exposed during the embryonic period

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Bisphenol A (BPA), is a ubiquitous chemical substance used in the synthesis of polycarbonate plastic and epoxy resins, inserted in a variety of everyday products, such as plastic bags and containers, thermal papers, cans for food and beverages, and several other materials. BPA is identified as an endocrine disruptor, in which several studies propose a relationship between exposure to BPA and the emergence of adverse health effects, such as cancer, infertility, diabetes and obesity, among others. Increasing evidence indicates that exposure to BPA is related to a variety of disorders in the reproductive system, which are being studied. The present study aimed to evaluate the effect of embryo exposure to BPA on the development of *Drosophila melanogaster.* For this study, virgin males and females were divided into 3 groups: 1) Control (standard diet only); 2) BPA 0.5 mM and 3) BPA 1 mM (respective concentrations of BPA mixed with the standard diet). After copulation, the progenitors were removed and the treatment medium containing the embryos was preserved for the following evaluations: a) average of viable eggs, b) hatching percentage in relation to the average number of eggs laid, c) eclosion rate of pupae. Flies exposed to different concentrations of BPA did not show a statistical difference in the percentage of viable eggs compared to the average number of eggs laid. Flies exposed to BPA 1 mM showed a decrease in the eclosion rate of the pupae when compared to the control group. Our data suggest that exposure to BPA does not interfere with embryo fertility, but exposure during larval development influenced the complete development of the fly, as seen by the decrease in pupal and eclosion rates

Keywords: Drosophila melanogaster, Eclosion, Bisphenol A
Supported by: Unipampa, CAPES and CNPq

FB.10 - Comparative transcriptomics among archaea to search for circular RNAs

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Circular RNAs (circRNA), i.e. RNA molecules with no 3' nor 5' loose ends, are pervasive in eukaryotes and progressively being understood not as mere splicing by-products but rather probably functional molecules. In bacteria, on the other hand, circRNAs were not consistently reported. Therefore, archaea, interesting prokaryotes withstanding features from both eukaryotes and bacteria, are well placed organisms to provide insights on evolutionary paths that established circRNAs. A specialized RNA-seq protocol, introducing an RNase R treatment sample preparation step before deep sequencing, is commonly used to search for circRNAs in a high-throughput fashion (Circ-seq). Among archaea, public Circ-seq data is available for *Saccharolobus solfataricus*, *Sulfolobus acidocaldarius* and *Pyrococcus abyssi*, covering only the *Crenarchaeota* and *Thermococci* clades. We performed Circ-seq experiments in *Halobacterium salinarum*, a prominent extremophile model organism representing the *Stenosarchaea* group. We implemented a bioinformatics pipeline, named Monark-seq, that explores high-quality non-aligned reads from regular RNA-seq pipeline genome alignments searching for circRNAs signatures which also survived RNase R digestion. Preliminary data yielded 763 putative redundant circRNAs signatures coming from ~80 unique genes in *H. salinarum* NRC-1 strain, some of which were observed in *S. solfataricus* and *P. abyssi*: $tRNA^{Trp}$ and both 16S and 23S rRNAs. Further analysis will probably reveal conserved sequences that may shed light on functional and biogenesis aspects of circRNAs.

Keywords: Archaea, Circular RNAs, RNase R
Supported by: FAPESP, FAEPA and CAPES

FB.11 - AQP3 and AQP5 silencing promotes changes in cell-cell adhesion and biomechanical properties of pancreatic cancer cells

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Aquaporins (AQPs), a family of transmembrane proteins, are responsible for the bidirectional transfer of water and small solutes across cell membranes in different human tissues. AQPs have important physiological functions in both exocrine and endocrine pancreas. AQP3 and AQP5 are known to be overexpressed in exocrine pancreatic cancer, playing key roles in cell migration, cell proliferation and invasion. Our study aimed to evaluate changes in the biophysical, biomechanical and morphological properties of pancreatic cancer cells, as well as their changes upon silencing of AQP3 and/or AQP5. Our results show that silencing AQP3 and AQP5 had implications on cell migration, with the silenced cells showing slower recover of wounded area. We assessed membrane fluidity of the different cells using multi-photon scanning fluorescence microscopy to measure laurdan generalized polarization (GP). Silenced AQP5 and AQP3/5 cells (combined silencing of both AQPs) showed lower GP values, meaning that they have higher membrane fluidity. Using atomic force microscopy (AFM), we evaluated biomechanical and morphological properties. AQP5 and AQP3/5 silenced cells showed to be more elastic than the control. Furthermore, these silenced cells also showed to be smaller, with lower volume, higher height and with a higher surface roughness, when compared with the control cells. Through cell-cell adhesion measurements conducted by AFM, we also saw that the work and maximum force necessary to detach two cells were lower in AQP-silenced cells than for the control, showing that these AQPs have implications on cell-cell adhesion. These findings suggest AQP3 and AQP5 contribution for cell migration and cell-cell adhesion. Water channels play a significant role in tumor development. Our findings provide new insight into possible strategies toward the development of antitumor therapies.

Keywords: Aquaporin-3, Aquaporin-5, Pancreatic Cancer

GA - Arthropods and Mechanobiology

GA.01 - Effect of mealworm meal on immunological parameters, digestive enzymes and midgut microbiota of the shrimp *Litopenaeus vannamei*

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Shrimp farming is one of the most profitable sectors of aquaculture, and the shrimp *Litopenaeus vannamei* is one of the most cultivated species worldwide. The nutrition of species is a great hindrance of shrimp farming, due to its high cost, in particular due to the use of fishmeal as one of the components in the feeds. Many studies have been carried out to strength the use of insect meal in shrimp feed without financial losses.The main goal of the present work was to analyze if the substitution of fish meal (TM-0) by mealworm meal (TM-25, TM-50, TM-75 and TM-100) can influence immunological parameters, digestive enzymes and midgut microbiota of *L. vannamei.* Five diets consisting of 0%, 25%, 50%, 75% and 100% of fishmeal replacement by MLM (denominated TM-0, TM-25, TM-50, TM-75 and TM-100) were performed during six weeks of culture. All diets were formulated on a digestive basis to provide 300 g kg-1 protein digestible and 3000 cal kg-1 energy digestible. The total count of hemocyte, intestinal microbiota, total protein concentration, phenoloxidase activity in haemolymph and the activities of trypsin, chymotrypsin, lipase and α-amylase were quantified. The total count of hemocytes, protein concentration, phenoloxidase activity in haemolymph were not significantly altered when TM-0 was substituted by the insect meals ($p > 0.05$). However, the agglutinating activity of *L. vannamei* serum against dog erythrocytes was higher in the shrimp group fed with TM-0. The results of the absolute and specific activities of trypsin, chymotrypsin, lipase and α-amylase demonstrated no significant differences between treatments. The shrimp midgut microbiota profile was similar in bacteria of the genus: Pseudoalteromonas, Rubritalea, Ruegeria, Tenacibaculum and Vibrio. These results support that the mealworm meal can be utilized as protein source for *L. vannamei* without any loss of digestive capability.
Keywords: Digestion, Aquaculture, Entomophagy. **Supported by:** Capes

GA.02 - iTRAQ-based quantitative proteomic analysis of the midgut from the soybean caterpillar *Anticarsia gemmatalis* in the presence of Bacillus thuringiensis Cry1Ac toxin

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Anticarsia gemmatalis is considered one of the main defoliating pest of soybeans in Brazil, being an important target of pest control. *Bacillus thuringiensis* is a Gram-positive bacterium, with wide global dispersion, characterized by the production of Cry toxins during the stationary phase of its growth (sporulation), to which an entomopathogenic effect is attributed. That is why this microorganism is used as a biopesticide to control several agricultural pests, including *A. gemmatalis*. However, insects have developed resistance to this control. This work aims to obtain the quantitative proteome of the midgut epithelium of *A. gemmatalis* after challenged or not with the toxin Cry1Ac of *B. thuringiensis*. A bioassay was performed to calculate the lethal concentration (LC50) of *B. thuringiensis*, using a total of 35 caterpillars in seven different concentrations of the sporulated bacteria, monitoring the survival rate for 96 hours. Midguts were dissected and protein extraction from epithelial tissue was performed using S-Trap column (Protifi®) protocol. Trypsin (Promega®) was used for the digestion step. After an iTRAQ labeling method, a fractionation step by reverse phase chromatography was applied and samples were analyzed in Q-exactive Plus (ThermoScientific®) mass spectrometer. Protein identification was performed using Proteome Discoverer 2.4 (ThermoScientific®). Bioassay results showed a LC50 of 0.073 mg/mL using *B. thuringiensis* sporulated bacteria against *A. gemmatalis*. A total of 2,990 proteins from 24h and 48h exposed caterpillars were identified by the bottom-up proteomics approach. A deeper proteome analysis will be performed since there is no description for this insect midgut. To overcome this difficulty, we plan to identify proteins by de novo sequencing and homology search protocol, used to describe proteomes of organisms with unsequenced genomes, comparing up and down-regulated proteins after *B. thuringiensis* infection. Proteomics approach can elucidate the mechanisms involved in the response of *A. gemmatalis* during *B. thuringiensis* infection.
Keywords: Anticarsia gemmatalis, Bacillus thuringiensis infection, Proteomics
Supported by: CAPES and FAPERJ

GA.03 - The cytotoxic effect of *Amblyomma sculptum* tick saliva over cancer cell lines is associated with ΔΨm loss and cleavage of caspases-3 and caspase-9.

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Animal secretions are promising sources of bioactive molecules. The crude saliva of *Amblyomma sculptum* (Fabricius, 1787) has demonstrated cytotoxic effects on different tumor cell lines, with no cytotoxicity in non-tumoral cells. However, little is known about the mechanisms involved in those activities. Here, we elucidate the effect of *A. sculptum* crude saliva on activation of programmed cell death pathways in tumoral cell lines. The crude saliva (SL) was collected by the Butantan Institute using microcapillary tubes and dopamine stimulation. Breast cancer (MDA-MB-231), colorectal adenocarcinoma (HCT-116), and neuroblastoma (SH-SY5Y, SK-N-SH and Be(2)M17) lineages were treated with 10% of the SL for 72 hours and analyzed to detect apoptosis, loss of mitochondrial potential and caspase cleavage. 5-Aza-2-deoxycytidine (AZA) demethylating agent known to increase caspase-8 expression and the pan-caspase-inhibitor Q-VD-Oph were tested in combination with SL. Protein expression was analyzed by western blotting. To evaluate the role of antiapoptotic proteins and mitochondrial potential control, cells overexpressing Bcl-XL and Mcl-1 recombinant proteins were generated by lentiviral infections. Statistical analyses were performed using GraphPad-Prism6.0. Apoptosis induction was observed after SL treatment on tested cell lines. Protein expression profiles showed that neuroblastoma lineages are deficient in initiating caspases and death receptors, suggesting SL activity to be independent of extrinsic apoptotic triggers. Which was supported by the fact that caspase-8 recovered expression with AZA did not affect cell death. QVD, however, was able to entirely protect cells against SL. Overexpression of Mcl-1 and Bcl-XL was able to prevent apoptosis and mitochondrial potential loss. Caspase-3 cleavage was no longer present in the generated cell lines, as well as laminin A/C and caspase-9 cleavage. Therefore, we provide evidence that *A. sculptum* crude saliva induces intrinsic apoptosis in tumor cell lines by mitochondrial potential loss and consequent cleavage of caspase-9 and caspase-3. **Keywords:** Tick saliva, Antitumoral, Intrinsic apoptosis. **Supported by:** CAPES/COFECUB

GA.04 - Protein profile of *Tityus paraguayensis* venom and hemolymph

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Tityus paraguayensis scorpion is endemic in Mato Grosso do Sul. The chemical composition of the scorpion venom can vary between species and individuals, and season. Another potential source of molecules in scorpions is the hemolymph. Several antimicrobial peptides (AMPs) like defensins and open-end cyclic peptides have been described in scorpion hemolymph. This study aimed to determine the protein profile of hemolymph and venom from *T. paraguayensis* scorpion. Protein concentration was determined by the Bradford method. In order to analyze the protein profile, the SDS-PAGE electrophoresis was realized under reducing conditions. The concentration of venom protein measured was 3.54 mg/mL. The electrophoretic profile of venom revealed the presence of seven proteins with different sizes, ranging from 150 to smaller than 10 kDa. The most abundant proteins in the venom have 75 kDa, 70 kDa and 10 kDa. The protein of 10 kDa suggests the presence of low molecular weight proteins or peptides, which are described in scorpion venoms as responsible for the toxicity of the venoms. On the other hand, 70 kDa and 75 kDa proteins correspond to hemocyanins, which are abundant protein complexes in arthropod hemolymph, being involved in gas exchange. To hemolymph, the concentration of plasma fraction measured was 4.23 mg/mL, while hemocytes fraction showed the lowest concentration (1.12 mg/mL). Both hemolymph fractions analyzed revealed a predominant band around 75 kDa. These results demonstrate a high presence of hemocyanin in hemolymph and venom. This study demonstrated that venom and hemolymph of *T. paraguayensis* not only contains hemocyanin but other proteins, which can serve as a starting point for future research facilitating the identification of the venom and hemolymph composition of scorpions.

Keywords: proteins, peptides, scorpion. **Supported by:** CNPq, FUNDECT and UFMS

GA.05 - Acoustofluidic Interferometric Device (AID) for cells' mechanical characterization

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Here we present a high throughput single cell physical characterization device intended to address the growing need for marker-free tools for biomedical applications. Intended for real time cells Optical Thickness (OT) assessment We designed and implemented a microfluidic chip integrating a Fabry-Perot resonator which allows to measure the OT profile of cells flowing through it with high throughput. To ensure the reliability of the measurement, cell trajectories should be constrained as much as possible on the same plane. A piezoelectric transducer was coupled to the chip, tuned to induce an acoustic standing wave in the chip, thus providing vertical alignment of floating cells. The same experimental setup has been exploited towards cellular mechanotyping. In fact, the size distribution collected with the system depends on the power applied to the acoustic trap, hence, it can be related to the deformability of the cell. Comparing the distribution of elasticity collected at very different values, is possible assess the overall mechanical properties with high statistical relevance aimed at measuring drugs effect or physiological and pathological condition response on this parameter. The analysis of the perturbation enables the assessment of the cell's optomechanical properties. Measurement of Algae and Yeast cells' deformability has been carried out to test the instrument's performance and compared to the equivalent perturbation introduced by Microgel beads and Polystyrene spheres as controls. The results show a change in the optomechanical properties of the Algae, Yeast, and Microgel. Notoriously, the Polystyrene sample remains virtually unchanged, as expected since Polystyrene is much stiffer than a cell and cannot be deformed by the instrument's pressure field. These results show that the acoustofluidic technique presented here is useful to detect and measure different optomechanical properties which, potentially, can be used as label-free biomarkers in clinical diagnosis. Corresponding author: julian.mejia_morales@ugent.be
Keywords: Microfluidics Cytometry, Cells Mechanics, Acoustofluidic Interferometric Device

GA.06 - Project: Biochemical and biological characterization of venom and hemolymph from scorpions *Tityus mattogrossensis* and *Tityus confluens*

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Scorpions are an order of animals that cause poisoning due to their venom and have been considered a public health problem in several countries as Brazil. Both scorpion venom and hemolymph are important for biochemical, physiological and pathological studies, as well as promising for the development of new biotechnological and pharmacotherapeutic product. The present project aims to evaluate the biochemical and biological characteristics of the venom and hemolymph of from scorpions *Tityus mattogrossensis* and *Tityus confluens*. In order to understand and characterize the effects of venom and hemolymph of scorpions, their effects on the activity of enzymes (Na^+, K^+)-ATPase and Ca^{2+}-ATPase will be evaluated. The proteolytic enzymes, phospholipase A2 and fibrinogenolytics activities will also be investigated. Furthermore, the cytotoxicity of venom and hemolymph against normal and tumor cells, and *T. cruzi* epimastigote will be assayed. The dates will be validated by statistical analysis and it is expected to improve the knowledge about these scorpions present in Mato Grosso do Sul, as well as to identify compounds with pharmacological or biotechnological potential. In addition, to contribute for the understanding of the role of these compounds in the clinical overview, promoting a more appropriate treatment of scorpionic accidents.
Keywords: Enzymes, scorpion, Tityus sp.

GA.07 - Construction of a *Rhipicephalus microplus* salivary peptide library on the surface of filamentous phages

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Rhipicephalus microplus is a tick species present in Brazil and well known to parasite cattle. High levels of parasitism can seriously affect the animals, resulting in reduced milk and meat production and causing substantial economic losses to the livestock. The control of ticks in animals is carried out mainly by using chemical acaricides from different families, but the indiscriminate use of these agents can be responsible for the emergence of resistant tick populations and the environmental contamination. Thus, it is necessary to search for alternative control methods that could be employed in herds and contribute to the reduction of parasitism levels by ticks. The main objective of the present study is to identify immunogenic targets that could be useful in control of *R. microplus* through the phage display technique. Firstly, salivary glands from partially engorged tick females were dissected and the total RNA was extracted. A cDNA library was synthetized, amplified by PCR and fragmented by a restriction enzymes cocktail. Then, purified DNA fragments were cloned into the pHORF3 phagemid. A pool of salivary glands from five female ticks (mean weight = 55mg) was used in the cDNA library construction. The cDNA fragments library was used to transform *E. coli* TOP10F' competent cells. Transformation titer of $2{,}6 \times 10^{4}$ and insert rate of 63% were observed, while most of the cloned inserts ranged from 100 to 300 bp. cDNA fragments library will be packaged in M13 phages, that will express and present the salivary peptides on its surfaces. Then, by the phage display technique, a screening of the most reactive M13 phages against IgG from tick-resistant cattle will be performed. It is expected that the selected immunogenic peptides could be useful for the development of an effective vaccine against the *R. microplus* parasitism.

Keywords: Phage display, *R. microplus*, salivary glands
Supported by: FAPESP

GA.08 - *Tityus paraguayensis* venom increases the K^+-phosphatase activity of the enzyme (Na^+/K^+)-ATPase from rat kidney

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Scorpion venoms have a great chemical diversity, and several biological activities have been described. However, there are a few studies about some species, such as the endemic scorpion *Tityus paraguayensis*. (Na^+/K^+)-ATPase is important to maintain the osmotic balance for the transport of ions through the membrane and one of the proteins that was shown to interact with (Na^+/K^+)-ATPase in the presence of ouabain is the so called melittin like protein from bee venom. Thus, this study aims to evaluate the effect of the venom from scorpion *Tityus paraguayensis* on the (Na^+/K^+)-ATPase enzyme. The venom was obtained by an electrical stimulus applied to the scorpion's telson. After this, the venom was diluted in distilled water and centrifuged, followed by lyophilization of the supernatant. Bradford assay was used for protein quantification. To evaluate the K^+ -phosphatase activity of the enzyme (Na^+/K^+)-ATPase, reactional medium was prepared with the substrate pNPP, in the presence or the absence of ouabain. The specific activity of the enzyme was obtained by the difference between in absence and presence of ouabain. The reactional medium was incubated for 50 minutes at 37 ^{o}C with 50 µg of rat kidney homogenate with or without scorpion venom. An increase of 9% and 20% in K^+ -phosphatase activity was observed in the presence of 1 and 10 ug of venom, respectively, when compared to the negative control. These results indicate the presence of components in the venom of *Tityus paraguayensis* that can modulate the (Na^+/K^+)-ATPase activity, requiring further studies for isolation and characterization of these compounds.

Keywords: ATPase, pNPP, scorpion
Supported by: CNPq, CAPES, FUNDECT and UFMS

GA.09 - Deformation-dependent relaxation induced by local viscoelasticity in DNA solution

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Micro-rheology has been widely used to measure viscoelasticity of a cell and cytoplasm. To understand the highly complexed viscoelasticity, fundamental viscoelastic properties of biopolymer solutions or gel are needed. The viscoelastic properties of DNA solution have been investigated to clarify concentration, length, and topological effects on its viscoelasticity. However, there is little knowledge to explain nonlinear relaxations such as double-exponential or power-law relaxation observed in dense DNA solution. Since there is no crosslinking between DNA molecules, the viscoelastic properties are sensitive to the degree of deformation. In this study, we focused on the effect of the degree of deformation on microstructure in dense DNA solution. We used 0.6 mg/ml Klenow-fragment-treated λ-phage DNA. We observed the relaxation process of a bead after a movement at a certain distance xm at a constant speed (1.0 um/s) by optical tweezers.xm corresponds to the degree of deformation, and we changed the xm from 0.4 to 6.5 um. We observed that the relaxation changed from a double exponential to a power law with an exponent of -0.5, as the degree of deformation increased. We found that these two nonlinear relaxations can be explained by a simple viscoelastic model with considering fluctuation of viscosity. In our model, viscous force acting on the bead was distinguished from that acting on DNA mesh structure, which enabled us appropriate evaluation of viscoelasticity. The elasticity was independent of xm, and it was equivalent to that of mesh structure. On the other hand, the viscosity increased as increasing xm, resulting from compression of the mesh structure. Micro-rheology focusing on relaxation process could give novel insights to evaluate the viscoelasticity of dense polymer solution and a cell.
Keywords: DNA solution, microrheology, viscoelasticity

GA.11 - Correlation of cellular traction forces and dissociation kinetics of adhesive protein zyxin revealed by multi-parametric live cell microscopy

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Cells exert traction forces on the extracellular matrix to which they are adhered through the formation of focal adhesions. Spatial-temporal regulation of traction forces is crucial in cell adhesion, migration, cellular division, and remodeling of the extracellular matrix. In this work, we present an approach based on a combination of several microscopies and quantitative data analysis that allowed us to explore the correlation between the generation of traction forces and zyxin dynamics at focal adhesions. To this end, we combine techniques such as traction force microscopy (TFM), fluorescence recovery after photobleaching (FRAP) and fluorescence correlation spectroscopy (FCS), in addition to the fabrication of adjustable stiffness polyacrylamide hydrogels and the characterization of their elasticity by force spectroscopy using an atomic force microscope (AFM). By cultivating cells on polyacrylamide hydrogels of different stiffness we were able to investigate the effects of substrate stiffness on the generation of cellular traction forces by TFM, and characterize the molecular dynamics of the focal adhesion protein zyxin by FCS and FRAP. As the rigidity of the substrate increases, we observed an increment of both, cellular traction generation and zyxin residence time at the focal adhesions, while its diffusion would not be altered. Moreover, we found a positive correlation between the traction forces exerted by cells and the residence time of zyxin at the substrate elasticities studied. This correlation persists at the subcellular level, even if there is no variation in substrate stiffness, revealing that focal adhesions that exert greater traction present longer residence time for zyxin. A key advantage in the approach presented here lies in the possibility of an integral and multiparametric single cell analysis. Our results provide further evidence reinforcing the mechanosensitive properties of zyxin, pointing it out as a key protein for cellular traction forces.
Keywords: imaging, traction forces, zyxin
Supported by: UBA, ANPCyT, CONICET

GA.12 - Non-invasive force measurement for axonal transport by kinesin and dynein

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Neuronal morphology necessitates particularly fast cargo vesicle transport for efficient communication between the cell body and distal processes. Kinesin superfamily proteins and cytoplasmic dynein haul cargo vesicles, such as synaptic cargos and mitochondria, anterogradely toward the terminal and retrogradely toward the cell body. The in vivo driving force produced by motor proteins acting on cargo vesicles was investigated through non-invasive force measurements based on non-equilibrium statistical mechanics. Using the method, by inferring the driving force from the fluctuating motion of transported cargo, the number of motor proteins carrying a single cargo was estimated. In this research, the number of motors was estimated for many kinds of cargo vesicles in the cases of healthy neurons and disease neurons. Primary culture of neurons was performed. After culture for 4–7 days, the neurons were transfected with the plasmid vector to label cargo vesicles. Cargo movement was observed with a fluorescence microscope. Images were obtained with an sCMOS camera at 100 frames/s. The center position of each cargo vesicle was determined from the recorded images using custom software. The force index χ was defined using the idea of the fluctuation theorem as $\chi=\ln[P(\Delta X)/P(-\Delta X)]/\Delta X$. Using the force index χ, the number of motors carrying a single cargo was estimated as 1–4 anterograde and 1–3 retrograde motors for endosome transport in mice dorsal ganglion neuron, 1-6 anterograde and 1-6 retrograde motors for synaptic cargos in mice hippocampal neurons, 1–3 anterograde FPUs for synaptic cargos in motor neurons of Caenorhabditis elegans. We found that the number of motors was changed for disease neurons. Because multi-motor cooperativity contributes to the stable and long-distance transport of materials along axons to maintain neuronal activity, the number of motors hauling a cargo is considered to be a reasonable indicator of healthy neuronal activity.

Keywords: molecular motor, axonal transport, non-equilibrium physics
Supported by: Japan Science and Technology Agency

GB - Glycobiology

GB.02 - Anticoagulant activity of a partially hydrolyzed and chemically sulfated xylan from açaí *(Euterpe oleracea)*

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Thromboembolic events are developed due to changes in the mechanisms of hemostasis and as therapeutic alternatives, heparins stand out, which are naturally sulfated polysaccharides. But, despite effective, these drugs have limitations of use. In this work, the homoxylan from açaí pulp (Euterpe oleracea Mart.), with uronic acid content of 2.8%, was used in order to obtain a chemically sulfated derivative with linear structure and low molecular weight, as an alternative to low molecular weight heparins (LMWH). Native xylan was partially hydrolyzed at three different times (30, 60 and 90 min), at 100 °C, using 0.5 M HCl, to obtain low molecular weight xylans (LMWX). LMWX were chemically sulfated for 2 h, at 50 °C, at a molar ratio of 9:1 (SO3.Pyr:OH on the polysaccharide), and the DS and the ability of the samples to alter the normal aPTT of citrated sheep plasma were evaluated. HSQC and HPSEC analyses were performed for structural elucidation and molecular weight evaluation. HSQC analyses obtained from LMWX showed 13C/1H correlations characteristic of β-D-xylan (1→4)-linked. The partially hydrolyzed sulfated xylans (LMWXS) showed DS between 0.35 and 0.39. Dose-response curves showed that for each increase of 1 µg/mL of LMWXS, aPTT increased around 14.72 to 18.30 s, an anticoagulant effect greater than that observed for enoxaparin, a LMWH. LMWXS had homogeneous elution profiles in HPSEC, with molecular weights higher than 4.786 x 104 g/mol. However, previous studies have shown that sulfated polysaccharides with a molecular weight around 1 x 104 g/mol are suitable for use as subcutaneously administered anticoagulants, as occurs with LMWH. Despite the remarkable anticoagulant activity of LMWXS, it is still necessary to reduce the molecular weight of LMWX in order to obtain a compound suitable for subcutaneous use, such as LMWH.
Keywords: aPTT, chemical sulfation, polysaccharides
Supported by: CAPES and CNPq

GB.04 - Effect of oxidative stress in syndecan-1 of endothelial cells.

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Oxidative stress is defined as a state of overproduction of reactive oxygen species (ROS) that results in pathophysiological changes. Numerous diseases are associated with oxidative stress, such as cardiovascular events that are the first cause of death globally. In vascular diseases, oxidative stress seems to affect glycocalyx. This endothelial dysfunction is one of the first changes that give rise to atherosclerosis. Syndecans are a family of heparan sulfate proteoglycans and are present in the glycocalyx of various tissues. The decrease of *syndecan-1* in vessels leads to cell stiffness, alteration in microvascular tone, leucocyte adhesion, and thrombosis. Analyze *syndecan-1* expression during oxidative stress. ECV-340, a human endothelial cell line, was treated with hydrogen peroxide (exogenous ROS) in different doses and incubation times. Gene expression analysis was performed using qPCR. In addition, ECV-340 was treated with an inhibitor of glutathione synthesis that raises endogenous ROS, L-buthionine sulphoximine (BSO), in different doses. The increase of ROS was confirmed by 2',7'–dichlorofluorescein diacetate. Besides, the *syndecan-1* was analyzed by flow cytometry. There was a dose-dependent decrease of *syndecan-1* mRNA levels with 3 and 18 hours of hydrogen peroxide incubation. The *syndecan-1* also decreased with BSO treatment. The flow cytometry confirmed the decrease of *syndecan-1* in the cell surface. It is well known that ROS raises sheddases levels; however, our results demonstrated that ROS also may alter *syndecan-1* gene expression. This study shows some perspective about the alteration of *syndecan-1* gene expression by ROS. Oxidative stress seems to alter gene expression of *syndecan-1* in the ECV-340 cell line.
Keywords: Proteoglycans, Reactive oxygen species, Vascular endothelium
Supported by: CNPq, FAPESP, CAPES.

GB.05 - Analysis of genes that co-express with versican in invasive breast carcinoma

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Breast cancer is one of the major public health problems worldwide, as it not only has a high mortality rate, but is also the most incident in the world among women. Understanding the mechanisms of carcinogenesis and tumor progression is essential to mitigate this disease. Versican (VCAN), a proteoglycan present in the extracellular matrix (ECM), is not expressed in normal breast tissue, but is largely produced by the stroma in the tumor environment, showing the importance of VCAN in breast tumor carcinogenesis. It can be cleaved by proteases, such as matrix metalloproteinase-2 (MMP2), forming two active fragments: the G1 fragment that can modulate the cell cycle, and the G3 fragment that binds to growth factors and other ECM molecules. Although VCAN is important in carcinogenesis, its gene expression regulation has not yet been fully elucidated. Investigate the genes that co-express with the VCAN gene to give some insight into VCAN regulation. The study was conducted using Microarray and RNA-Seq data from two distinct invasive breast carcinoma databases, The Cancer Genomic Atlas (TCGA), Nature 2012 (n=460) and Metabric, Nature 2012/2016 (n=1904). The data was analyzed using the cBioPortal software. Regardless of breast tumor subtype or staging, linear regression analyses showed a statistically significant correlation between MMP2 and VCAN gene expression in invasive breast carcinoma tissues, both in the TCGA database [$p<0.001$; $q<0.001$; $R=0.846$], and in the Metabric database [$p<0.001$; $q<0.001$; $R=0.701$]. The mechanism of substrate and enzyme being mutually regulated is very common in various cells and tissues. Confirming bioinformatics analysis, qPCR with different breast cancer cell lines was performed (MCF-7, SKBR3, MDA-MB-231). These results give a better understanding for elucidation of VCAN modulation, since MMP2 and VCAN genes show statistically significant coexpression in breast neoplasm.
Keywords: bioinformatics, breast cancer, proteoglycans. **Supported by:** CNPq, CAPES, FAPESP

GB.06 - Dermatan sulfate: antithrombotic effects and stimuli of heparan sulfate proteoglycan synthesis from endothelial cells

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Dermatan sulfate (DS) is an anionic, linear, and structurally complex polysaccharide belonging to the glycosaminoglycans (GAG) class, found in a wide variety of tissues in many species attached to a core protein as proteoglycans (PG). DSPGs have been implicated in various cellular processes and cell-matrix interactions. DS chains may also influence the coagulation process, binding to serum proteins and inhibiting thrombin (interacting with HCII). This study aimed to investigate DS effects upon coagulation hemostasis. DS from porcine skin (PS) and bovine cornea (BC) extracted by proteolysis and purified by anion exchange chromatography were characterized by agarose gel electrophoresis, molecular mass, chemically (hexosamine, uronic acid, and sulfate contents), and spectroscopically (IR, RAMAN, and RMN). The purified polysaccharides were evaluated in experimental animal models of thrombosis and the stimulation of heparan sulfate proteoglycan synthesis by endothelial cells in culture. The purified polysaccharides have a distinct polymeric structure. BC-DS showed the highest molecular mass and lower sulfate: hexosamine ratio (DS-BC=0,802, DS-PS=1,189), also it seems to have the highest content of IdoA when compared with PS-DS (GlcAC/HEX: DS-BC=0,619, DS-PS=1,109). Although the spectroscopical analysis of the purified carbohydrates showed similar spectra, it is possible to find some subtle differences that might be influenced by the chemical environment on each of their polymeric structure. No other contaminants were identified on the samples. DS-BC and DS-PS showed similar effects on coagulation, with BC-DS ceasing thrombi formation with a lower dose (50µg/g) compared to DS-PS (75µg/g). Both GAGs were capable of stimulating the synthesis of endothelial HSPG secreted to the medium. We highlight the findings on antithrombotic properties of DS from PS and BC, and presented a new result regarding its properties on the endothelial cell wall, stimulating the synthesis of HS, which have been found to promote angiogenic responses of endothelial cells beyond its antithrombotic properties.
Keywords: Dermatan sulfate, Thrombosis, Coagulation

GB.07 - Alteration on glycated human erythrocyte by *in vitro* action of anesthetics: propofol, remifentanil, and vecuronium

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Glycation refers to slow, non-enzymatic, and spontaneous reactions between the amino groups of proteins and the carbonyl group of a reduction sugar, like glucose. In pathological conditions, such as diabetes, the glucose level in the blood is high. Due to this hyperglycemia, glycation occurs on the red blood cells (RBC), resulting in possible alterations of erythrocyte viscoelasticity. Several authors have demonstrated that anesthetic drugs commonly used in surgery (propofol, remifentanil, and vecuronium) may affect the stationary viscoelastic parameters of red blood cells from healthy donors. This work aimed to evaluate the hemorheological effect of anesthesia in hyperglycemia. Therefore, RBCs from healthy donors were incubated with glucose solutions 0,5% v/v for 5 hours at 37°C under constant stirring. After the glycation procedure, the RBCs were incubated separately and combined with propofol (P, 4 µg/ml whole blood), remifentanil (R, 10 ng/ml plasma), and vecuronium (V, 0.15 µg/ml plasma) for 30 minutes at 37°C under constant stirring. The Reómetro Eritrocitario was used to determine the viscoelastic parameters, obtaining the erythrocyte elastic modulus (µ), surface viscosity (η), and deformability index (DI). Statistical analysis shows a significant decrease of µ for RBC incubated with R and RV anesthetics ($p<0.05$). Nevertheless, µ significant increase for RBC treated with P and PR ($p<0.05$). The η values increase in RBC treated with PR and PV ($p<0.05$), and V ($p<0.001$). The DI was increased for RBC, glycated and no glycated, when treated with R and RV ($p<0.01$). However, DI decreases for RBC glycated and incubated with P ($p<0.05$). These results suggest an interaction of these anesthetics with the RBC, which could alter the viscoelastic parameters of the erythrocyte membrane at different levels. Consequently, these results would be a contribution to the prevention of complications in surgical procedures.

Keywords: anesthetic drugs, glycated erythrocyte , viscoelastic parameters
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GB.08 - New bovine intestinal heparin derivated molecule with low anticoagulant activity and its anti-tumoral activity

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During the last few years, heparin's biological effects have been incorporated, which go beyond its anticoagulant activity, such as anti-inflammatory, antiviral, and anti-tumor effects. However, its use for these purposes is limited due to its potential hemorrhagic effect. In this context, heparinoid molecules have been researched in order to obtain molecules with low anticoagulant activity and other therapeutic effects. With this in mind, in our laboratory we were able to purify a fraction of intestinal bovine heparin with low anticoagulant activity (LABH). In the present work, our objective is to test this molecule for an antitumor activity. link the ectopic tumor growth model with Lewis lung carcinoma developed on the back of C57BL/6 mice treated with daily injections at a dose of 8mg/kg subcutaneously for 4 weeks. Treatment with LABH reduced: a) cachexia, b) tumor complication, c) death and delayed tumor growth. LABH showed anti-tumor action in this animal model. Further studies are necessary in other models and at other doses in order to investigate the full potential benefit of this molecule.

Keywords: heparin, cancer, coagulation
Supported by: FAPERJ and SENAI

GB.09 - Extraction, isolation, characterization and biological activity of sulfated polysaccharides present in ascidian viscera *Microcosmus exasperatus*

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Ascidians are marine invertebrate tunicates that synthesize sulfated glycosaminoglycans (GAGs) within their viscera. Ascidian GAGs are considered analogues of mammalian GAGs and possess great potential as bioactive compounds, presenting antitumor and anticoagulant activity. Due to their clinical potential, it is important to understand how ascidian GAGs are produced, their function within the ascidian organism and how they keep their bioactivity after extraction and purification processes. Mammalian heparin is one of the main compounds used to treat thrombosis and related diseases due to their anticoagulant activity. However, heparin presents adverse effects, in this context, ascidian GAGs are proposed as good alternatives for mammalian heparin and, therefore, should be carefully studied. Our main objectives are to study the ascidian *Microcosmus exasperatus* regarding GAGs composition, structure, distribution within the viscera and also its biological activity. Ascidians were collected by free diving. GAGs were extracted by proteolytic digestion and purified by ion-exchange liquid chromatography and characterized by agarose gel electrophoresis and enzymatic treatments. Anticoagulant activity was evaluated by aPTT assays. Cell cytotoxicity was evaluated by MTT assays using two tumor cell lines (LLC and MC-38). Tumor cell migration activity was evaluated *in vitro* by wound healing assays. Our results show that *M. exasperatus* presents three distinct polysaccharides. These polysaccharides were fractionated into two fractions named PS 1 and PS 2. *M. exasperatus* produces a low anticoagulant dermatan sulfate (PS 2) and a heparin-like (PS 1) compound, which, interestingly, is not susceptible to heparinases treatment and does not present significant anticoagulant activity. PS 2 shows mild cytotoxicity in LLC tumor cells when in combination with manganese, however it has no effect on the invasive potential of LLC and MC-38 tumor cells. As a conclusion, we hope to establish *M. exasperatus* GAGs as suitable compounds for future preclinical studies in cancer and vascular disease areas.
Keywords: Microcosmus exasperatus, sulfated glycosaminoglycans, ascidians
Supported by: CNPq, FAPERJ, CAPES, Fundação do Câncer, UFRJ and IFRJ

GB.10 - Optimization of anticoagulant activity of chemically sulfated citrus pectin

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Sulfation and other chemical modifications of polysaccharides can lead to better anticoagulant activity, which is important in searching alternatives to heparin. Citrus pectin (CP) has great potential for this purpose. Therefore, this research aims to optimize chemical sulfation and carry out other modifications in the CP structure in order to understand and further improve its performance. Commercial CP was subjected to dialysis and partial acid hydrolysis, obtaining high molecular weight pectin (CPHW – 298,8 kg/mol) and low molecular weight pectin (CPLW – 7,53 kg/mol). Both fractions were subjected to optimization of chemical sulfation, modifying the molar ratio of sulfation agent to hydroxyl group on the polysaccharide (ηSO3-Pyridine/OH), the total reaction volume to weight of sample (Vt/w) and the reaction time. Anticoagulant activity was evaluated by the ability of derivatives to increase aPTT. The high and low molecular weight sulfated derivatives with the best anticoagulant activities, CPHWS1 and CPLWS1, were obtained with ηSO3-pyridine/OH=9, Vt/w=100 mL/g, and 8 h and 4 h of reaction, respectively. Subsequently, CPHWS1 and CPLWS1 were carboxyl-reduced (CR; 1 and 4 cycles each), resulting in lower anticoagulant activities, reinforcing the role of carboxyl groups in bioactivity. The sulfated and carboxyl-reduced samples were resulfated (S2), using optimized parameters. The resulting derivatives showed better anticoagulant performances, indicating that replacement of carboxyl by sulfate groups favors anticoagulant activity. The derivatives that showed the best results were CPHWS2CR4 (aPTT at 40 µg/mL=226,2 s; DS=1,27) and CPLWS2CR1 (aPTT at 50 µg/mL=187,1 s; DS=1,30). Interestingly, they were able to inhibit FIIa and FXa without antithrombin, with the effect being increased in the presence of antithrombin, especially on FIIa. New approaches for the development of citrus pectin-based anticoagulants were presented. The optimization of chemical sulfation and the other changes made in CPHW and CPLW have shown to influence their anticoagulant activities. **Keywords:** Anticoagulant activity, Citrus pectin, Polysaccharides. **Supported by:** CNPq

GB.11 - Anticoagulant activity of partially hydrolyzed, oxidized and chemically sulfated guar gum

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Heparin is a naturally sulfated polysaccharide widely used as anticoagulant. Despite its importance, it has some disadvantages such as its high cost, side effects, and risk of contamination. Therefore, researchers seek development of alternative anticoagulants. In the case of polysaccharides, the sulfation process is important to promote anticoagulant activity, but little is known about the importance of uronic acids, which are also present in heparin structure. Guar gum, when partially hydrolyzed and chemically sulfated, showed promising anticoagulant effect in previous studies. Therefore, this paper aimed to evaluate whether the presence of uronic acids in partially hydrolyzed and chemically sulfated guar gum would contribute to the anticoagulant action. Thus, native guar gum (GG) was submitted to partial acid hydrolysis, giving rise to GGH (Mw = 1.47 x 10^4 g/mol). Methylation and HSQC analyses confirmed the galactomannan structure. GGH was oxidized in a pH 10 buffered system using the oxidant 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) and co-oxidant chloroisocyanuric acid (TCCA), giving fractions with low (GGHOP) and high (GGHOT) degree of oxidation according to the amount of co-oxidant used. All fractions were chemically sulfated and the derivatives evaluated for anticoagulant activity using aPTT, PT, TT assays. The anticoagulant effect was inversely proportional to the degree of oxidation, with the sulfated but not oxidized derivative GGHS1 showing considerably better results. It is noteworthy that GGHS1 showed better anticoagulant activity than the low molecular weight heparin enoxaparin. All fractions had a similar mechanism of action, inhibiting mainly FIIa, especially in the presence of antithrombin. In addition, they were able to respond to the protamine antidote. These results indicated that the presence of uronic acids (after the oxidation process) in partially hydrolyzed and chemically sulfated guar gum decreases its anticoagulant activity, reinforcing the anticoagulant potential of GGHS1.
Keywords: Anticoagulant activity, Selective oxidation, Guar gum
Supported by: CNPq

GB.12 - New bovine intestinal heparin derivated molecule with low anticoagulant activity and its anti-metastatic activity

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Evidence has emerged that the biological effects of heparin go beyond the anticoagulant activity. Studies have demonstrated anti-inflammatory, anti-viral, anti-tumor effects. However, it is not possible to use it for these therapeutic purposes due to its potential hemorrhagic effect. In view of this, new molecules have been researched in order to bypass this obstacle. Our laboratory we were able to purify a low anticoagulant activity fraction of bovine intestinal heparin (LABH). In this work, our goal is to test this molecule for anti-metastatic activity. Lung metastasis model induced by intravenous injection of Melanoma cells in C57BL/6 mice previously treated with a single dose of LABH (2, 4, 8 or 20 mg / kg) or saline, subcutaneous administered one hour before the melanoma cells injection. After 4 weeks of follow-up, the lungs were weighed and the metastatic foci counted. Results: Treatment with LABH reduced: a) cachexia, b) mortality and c) amount of lung metastasis. Treatment with LABH reduced: a) cachexia, b) mortality and c) amount of lung metastasis. LABH showed anti-metastatic action in this experimental model. Further studies in other models and other doses are necessary in order to investigate the full potentiality of the benefit of this molecule.
Keywords: heparin, cancer, coagulation
Supported by: FAPERJ and SENAI

GB.13 - Polysaccharides isolated from *Piper regnelli* (Pariparoba) leaves: structural characterization and antinociceptive activity

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Piper regnellii is a medicinal plant popularly known as pariparoba and is widely used to treat pain, inflammation, ulcer among others. Amongst the bioactive molecules isolated from plants are the polysaccharides, biopolymers with biological potential to treat many conditions and diseases, as described in scientific literature. Little is known about *P. regnellii'* polysaccharides. This study aims to extract, purify and characterize the polysaccharides of the plant leaves and evaluate its antinociceptive activity. From the aqueous extract of *P. regnellii* leaves (decoction; 15 minutes under boiling), followed by a number of purification processes, such as ethanolic precipitation, freeze thawing and ultrafiltration, was obtained a purified polysaccharide fraction, named PR30R (fraction retained in membrane with 30 kDa cut-off). The elution profile in HPSEC of PR30R was found to be homogeneous, with a calculated molar mass of 164.000 g/mol. The monosaccharide composition analysis by GC-MS revealed that PR30R was mainly constituted by galactose (51.5%), arabinose (24.9%) and galacturonic acid (11.3%). NMR and methylation analysis showed that PR30R is constituted by a type II arabinogalactan (AGII), with a backbone of β-D-Galp-(1→3) units, O-6 substituted by β-D-Galp-(1→6) side chains, which are O-3 substituted by non-reducing ends of α-L-Araf, and by a type I rhamnogalacturonan (RGI), composed of a backbone of α-D-GalpA-(1→4) units intercalated by α-L-Rhap-(1→2). By integrating the NMR spectra signals of methyl and non-methyl esterified galacturonic acid units, we were able to calculate the degree of esterification of the RGI as 70.5%. Finally, PR30R was evaluated for its antinociceptive activity, using the acetic acid-induced writhing model in mice. We found that the fraction, at a dose of 0.1096 mg/kg, was able to reduce the number of abdominal writhes by 56% when compared to vehicle group, confirming the contribution of *P. regnellii* polysaccharides to the popular use of the plant leaves tea to treat pain.

Keywords: Antinociceptive Activity, Piper regnellii, Polysaccharides
Supported by: CNPq

GC - Microorganisms and Pathogens

GC.01 - HOST IMMUNE RESPONSE PARTICIPATES IN MALARIA ACUTE KIDNEY INJURY: ROLE OF CD8+ T CELLS

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Severe malaria is attributed to, *Plasmodium falciparum* infection and entails different pathologies caused directly by parasite infection and host immune response. Malaria acute kidney injury (MAKI) is characterized by glomerular and tubular damage. This process is attributed to oxidative stress and obstruction of renal microvasculature by aggregates of parasitized erythrocytes. Overactivation of the host immune response is also postulated, however its precise role in MAKI is still unknown. The objective of the work was to evaluate the participation of T cells in MAKI pathogenesis. For this, we performed adoptive transfer of splenocytes-derived T cells from C57Bl6 mice infected with *P. berghei* ANKA to healthy acceptor animals. Renal function as well as homing and immune response were assessed. Adoptively transferred T cells induced proteinuria (2-fold) and increased UPCr (protein and creatinine ratio; 2.3-fold). Markers of glomerular injury, creatinine clearance, plasma creatinine and plasma urea, did not change. However, we observed an increase in γGT activation in urine (1.6-fold), a marker of renal tubular damage. These results indicate that malaria-responsive T cells induce renal tubular damage without glomerular involvement. Accordingly, there was a remarkable increase in T cell homing to the kidneys, as well as spleen and brain. Moreover, we observed increase in renal proinflammatory cytokines INFγ (2-fold), IL-17 (1.3-fold) and IL-6 (1.5-fold). FACS analysis revealed an increase in the frequency of CD8+ T cells in the kidney, which was accompanied by increased expression of perforin in the renal cortex (1.7-fold). These results indicate that CD8+ T cells are activated during malaria infection and can migrate to the kidney besides brain and spleen. In the kidney, perforin production has a role in inducing renal tubular damage. This work adds new insights into the pathogenesis of MAKI describing it as a consequence of host exacerbated immune response.

Keywords: malaria, kidney injury, immune response

Supported by: Faperj, CNPq e Capes

GC.02 - Effects of Natural Compounds on the Peroxidase Activity of AhpCs from Pathogenic Bacteria

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The resistance of pathogenic bacteria to various antibiotics has increased worldwide in recent years, requiring new antibacterial compounds. Recently, some studies have shown that different antibiotics have the convergent capacity to produce reactive oxygen species (ROS), which and together with the host's oxidative defenses contributes to pathogen annihilation. Bacterial pathogenic cells have several antioxidant enzymes can decompose the ROS and minimize the host defenses and antibiotic oxidant properties. The thiol peroxidase AhpC is considered the major hydroperoxide scavenger in some bacteria species and may represent inhibiting targets to combat bacterial infections. The high reactivity of AhpC relies on a very reactive cysteine (C_P) together with two polar residues (Thr/Ser and Arg) makes up the catalytic triad. The objective of this work is to identify natural compounds can interfer with the AhpC from Pseudomonas aeruginosa (PaAhpC). PaAhpC was recombinantly expressed and purified by IMAC. The effects of four compound on PaAhpC activity were evaluated by the NADPH oxidation. The results revealed that a compound, named here as CN-ABP1, acted as an PaAhpC inhibitor since decreased significatively the initial decomposition rates (v0PaAhpC = 0.22 ± 0.01 μs^{-1} and v0PaAhpC+CNABP1 = 0.09 ± 0.02 μs^{-1}) and total NADPH consumption after 300sec (PaAhpC = 93µM and CN-ABP1 = 24.30µM, approximately). To better understand this result, we performed molecular docking analysis and our results indicate that CN-ABP1 can be stabilized in the microenvironment of the PaAhpC active site (ΔG = -6.3 to -7.4kcal/mol) by several hydrophobic and polar interactions, including the amino acids from the catalytic triad. Our results also suggest that the inhibition occurs by a Michael addition between an α, β unsaturated carbonyl system of the natural compound and the sulfur of the CP AhpC. Approaches for determining IC_{50} and evaluating the effects of CN-ABP1 on bacterial cells (MIC_{50}) are underway.
Keywords: Inhibitor , Natural compounds, Peroxiredoxins
Supported by: FAPESP.

GC.03 - CHARACTERIZATION OF THE ORAL VIROME IN HEALTH AND PERIODONTITIS

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The oral cavity encompasses a highly complex microbiome composed by a wide variety of microorganisms (bacteria, fungi, protozoans and viruses). The interactions between the different components of the oral microbiota can trigger inflammation in the periodontium, leading to the development of periodontitis, a disease that causes significant morbidity at national and global levels. In this sense, this project aims to study the viral genetic material (viroma) present in the oral cavity of healthy individuals and patients harboring periodontitis. For this purpose, 27 saliva samples from healthy (n = 13) and periodontitis individuals (n = 14) were collected and their genetic material was extracted and quantified. The metagenome was submitted to next generation DNA sequencing on the Ilumina Hiseq2500 platform. After nucleotide sequence quality control, reads were paired into larger contiguous sequences (contigs) using the PEAR program, and later annotated with the Kaiju program coupled with the NCBI Refseq virus reference database. Taxa quantification and tabulation was performed using R program packages. The two hundred most abundant taxa were submitted to Linear Discriminant Analysis (LDA) through the LefSe software, on the Galaxy/Hutlab platform. Overall, the majority of viruses corresponded to bacteriophage species belonging to the Myoviridae and Siphoviridae families. Especifically, Streptococcus spp., Enterococcus spp. and Eggertella spp. phages were most prevalent in the healthy group (LDA ≥ 3.0), while Escherichia spp. and Bacillus spp. phages along with microalgae and protozoan viruses (such as Chrysochromulina ericina, Only Syngen Nebraska, Cafeteria roenbergensis, and Acanthamoeba polyphaga viruses) were most abundant in the periodontitis group (LDA ≤ -3.0). The results helped us gather a better understanding of the nature of the oral virome, revealing previously unreported viruses associated with disease onset and homeostasis. All in all, we believe that the taxonomic units denoted here may represent important biomarkers for future research on oral microbial ecology. **Keywords:** Oral, Virome, Virus.
Supported by: Fapeam

GC.04 - Combination effect of isoobtusilactone A and benznidazole on *Trypanosoma cruzi*

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Isoobtusilactone A is a butanolide obtained from the active extract of the xylopodium of *Aiouea trinervis* Meisn. (Lauraceae), which grows in the Cerrado biome of Mato Grosso do Sul, Brazil. This compound has already shown lethal effect and a fast action mechanism on *Trypanosoma cruzi*, the etiological agent of Chagas Disease. The current treatment of Chagas Disease in Brazil is done with benznidazole, a drug known by its several side effects and its barely activity on the chronic stage of infection. For being the main approved drug for use in Brazil, benznidazole still being target of many studies, which seek its effects under combinations with other drugs. The aim of this work was to avaliate the combination effect of isoobtusilactone A and benznidazole on epimastigotes forms of *T. cruzi*. A combination assay was done with different proportions (1:3; 1:1; 3:1) and concentrations (2 µM – 12 µM and 5 µM – 60 µM) of isoobtusilactone A and benznidazole, respectively, in 96-well plates during 24h at 28ºC. Viability after was determinated through MTS assay. The results showed that proportions belonging to IC_{90} had synergistic effect (CI < 1), and all others concentrations belonging to IC_{25-75} had almost additive effect (CI = 1) or antagonist effect (CI > 1). To achieve a synergistic effect, the two drugs needed to be administered in higher concentrations. Despite that, isoobtusilactone A was able to reduce up to 5 times (DRI) the amount of benznidazole needed to reach IC_{90}, which can influence the reduction of side effects attributed to benznidazole. The current work is unprecedented in evaluating the effect of combining a butanolide with benzonidazole and has reinforced our previous data that isoobtusilactone A holds promise in the search for new trypanocidal agents.

Keywords: Benznidazole, Combination assay, *Trypanosoma cruzi*

GC.05 - PROJECT: The *in vitro* role of *Trypanosoma evansi* Rad51 in hydrogen peroxide resistance

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Trypanosoma evansi causes the “Surra” disease in animals like equines, cattle, sheep, goats and pigs. This sickness present non-specific clinical signs such as weight loss, anorexia, anemia, drop in milk production and reproductive disorders. Homologous recombination (HR) is able to repair DNA double-strand breaks and damaged replication forks. In HR repair, in which RAD51 protein plays important roles, a homologous DNA sequence serves as a model for restoration of lost sequence information at the break site. Regarding RAD51 in trypanosomatids, one study showed that overexpression of RAD51 in *Trypanosoma cruzi* promoted increased resistance against hydrogen peroxide treatment (a potent cytotoxic agent). Thus, this work aims to explore TevRad51 (Rad51 protein from *T. evansi*) in DNA repair and cell survival in *T. evansi*. (1) Clone and express the *TevRad51* gene; (2) Develop antibodies against the TevRad51 protein and perform immunofluorescence assay; (3) Establish *in vitro* culture of *T. evansi*; (4) Perform *in vitro* cytotoxicity assay with *T. evansi* and evaluate the *TevRad51* expression using the developed antibodies. *TevRad51* gene was inserted in pGEM-T Easy and will later be inserted in pET-28 a (+) vector for protein expression in *Escherichia coli*. Rats will be immunized with the TevRad51 protein for production of antibodies. *TevRad51* expression in *T. evansi* during cytotoxicity assay will be evaluated using the developed antibodies. Temporary *T. evansi* culture was standardized with Ham’s F12 and DMEM culture mediums. The *T. evansi* survive for up to 8 days in temporary *in vitro* culture established. Colony PCR showed positive colony for *TevRad51* after ligation gene in pGEM-T Easy vector and bacterial transformation by electroporation. The cytotoxicity assays and *TevRad51* expression evaluation will contribute for understanding TevRad51 role in *T. evansi* survival.

Keywords: DNA repair, TevRad51 , *Trypanosoma evansi*
Supported by: FAPESC (Edital nº 27/2020), CNPq.

GC.06 - A Family of T6SS Antibacterial Effectors related to L,D-transpeptidases Targets the Peptidoglycan
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Type VI secretion systems (T6SSs) are contractile nanomachines widely used by bacteria to intoxicate competitors. *Salmonella* Typhimurium encodes a T6SS within the *Salmonella* pathogenicity island 6 (SPI-6) that is used during competition against species of the gut microbiota. Characterize a new SPI-6 T6SS antibacterial effector containing DUF2778 (STM14_0336) and its cognate immunity protein containing DUF2195 (STM14_0335). Bioinformatic analyzes of the SPI-6 T6SS cluster revealed a putative effector and immunity protein pair. Effector was cloned with or without N-terminal PelB periplasmic localization sequence and its toxicity was analyzed by microscopy. Searches for DUF2778 homologs at NCBI nr database established its evolutionary relationship. Effector was expressed as recombinant protein for enzymatic assays using muropeptides as substrates and analyzed by RP-HPLC coupled to MS. STM14_0336, renamed Tlde1 (T6SS L,D-transpeptidase effector 1), was toxic in target-cell periplasm. Its toxicity was neutralized by co-expression with immunity protein Tldi1 (T6SS L,D-transpeptidase immunity 1) (STM14_0335). Time-lapse microscopy revealed that intoxicated cells displayed altered cell division, swelling and lysis, indicating cell wall damage. Bioinformatics analysis showed that DUF2778-containing proteins comprise a superfamily evolutionarily related to L,D-transpeptidases that further divided into three families (Tlde1a, Tlde1b, Tlde1c). Point mutations on conserved His121 and Cys131 residues eliminated toxicity. Co-incubation of purified Tlde1 and peptidoglycan tetrapeptides showed that Tlde1 displays L,D-carboxypeptidase activity,cleaving GM-tetrapeptides between *m*DAP[3] and D-Ala[4]. Results suggest that Tlde1 promotes depletion of acceptor GM-tetrapeptides, thus preventing formation of new crosslinks and weakening the peptidoglycan mesh structure. Tlde1 comprise a new family of antibacterial effectors with L,D-carboxypeptidase activity evolutionarily related to L,D-transpeptidases. DUF2778 superfamily is widespread in Proteobacteria.
Keywords: peptidoglycan, toxin, T6SS. **Supported by:** FAPESP

GC.07 - A New Synthetic Antimicrobial Peptide Bioinspired from a Plant Protein is Active against Staphylococcus saprophyticus biofilms
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Bacterial resistance is the cause of overwhelming number of deaths, a public health problem. One of the causes of bacterial infections, mainly in the urinary tract, is *Staphylococcus saprophyticus*. Furthermore, *S. saprophyticus* biofilms are naturally resistant against the available antimicrobial therapies. Antimicrobial peptides (AMP) are short-chain amino acid molecules with a broad spectrum of activity. Considering the urgent need for discovery of compounds with antibiofilm activity, we investigated antibiofilm properties of a new AMP, designed from the *Inga laurina* trypsin inhibitor (ILTI) amino acid sequence, named KWI-19. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of KWI-19 against *S. saprophyticus* ATCC 49453 was evaluated according to CLSI Protocol, using a KWI-19 solution prepared in a sterile 0.9% NaCl solution and serially diluted (from 10 to 0.02 μM). The positive control was carried out with vancomycin (from 87 to 0.17 μM), the negative control was prepared with MH broth and bacterial suspension. The effects of KWI-19 on the inhibition of *S. saprophyticus* ATCC 49453 biofilm formation and the eradication of mature biofilm were also evaluated. The biofilm viability was quantified as a percentage of the total number of viable colony-forming units (CFU). KWI-19 presented MIC and MBC of 1.25 and 2.5 μM, respectively, while vancomycin presented MIC and MBC of 0.68 μM and 1.36 μM, respectively. The peptide inhibited 40.6% and 46.6% of the biofilm formation at 1.25 and 12.5 μM, respectively. KWI-19 eradicated 48% and 57.9% of 24-h mature biofilms at 1.25 and 12.5 μM, respectively. KWI-19 inhibited the biofilm formation better than vancomycin at MIC, suggesting the potential use of AMP to control biofilm infections. Therefore, KWI-19 showed antibiofilm potential to control *S. saprophyticus*. The potential of KWI-19 to control infections through *in vivo* models can be investigated in the future. **Keywords:** peptide, antibacterial, antibiofilm. **Supported by:** FUNDECT, FINEP, CNPq and CAPES

GC.08 - Antioxidant activity and antimicrobial effect of lyophilized extract of Macrocybe titans giant mushroom

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Over the past few decades, the rise and spread of bacterial and fungal resistance to most clinically available antimicrobial agents, has reached alarming rates. In Brazil, the macrofungus *Macrocybe titans* was recently reported and there are no records of its antimicrobial potential. Based on that, this work aimed to evaluate the antimicrobial and antioxidant activity of the lyophilized aqueous extract of *M. titans* on ATCC strains of pathogenic bacteria and fungi, *Bacillus cereus* 10876, *Salmonella Typhimurium* 14028, *S. enteridiidis* 13076, *Listeria monocytogenes* 19111, *Escherichia coli* 25922, *E. coli O157:H7* 43888, *Pseudomonas aeruginosa* 27853, *Staphylococcus aureus* 6538, *B. subtilis* subspecie *Spizizinni* 6633, *Klebsiella pneumoniae* 13883, *Candida albicans* (INCQS 40006), *C. krusei* (INCQS 40147), *C. parapsilosis* (INCQS 40038), *C. glabrata* (INCQS 40136) *C. tropicalis* (750) and one clinical isolate of *Trichosporon* spp. Aqueous extracts of the mushroom were prepared from dried fruting bodies, grounded and diluted in water and then lyophilized. The antimicrobial and antifungal effects were evaluated by the disk-diffusion method, based on CLSI M2-A12 Vol. 35, No 1 and CLSCI, M44-A2 protocols. Antioxidant potential was determined by scavenging free radicals (DPPH), metal reducing power (FRAP), phenolics and flavonoids. The results obtained with the use of the aqueous extract of *M. titans* demonstrated a great antimicrobial potential, significantly inhibiting ten bacterial as well as the six yeast tested strains. It was also verified that the extract presented a high rate of phenolic compounds, flavonoids and antioxidant activity. Thus, the results allowed us to state that the aqueous extract of *M. titans* presented high potential as an antimicrobial agent, requiring the continuation of the study of purification and structural elucidation of its chemical components.
Keywords: Macrocybe titans, disk-diffusion, antimicrobial activity

GC.09 – PROJECT: The effect of diaryl disulfides on the replicative and infective forms of *Trypanosoma cruzi*

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Diaryl disulfides represent a class of synthetic compounds, which have shown an inhibitory action on tubulin protein synthesis in several human neoplastic cell lines, including the MCF-7 lineage. Our Research Group has been studying these compounds and recently it was found that they also have inhibitory activity on replicative forms (epimastigotes) of *Trypanosoma cruzi*, the etiologic agent of Chagas Disease (CD), and reduced cytotoxicity on Vero cells. Currently, the only drug available in Brazil for the treatment of CD is Benznidazole, but although this drug has been used for approximately five decades, it does not have a fully elucidated mechanism of action, it has several side effects and reduced efficacy in the treatment of the chronic phase of the disease. Due to the high toxicity that this drug has, combined with the lack of therapeutic alternatives, it is necessary to search for new bioactive compounds to combat this neglected disease. Therefore the objective of this work will be to evaluate the effect of diaryl disulfides on the replicative and infective forms of *T. cruzi*. For that, five compounds of the diaryl disulfides class will be tested in order to evaluate their trypanocidal or trypanostatic effect, through the recovery assay. Epimastigote forms will be treated during 4h of incubation with high concentrations of compounds. After removing the drugs, the growth of the parasites will be monitored through daily counts, in a Neubauer chamber, up to 168 h. The effect of these compounds against the trypomastigote form will also be tested. Analyzes will be performed with the aid of Graph Pad Prism 7 and Microsoft Excel 2013 software. It is hoped that this work can contribute more information about the effects of diaryl disulfides on replicative and infective forms of *T. cruzi*, in addition to suggesting possible mechanism of action.
Keywords: Diaryl disulfides, *Trypanosoma cruzi*, trypomastigotes

GC.10 - Can a *Proteus mirabilis* exotoxin cause neuroinflammation? Evidence of a new role for the urease of *P. mirabilis*.

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Proteus mirabilis is a gastrointestinal bacillus and an opportunistic uropathogen. The infection for *P. mirabilis* typically leads to formation of stones in the urinary tract and catheter-associated urinary infections. The urease of *P. Mirabilis* (PMU) produces ammonia that induces alkalinization of the urine leading to precipitation of urinary salts and formation of stones, which protect the entrapped bacteria. Proteus spp. have been described also as the causative agent of several diseases such as several types of meningitis and is currently associated with Parkinson's disease. Better understanding of the contributions of PMU not only to urinary infections, but also to the extra-urinary pathologies associated with this bacterium. All assays were performed with three cell lineages: human neuroblastoma SH-SY5Y, murine microglial BV-2 and human embryonic kidney HEK293 and three doses of PMU were used: 63, 126 and 252 nM with incubation of 6 or 24 h. We evaluated the metabolic activity with MTT assay. The amount of intracellular calcium ($[Ca^{2+}]i$) and the release of reactive oxygen species (ROS) were analyzed by Fluo4 and CM-DFFDA fluorescent probes, respectively. PMU was labeled with Texas Red to study the interaction of the protein with cells. Nuclear localization sequence analyzes were performed using cNLS mapper. Our results showed that PMU did not alter cell viability in all cell lineages tested. In SH-SY5Y cells PMU induced the increase of $[Ca^{2+}]i$ in both times tested while BV-2 showed a decrease in $[Ca^{2+}]i$ while HEK293 cells were not altered. PMU induced an increase of ROS production in SH-SY5Y and HEK293 cells while no effect was seen for BV-2. Texas Red-labeled PMU was present inside the cytoplasm of all cell lineages, possibly interacting with the nucleus as well. *In silico* analyzes show the presence of a nuclear localization sequence in PMU. PMU may induce neuroinflammation and contribute to extra-urinary pathologies.

Keywords: Neurotoxin, Parkinson's Disease, Proteus mirabilis. **Supported by:** Fapergs, CNPq and CAPES

GC.11 - Investigation of pro and anti-apoptotic modulators during infection by pathogenic protozoa such as *Trypanosoma cruzi* and *Leishmania amazonensis*.

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Trypanosoma cruzi is the etiologic agent of Chagas disease. Leishmania sp. is the etiological agent of leishmaniasis, being *Leishmania amazonensis* causing the cutaneous manifestation. We believe that both parasites appropriate anti-apoptotic proteins such as Galectin-3 (Gal-3) to subvert the cell death. The project aims to investigate the cell signaling pathways responsible for modulating apoptosis in cells infected by *L. amazonensis* or *T. cruzi* with the participation of the Gal-3 protein. Using HeLa lineage silenced for Gal-3 infected with *T. cruzi* and mouse peritoneal macrophages from C57/Black C6 Wild type(WT) and KO Galectin-3 (KOGal-3) infected with *L . amazonensis* assays with TMRE, PI and DHE labeling were performed. The protein level of proteins that regulate apoptosis such as Survivin, XIAP, cIAP-1, Bax and Bcl-2 were analyzed by western blotting during the infections. Another assay performed was RNAseq with mRNA analysis of 100 different genes selected using the Iseq. In the TMRE labeling assays there was a reduction and in PI labeling there was an increase in cells which presents reduction gal- 3 levels for both infections, in the DHE assay there was no change in during infection with *T. cruzi*, however there was greater staining with DHE in the peritoneal macrophages of KOGal-3 mice. In the western blotting assay, there was an alteration in the protein levels of IAPs throughout both infections, especially when compared to cells with a reduced level of gal-3, whereas for Bax and Bcl-2 there was no change during *T. cruzi* infection, but there was change during infection by *L. amazonensis*. During the RNAseq assay it showed particular patterns during each of the infections, demonstrating the importance of Gal-3. We believe that parasite appropriates of the signaling pathways of the host cell in favor of positively regulating the functions of Gal-3 related to survival and apoptosis inhibition. **Keywords:** Apoptosis, *Trypanosoma cruzi*, *Leishmania amazonensis*

Supported by: FAPERJ e CNPq.

GC.12 - Gene expression analysis of putative Pho-regulon genes in *Vibrio cholerae*.

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Cholera is a life-threatening waterborne gastroenteritis caused by the Gram-negative bacterium *Vibrio cholerae*, naturally occurring in freshwater, estuarine and marine ecosystems worldwide. It can be found free-living or in association with different types of aquatic fauna, flora and certain cyanobacteria species. Natural aquatic environments are generally poor in nutrients such as phosphorus, an essential element for all forms of life. Thus, *V. cholerae* survival in such media depends on its ability to cope with phosphorus starvation. Most organisms use inorganic phosphate (Pi) as phosphorus main source and respond to Pi deficiency by expressing genes collectively known as the Pho regulon. These genes are under transcriptional control of the PhoR/PhoB, a two-component regulatory system. Under low Pi levels, PhoR gets phosphorylated and then transfers the phosphate group to PhoB. PhoB~B binds to Pho-boxes, in the regulatory of the Pho regulon genes, to regulate their expression. In previous research, using bioinformatic tools, our group found putative PhoB binding sites in the regulatory regions of many *V. cholerae* genes, raising the hypothesis they could be members of the Pho regulon. In this work, we selected five of those genes for further study to evaluate which can be a Pho regulon gene. The selection was based on their putative function, position of their Pho-box sequences and similarity to the consensus Pho-box sequence. Using quantitative PCR, we analyzed the expression of those genes in *V. cholerae* cells, grown under low and high Pi level. Two genes, *vc1592*, involved in the metabolism of the second messenger cyclic di-GMP (c-di-GMP) that controls a wide range of cellular processes in bacteria and *vc2488*, a putative lipid phosphatase of the PAP2 family, were differentially expressed under low Pi levels. This findings suggest that these genes might be new members of the Pho regulon of *V. cholerae*.

Keywords: Phosphate, Vibrio, Pho regulon
Supported by: CAPES

GC.13 - Comparative genomics of seven *V. parahaemolyticus* strains isolated in the Brazilian territory

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Vibrio parahaemolyticus is a halophilic bacterium found in marine and estuarine environments, free living or associated with biotic or abiotic surfaces. Considered one of the main pathogenic species to humans among, it can cause acute gastroenteritis, through ingestion of contaminated raw fish and/or seafood, and systemic infection. Many virulence factors have been described for this species, including the hemolysins TDH and TRH, type III (T3SS) and type VI (T6SS) secretion systems. Investigate other factors that might be involved in the pathogenicity of this species. With the increasing number of reports of *V. parahaemolyticus* in the environment and in clinical specimens in Brazil, we also want to investigate their genomic potential. In this work, seven Brazilian isolates of *V. parahaemolyticus* were analyzed. Three were clinical isolates, and four were from environmental sources. The isolates were cultured, their chromosomal DNAs were isolated and libraries were prepared for sequencing on the MiSeq platform (Illumina). FastQC and Trimmomatic were used to evaluate sequence quality, for genomic assembly Spades v.3.8.1 and the Contiguator were used, and the RAST server was used for annotation. More than 65 million reads were obtained for the seven genomes, resulting in two circular chromosomes per strain, with average sizes of 3.1 Mbp and 1.8 Mbp. An average of 2833 and 1609 CDS were annotated, respectively, in the chromosomes I and II. All genomes carried the *tlh* gene, but none had the *trh* gene. Gene clusters for T3SSI and T6SSII were found in all strains, but only the clinical isolates carried the gene *tdh*, and gene clusters for T3SSII and T6SSI. In summary, these results emphasize the genomic diversity of *V. parahaemolyticus* in the Brazilian territory. However, further comparative genomic analysis could provide a better understanding of their distinct pathogenic potential, environmental adaptation, and evolution.

Keywords: V. parahaemolyticus, Comparative genomics, Sequencing
Supported by: CAPES, CNPq, FAPERJ

GC.14 - Drug repurposing study based on enzymes from energy and lipid metabolism of *Trypanosoma cruzi*

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Trypanosoma cruzi is the etiological agent of Chagas disease, one of the main public health diseases of concern in Latin America. The available drugs for the treatment of Chagas disease are Benznidazole and Nifurtimox. Both drugs are more effective in the acute phase and severe side effects are reported. In this context, drug repurposing has become an important strategy, because it is a faster and less costly tool than the development of new drugs. The aim of this study was to select *in silico* approved drugs with potential activity against *T. cruzi* strain Dm28. On-line and free databases were used to perform the search and selection of drugs with potential action over homologous enzymes from energy and lipid metabolism of *T. cruzi*, based on the identification of drug targets with high similarity to pathogen genes. On TDR Targets database were selected 139 and 151 genes encoding enzymes from energy and lipid metabolism of *T. cruzi*, respectively. Subsequently, the amino acid sequence of the cited enzymes was obtained on TriTrypDB database. The sequences were inserted into DrugBank and Therapeutic Targets databases to identify homologous drug targets. Among the protein sequences with positive drug matches, only those with E-value *T. cruzi* strain Dm28 was performed using PUBMED, LILACS and Academic Google databases, resulting in 19 drugs for energy metabolism and 25 for lipid. The *in silico* analysis suggested that the selected drugs have activity against *T. cruzi*, with the advantage of being approved drugs for use in humans. In order to demonstrate the biological activity, *in vitro* tests are being carried out.

Keywords: Drug Repurposing, *In silico*, *Trypanosoma cruzi*
Supported by: FUNDECT, CNPq and UFMS

GC.15 - Annotation of two novel Saccharibacteria (TM7) genomes assembled from the oral metagenome

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Saccharibacteria (formerly TM7) is a phylum composed by small cell-size epibiontic bacteria that can inhabit various environments, including soil, water, plants, and animals. This phylum has also been associated with oral diseases in humans, including halitosis and periodontitis. Metagenome-assembled-genomes (MAGs) represent a novel bioinformatics approach with prominent contributions to microbial taxonomy and ecology. Given the scarcity of investigations targeting the Saccharibacteria phylum, this study aimed to retrieve Saccharibacteria MAGs from the oral cavity, in effort to shed additional light into their role in the oral cavity. To this end, saliva samples were collected from 13 healthy individuals and 14 periodontitis patients. Total DNA was extracted and subjected to Illumina Hiseq2500 sequencing. The recovered reads were assembled and the, GC content, integrity, coverage levels and genome size were assessed. Six distinct Saccharibacteria clones were identified, of which only 2 were further selected, based on 97% minimum completeness and 5% maximum contamination values. The assembled datasets were annotated via the IMG-JGI pipeline. In all, 874 and 866 genes were identified in clones RVB-004 and BGC-009, respectively. Of this total, annotation revealed 816 and 817 general protein encoding genes, 56 and 47 RNA genes, 2 regulatory and miscellaneous system genes, and 563 and 573 functional genes. Overall, the sequenced clones were proved to be genetically distinct, with BGC-009 being phylogenetically closest to *Candidatus Saccharibacteria* bacterium YM_S32_TM7_50_20 and RVB-004 to *Candidatus Saccharibacteria* bacterium oral taxon 955 strain FS17P. Both showed important virulence factors such as antibiotic resistance genes (*van, pen, mur, mra* and *mrc*), pleomorphism determinants (*glm*), immune response modulators (*imm*), efflux pumps (ABC transporters) and motility components (*pil, fli* and *fla*). In summary, the present study unveiled novel Saccharibacteria genomes in health and disease conditions, validating the importance of MAGs retrieval for characterization of previously unreported metabolic pathways in the oral cavity.

Keywords: Saccharibacteria, metagenome, periodontitis. **Supported by:** FAPEAM and PMBqBM

GC.16 - Antimicrobial properties of a new ultrashort peptide active against multi-resistant bacteria

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Antimicrobial peptides shared a differential mechanism of action when compared with traditional antibiotics. An expressive amount of research has been looked for ultra-short peptides, molecules with around 10 amino acid residues, able to control multi-resistant bacteria, without presenting side effects against eukaryotic cells. Thus, we describe the antimicrobial properties of USP_1, our first ultra-short peptide containing 8 amino acid residues. The peptide was chemically synthesized and assayed against multi-resistant Gram-negative bacteria, *E. coli* KPC+ and *Acinetobacter baumannii*; and Gram-positive *Staphylococcus aureus* methicillin resistant (MRSA) strains. The synergic effect of USP_1 and ciprofloxacin were assayed against *E. coli* KPC+. The ability to eradicate *A. baumannii* multi-resistant mature biofilms was also assayed. USP_1 did not display cytotoxic effects against murine macrophages RAW 264.7 or human erythrocytes up to 100 μM. The peptide showed a minimum inhibitory concentration (MIC) for MRSA ATCC33591 and MRSA ATCC43300 of 8 and 16 μM, respectively. For *E. coli* KPC+ CI001812446 and *A. baumannii* CI003321216, USP_1 showed a MIC of 16 and 64 μM, respectively. The peptide and ciprofloxacin showed a Bliss Synergy Score of 3.99 against *E. coli* KPC+, keeping the antimicrobial activity in concentration of ciprofloxacin reduced by 32-fold and 8-fold the concentration of USP_1. At MIC, USP_1 eradicated 38% of 24-h *A. baumannii* mature biofilm, impairing both biofilm structure and bacteria viability, results observed through fluorescence microscopy. When administered alone at the MIC, ciprofloxacin increased the *A. baumannii* biofilm by 12%. CD analyses showed that RK8 assumes a non-ordered structure in water, presenting ~1,28% of α-helix content. Otherwise, in presence of SDS micelles, the peptide assumed an α-helix structure. Further assays are investigating the resistance of USP_1 against peptidases, to evaluate it potential in preclinical assays. We conclude that USP_1 represents a promising strategy to development of a new antimicrobial agents against multi-resistant bacteria. **Keywords:** Antimicrobial peptide, biomimetic, rational design. **Supported by:** FUNDECT, FINEP, CNPq and CAPES

GC.17 - Genotypic diversity and pathogenic potential of clinical and environmental *Vibrio parahaemolyticus* isolates from Brazil

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Vibrio parahaemolyticus is a leading worldwide agent of acute gastroenteritis associated with the consumption of contaminated raw or undercooked seafood. They also cause wound infections that can lead to septicemia and death. Many virulence factors in different combinations have been identified in clinical and environmental isolates, including the hemolysins TDH and TRH, the adhesive factor VpadF, and pandemic strain-associated markers toxRSnew and orf8, suggesting that *V. parahaemolyticus* pathogenesis has not been fully elucidated. An increasing number of isolates have been reported in Brazil. However, few studies have characterized their pathogenic potential. Therefore, we analyzed the genotypic diversity of *V. parahaemolyticus* isolates from clinical and environmental sources in Brazil, focusing on virulence, pandemic markers, and pathogenic potential. *V. parahaemolyticus* strains recovered from human diarrheal stools (3, one in 1975 and two in 2001) and environmental sources (4, between 2008-2010) were investigated for the presence of virulence genes (trh, tdh, and vpadF), pandemic markers (orf8, toxRSnew), and with respect to their pathogenic potential in mice and *Galleria mellonella* larvae systemic infection models. Based on the presence of the genetic markers, all environmental strains were classified as non-pathogenic, while one clinical strain was pathogenic/non-pandemic and the other two were pathogenic/pandemic. All strains, except for the clinical pathogenic/non-pandemic, produced lethal infection in both infection models, regardless of source, serotype, and genotype. Based on mice and larval mortality rates, which were remarkably similar, the pathogenic/non-pandemic strain was classified as avirulent, while the others were considered of high or intermediate virulence. These findings demonstrate that *G. mellonella* larvae can be used as an alternative model to study the pathogenicity of *V. parahaemolyticus*. Moreover, they raise doubts about the use of traditional virulence markers to predict pathogenesis of the species and show that reliable models are indispensable to determine the pathogenic potential of environmental isolates considered non-pathogenic.
Keywords: Infection models, *Vibrio parahaemolyticus*, Virulence and pandemic markers
Supported by: CAPES, CNPq, FAPERJ

GC.18 - Pan-genome analysis of the difficult-to-identify and multi-drug resistant emerging pathogen *Corynebacterium amycolatum*

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Corynebacterium amycolatum, which is an emerging multidrug-resistant (MDR) opportunistic pathogen, is often misidentified at the clinical microbiology laboratory when using traditional bacterial identification methods. This has hampered the development of studies aimed at understanding the molecular mechanisms involved in the transition from colonization to the invasive MDR phenotype in clinical isolates. To perform a comprehensive pan-genomic analysis of MDR clinical isolates of *C. amycolatum*, to obtain information on the genetic factors contributing to infectivity and multidrug resistance in this species. We generated 08 preliminary genome sequences from clinical isolates of *C. amycolatum* MDR from Spain and Tunisia. In addition, 18 complete or draft genomes of *C. amycolatum* were retrieved from the NCBI. The Bacterial Pan Genome Analysis (BPGA) v.1.3 tool was used to perform pan-genomic analysis. Automated antimicrobial resistance (AMR) gene predictions were performed in the Comprehensive Antibiotic Resistance Database (CARD) and the National Database of Antibiotic-Resistant Organisms (NDARO). Furthermore, virulence-related genes were searched with VFanalyzer, and genomic islands were annotated with IslandViewer4. The species *C. amycolatum* presented an open pan-genome (α=0.854905) containing 3,280 gene families distributed among the central (n=1,690), accessory (n=1,121), and unique (n=469) genomes. Four out of nine identified antimicrobial resistance (AMR) genes are associated with resistance to aminoglycosides. In addition, with exception of the housekeeping gene *rpsL*, all other AMR genes are present in genomic islands, indicating extensive horizontal gene transfer (HGT). We identified 47 putative virulence factors, including 17 associated with iron acquisition and the SpaD-type pili. Species with an open pan-genome have an extended ability to acquire new genes through HGT. Consistently, we found AMR genes predominantly in genomic islands in *C. amycolatum* isolates. Our results also demonstrate that *C. amycolatum* has a metabolism particularly adapted to invasive infections.
Keywords: Genomic Island, Pathogenomics, Virulence factor. **Supported by:** FAPESB

GC.19 - Analysis of outer membrane vesicles (OMVs) produced by *Vibrio cholerae* under limitation and abundance of inorganic phosphate and its relationship to the pathogenicity

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Vibrio cholerae, like other bacteria, produces outer membrane vesicles (OMVs), which are released from their surface during growth. OMVs contain phospholipids, lipopolysaccharides, proteins of the cytoplasm, periplasm and of the outer membrane, as well as, DNA and RNA. *V. cholerae* inhabits phosphorous-poor aquatic media and colonizes the host intestinal tract, environments where it expresses various genes in response inorganic phosphate (Pi) limitation (Pho regulon). This information led us to verify if *V. cholerae* OMVs produced *in vitro* under Pi limitation, could carry factors essential to the pathogenicity of the bacterium. For this, *V. cholerae* was cultivated under Pi abundance and limitation and the OMVs were purified and quantified. About 2 times higher protein content was found in the OMV suspension from MGLP (MOPS, glucose, low [Pi]) than from MGHP (MG, high [Pi]), suggesting greater OMV production at low Pi concentration. Proteomic analysis of OMVs by SDS-PAGE and mass spectrometry showed that the electrophoretic profile and protein composition of *V. cholerae* OMVs are dependent on the Pi concentration in the culture medium. Similar results were observed with the bacterial cells grown in MGHP and MGLP. However, *V. cholerae* proteins in OMVs released in MGHP and MGLP fall into the same functional groups. Among the proteins specific to OMVs released in MGLP, several are products of regulon Pho genes and some are important for the pathogenicity of the bacterium. The lipid composition of OMVs was analyzed by mass spectrometry. OMVs released by *V. cholerae* in MGHP and MGLP have similar lipid profiles with high levels of ornithine lipids (OLs) that are distinct from those of the original cells. The pathogenic potential of these OMVs in the *Galleria mellonella* infection model showed different mortality rates, in agreement with our findings that the *V. cholerae* OMVs generated under Pi limitation carry virulence-related factors.
Keywords: outer membrane vesicles, proteomics, lipidomics. **Supported by:** cnpq

GC.20 - Genomic analysis of antimicrobial resistance in OXA-23-producing *Acinetobacter baumannii* strains in Brazil

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Resistance to carbapenems in *Acinetobacter baumannii* isolates has become a major public health problem. Among the mechanisms responsible for this phenotype, OXA-type carbapenemases are the main ones, especially OXA-23 in Brazil. Therefore, WHO has designated carbapenem-resistant *A. baumannii* a threat in human health, with critical priority and need new antibiotics to come. To identify the main mechanisms of antimicrobial resistance in OXA-23-producing *A. baumannii* genomes isolates in Brazil. Data corresponding to bacterial genomes were collected from the genome bank of the National Center for Biotechnology Information using the Pathogen Detection tool. The genome search was done in May 7th, 2021, using as a filter to select the genomes: genotype, $bla_{\text{OXA-23}}$; organism group, *A. baumannii*; location, Brazil; type of isolate, clinical; host, *Homo sapiens*. Data were interpreted through descriptive statistical analysis with simple frequency distribution using the Microsoft Office Excel® software. The resistance genes of 153 *A. baumannii* strains were found and analyzed. Isolates were collected between 2008 and 2020. Most of the isolates (98%) presented information about the type of clinical sample which was obtained, being mostly blood samples (33.3%), followed by respiratory tract samples (31.4%). Regarding the location of the insolates, 95% had this specification. The states of São Paulo (68%) and Minas Gerais (7.2%) stands out. A total of 1986 genes were found, with an average of 12.98 genes per isolate. Among the genes found, most were associated with aminoglycoside resistance (33%), followed by b-Lactam antibiotics (27%). Further the $bla_{\text{OXA-23}}$, the bla_{ADC}, *ant(3")-IIa* and *amvA* genes were present in all strains. In conclusion, OXA-23-producing *A. baumannii* strains in Brazil have the ability to carry several resistance genes associated with multiple drugs, thus genomic analysis is an important tool to observe the spread of gene resistance and diversity.

Keywords: *Acinetobacter baumannii*, Drug Resistance, Carbapenems
Supported by: CAPES

GD - Natural Products

GD.01 - Evaluation of the antioxidant activity of mandacaru (*Cereus jamacaru* D.C) from Caatinga of Bahia

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Cereus jamacaru (mandacaru) has great potential as a source of medicinal substances. However, despite the great use of this species by the Caatinga population, studies on the antioxidant potential of mandacaru are still scarce. Thus, the aim of this study is to evaluate the antioxidant activity and determine the content of total phenolic compounds in the cladode of *Cereus jamacaru*. The cladodes were placed to dry at room temperature, being later pulverized in knife mills. The pulverized material was submitted to extraction by maceration with different solvents: ethanol, ethyl acetate, hexane and hydro-ethanolic. The crude extracts were concentrated in a rotary evaporator, under reduced pressure, at temperatures of 40-45ºC. The solvent residue of each extract was removed by evaporation in an exhaust hood. The antioxidant activity was evaluated by the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical and the 2,2-azinobis-3-ethyl-benzothiazolin-6-sulfonic acid (ABTS) radical scavenging method and the content of phenolic compounds was determined by the Folin-Ciocalteau method. In the DPPH assay, the extracts in ethyl acetate (21.07 µgmL-1) and hexane (22.65 µgmL-1) exhibited higherantioxidant activity than the others extracts, without significant differences regarding the IC50 value. The hydro-ethanolic and ethanolic extracts showed higher antioxidant activity by the ABTS method (378.61 µMTrolox.g-1 and 214.24 µMTrolox.g-1, respectively), differing statistically ($p>0.05$) from the other extracts. The content of phenolic compounds showed a strong negative correlation with the DPPH antioxidant capacity for the extracts in ethyl acetate (97.23 mgEGA.g-1/r = 0.95) and hexane (100.36 mgEGA.g-1/r = 0.95), while the hydro-ethanolic (126.73 mgEGA.g-1/r = 0.95) and ethanolic (104.23 mgEGA.g-1/r = 0.99) extracts showed a strong positive correlation with ABTS. Mandacaru cladode extracts have shown potential to be used in future studies showing the bioactive properties related to traditional use.
Keywords: ethnopharmacobotany, cactace, medicinal plants

GD.02 - Phytochemical analysis and *in vitro* antioxidant and toxic activities of ethanolic extracts from Amazonian plants

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The Amazon has plant species with potential in the formulation of new drugs with antioxidant action. We sought to evaluate the chemical profile and antioxidant activity of ethanol extracts from leaves of *Garcinia macrophylla* Mart., *Tovomita macrophylla* Walp. (Clusiaceae); *Vismia japurensis* Reichardt (Hypericaceae); *Cnidoscolus chayamansa* McVaugh (Euphorbiaceae); *Trema micrantha* (L.) Blume (Cannabaceae); and *Cecropia concolor* Willd (Urticaceae). The extracts were obtained by cold maceration. The evaluation of chemical classes was performed using thin layer chromatography with an elution system containing ethyl acetate, hexane, and dichloromethane (1:4:5 ratio). UV light at 254 and 365 nm, as well as aluminum chloride, ceric sulfate, ferric chloride and Dragendorff's reagent were used as developers. The toxicity of the extracts was evaluated through the bioassay with Artemia salina Leach, and the total phenolic content was determined by the Folin-Ciocalteu method using gallic acid as standard, while the antioxidant activity was measured via the DPPH• radical scavenging technique, using ascorbic acid and quercetin as standards. The presence of phenolic compounds, flavonoids, terpenes and alkaloids was observed in all the extracts; however, these which were not sufficiently toxic to the microcrustacean *A. salina* at 1.0, 0.5, 0.25 and 0.125 mg/mL. Three species showed higher phenolic content and noteworthy antioxidant potential: *C. concolor*, *V. japurensis* and *G. macrophylla*, with EC_{50} values lower than those found for ascorbic acid and quercetin. The extract of *T. macrophylla* also showed promising results for antioxidant activity. The presence of phenolic compounds and flavonoids may explain the promising antioxidant activity in the extracts, since these substances are known for their ability to react with free radicals. Of the Amazonian species evaluated here, *C. concolor* showed outstanding antioxidant activity, despite this species having not previously been described in the literature as a source of antioxidant metabolites.

Keywords: phytochemical, antioxidant, C. concolor

Supported by: CAPES

GD.03 - Effects of Lectin-rich Fraction from *Moringa oleifera* Seeds on Survival, Biological Cycle Progress and Midgut Development of *Aedes aegypti*

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The *Aedes aegypti* is a vector mosquito of the etiologic agents of different arboviruses such as chikungunya, dengue, yellow fever, and zika. The control of this insect is crucial to mitigate the spreading if these diseases. Seeds of *Moringa oleifera* contain a water-soluble lectin (WSMoL) with larvicidal and ovicidal activities against this insect. The present work evaluated the effects of a lectin-rich fraction containing WSMoL on the survival and development of *A. aegypti* individuals. The insects were exposed to the fraction (0.05–0.6 mg/mL of protein) at the third larval instar for 24 h and development were followed for 9 days post-exposure. In addition, alterations in the midgut organization of treated larvae, pupae, and adults were investigated. The fraction induced the death of A. aegypti larvae along the post-exposure period. The mean survival time was reduced to 5.000±0.096 days in the treatment at 0.6 mg/mL, while in control there was no death along the 9 days. The fraction also delayed the developmental cycle, since 90% of the individuals reached the adult stage in control while no adults were present in the treatment with the fraction at 0.1 mg/mL during the 9-day period. The midguts of treated larvae and pupae showed disorganization and epithelial vacuolization, while in treated adults, the epithelium was underdeveloped compared to control. Unlike in control mosquitos, proliferating cells were not detected in treated larvae, and appeared in lower numbers in treated pupae than in control pupae. In conclusion, females that developed from larvae treated with lectin-rich fraction showed damage to midgut organization, which can be linked to the impairment of development and survival.
Keywords: dengue mosquito, insecticidal proteins, development
Supported by: FACEPE, CNPq and CAPES

GD.04 - Evaluation of the antimicrobial activity of protein extract from Inga laurina seeds (Fabaceae)

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Inga laurina (Fabaceae) is a tree species native to the Cerrado, widely distributed in Brazil. In its seed extract, ILTI (*Inga laurina* trypsin inhibitor) has been isolated, a peptidase inhibitor that has antifungal activity. Thus, this study aimed to evaluate the inhibitory activity of the protein extract from *Inga laurina* seeds, as well, as its antimicrobial potential. Seeds of *I. laurina* were dried and ground. The extraction of soluble proteins from the flour was performed with 0.1 M phosphate buffer + 0.15 M NaCl, pH 7.6, at 25°C, for 2 h, under magnetic stirring. After centrifugation, the supernatant was denominated crude extract (EB). Protein quantification was performed according to Bradford (1976). For the trypsin inhibition assay, the methodology of Erlanger *et al*. (1961) and polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to Laemmili (1970). Antimicrobial activity was determined by the broth microdilution method according to CSLI standards. The EB yield was 1.72% in relation to the initial dry mass of seeds. Protein quantification indicated a content of 89.69% of total proteins. The EB inhibited the enzymatic activity of trypsin, and the band of 20 kDa, approximately, in electrophoresis gel confirmed the presence of ILTI in the EB. For most of the yeast tested, EB was active at a concentration of 4 mg/mL, showing better activity against *Cryptococcus gattii*, at the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of 31.25 µg/mL, in addition to show good activity against *Candida albicans*, *Candida guillermondii*, *Candida krusei* and *Candida nivariensis*, with MIC and MFC of 250 µg/mL for this yeast. EB at a concentration of up to 4 mg/mL did not show activity for the bacterial strains tested. EB showed good antifungal activity, which would allow it to be used to develop a pharmaceutical form for this purpose. **Keywords:** antifungal, ILTI, ingá
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GD.05 - Investigation of Insecticide Properties of *Inga cylindrica* Trypsin Inhibitor against *Anagasta kuehniella* larvae

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Several molecules obtained from plant seeds display insecticidal properties, including trypsin inhibitors. The aim of the study was to investigate the insecticidal potential of a trypsin inhibitor purified from *Inga cylindrica* seeds (IcTI) against the Mediterranean moth, the insect pest *Anagasta kuehniella* (Lepidoptera: Pyralidae). IcTI was purified from *I. cylindrica* seeds through gel filtration and ion-exchange chromatography. The inhibitory activity against trypsin was assayed using the colorimetric substrate, benzoyl-arginine-p-nitroanilide (BAPNA) and bovine trypsin. For in vivo assays with *A. kuehniella*, neonate larvae were fed with diet (250 mg) containing different concentrations of IcTI (0; 0.5; 1; 1.5; and 2% w/w). Two independent assays were carried out in 10 replicates (n= 30). The larvae were kept in standard conditions (25± 2 ºC, 12 h photoperiod) for 30 days. Then, the larvae were weighted, the survival rate was determined, and the midguts were removed for enzymatic assays. The diet containing IcTI prompted a reduction in larval weight by 72%, 84%, 86%, and 88%, in comparison with control-fed larvae. The survival rate was reduced 33% among the treatments. Enzymatic assays are being carried out to investigate the physiological effects triggered by IcTI ingestion. Since peptidase inhibitors can be expressed in transgenic plants, the knowledge of effects of ingestion these molecules by insect pests could be applied as biotechnological alternative for pest control.
Keywords: Biotechnological, Pest Control, Trypsin Inhibitor
Supported by: FAPESP, CNPq and CAPES

GD.06 - Antifungal potential of *Macrocybe titans* aqueous extract on Candida albicans

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With the rapid emergence of antibiotics resistant microorganisms, the search for new effective therapeutic agents has become a favorable alternative in the fight against diseases caused by fungi, mainly *Candida* species. Thus, the present study aimed to verify the antifungal potential of the mushroom *Macrocybe titans* extracts, recently reported in Brazil and with few reports in the literature about its antimicrobial potential, against *Candida albicans* (ATCC 90028), as well as the effects on the morphological aspects of this yeast. The extracts used were obtained from *M. titans*, as three aqueous fractions (concentrated, filtered EfraMat-45 and EfraMat-22) and methanolic/ethyl acetate extracts (cold method and Soxhlet). The antifungal susceptibility was evaluated by broth microdilution, according to CLSI's M27-A3 protocol. Micromorphological aspects were analyzed by microculture on cornmeal agar supplemented with 1% Tween 80, Fluorescence Microscopy using Calcofluor White and propidium iodide, and Scanning Electron Microscopy (SEM). It was verified that EfraMat-45 showed the best results for Minimum Inhibitory Concentration – MIC (31.25 µg/µl) among extracts. Microculture analysis showed complete inhibition of yeast growth on the culture slides referring to MIC, in all aqueous extracts. Morphological changes were observed on the slides of sub-MIC, with an increased formation of elongated cells and chlamydospores absence, compared to control yeasts. Through SEM, it was possible to observe morphological changes and slight damages on the surface of cells treated with EfraMat-45 MIC, as well as the presence of elongated cells viewed by Fluorescence Microscopy using Calcofluor White. Propidium iodide staining revealed cell death on yeasts treated with the extract. EfraMat-45 showed absence of *in vitro* cytotoxicity. The results suggest that the aqueous extract of *M. titans* showed a very promising pattern as regarded to antifungal action, with cellular changes certifying cell death.
Keywords: Candida albicans, Macrocybe titans, natural products

GD.07 - Anticancer properties of *Tulbaghia violacea* regulate the expression of p53-dependent mechanisms in cancer cell lines.

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Tulbaghia violacea harv is a South African local herb that has been used as remedy for several alignment. The crushed leaves are currently used as a cure for sinus headaches while the bulb has been used as a remedy for tuberculosis. Recently, *Tulbaghia violacea* harv has been demonstrated to have androgenic and anti-cancer properties in vitro. The purpose of this review is to further evaluate the molecular mechanism of *Tulbaghia violacea* extracts in regulating cell death in various cancer cell lines. Three organic solvents namely, methanol, hexane, and butanol at 10g per 100ml were used as extraction solvents. Each cell line was treated with varying concentrations of the plant extract to identify the half-maximal inhibitory concentration (IC50). The IC 50 was later used to analyse if the extracts were inducing apoptosis using annexin V analysis. Furthermore, the molecular mechanisms by which apoptosis was induced was analysed by qPCR, western blots. All three extracts exhibited anticancer activity with the most cytotoxic being methanol extract. p53 expression was significantly increased in treated cells that correlated with increased caspase activity. The results point to possible activation of apoptosis following treatment with hexane extracts.

Keywords: apoptosis, p53, *Tulbaghia violacea*
Supported by: South African Medical Research Council

GD.08 - Development and Characterization of Lipid Nanocapsules with Mauritia flexuosa Pulp Oil as a natural anti-inflammatory

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The *Mauritia flexuosa* palm is found in Brazil mainly in the Cerrado region. The fruit of *M. flexuosa*, locally known as 'buriti', has a pulp layer with a color ranging from orange and red. Among the bioactive compounds, buriti pulp oil (BUPO) has a high content of carotenoids (CA) and fatty acids (FA). Recent studies with BUPO demonstrated an anti-inflammatory effect attributed to the high CA and FA content. However, these compounds are easily degraded and have low bioavailability. To prevent the degradation of these compounds and improve their bioavailability, technologies were developed, example: encapsulation in lipid nanocapsules (LNCs). LNC is a promising and ''simple'' technique used in the Drug Delivery System (DDS). The development of LNCs replacing synthetic oils with a natural oil with a potential natural anti-inflammatory agent is interesting and promising. Therefore, the objective of the work was to develop, characterize (size, polydispersion, zeta potential and particle concentration) and evaluate the anti-inflammatory effect of BUPO nanocapsules (NCBU). The NCBU were developed using the phase inversion method (liquid lipid, BUPO, nonionic surfactant, hydrogenated soy lecithin and NaCl in Milli-Q water). The anti-inflammatory effect was evaluated by the Carrageenan-induced paw edema method. The results showed NCBU with average sizes of 55.68 ± 0.37 nm, PDI of 0.162 ± 0.028, zeta potential of -25.50 ± 1.37 and particle concentration of 1.42 ± 0.03 $x10^{15}$ mL^{-1}. The NCBU had an anti-inflammatory effect for 4h and edema inhibition of up to 89.69%. The NCBU are viable for more characterization studies and another's *in vivo* biological evaluation.

Keywords: natural products, nanocapsules, carotenoids. **Supported by:** Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, 2021/01237-8); CNPq and CAPES

GD.09 - Exposure of male *Drosophila melanogaster* to chronic unpredictable mild stress causes oxidative damage: Role of the antioxidant γ-oryzanol

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Exposure to chronic unpredictable mild stress (CUMS) leads to the development of a depressive-like state in experimental animals such as *Drosophila melanogaster*. Evidence suggests that cellular oxidative damage may be related to the development of depressive-like behaviors caused by mitochondrial dysfunction. Therefore, the use of compounds such as γ-oryzanol (ORY) that have a portion of ferulic acid that gives it antioxidant potential is of great value. Our aim was to evaluate the effect of ORY on oxidative stress caused by CUMS in male *Drosophila melanogaster*. Flies were divided into 4 groups: (1) Control, (2) CUMS, (3) ORY (25 μM), (4) ORY (25 μM) + CUMS. For CUMS the flies were subjected to stress by cold, heat, starvation, sleep deprivation for 10 days. At the end, the survival rate was evaluated, and measurements were made of the levels of reactive species (RS) in the head and body of the flies and the preliminary evaluation of the oxygen flux by high-resolution respirometry (HRR). As a result, we can observe an increase in RS levels only in the heads of flies exposed to CUMS compared to the control group. There was also an increase in mitochondrial oxygen flux in the HRR, mainly between complexes I and II in the ORY groups alone and treated group when compared to the CUMS group, where there was a decrease in the respiratory rate and a possible mitochondrial compromise. The observed oxidative stress presumably is a contributing factor to the reduced survival rate in the CUMS group. It is concluded that ORY had a beneficial effect on the oxidative stress caused by CUMS, and has the potential to prevent a possible breakdown of cellular energy, thus avoiding the triggering of depressive-like behavior in *Drosophila melanogaster*.
Keywords: Antioxidant, CUMS, Drosophila.
Supported by: FAPERGS, CNPq and CAPES

GD.10 - Antinociceptive Effect of *Schinus terebinthifolia* Raddi. (Anacardiaceae) Leaf Lectin in Mice

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Pain control remains a clinical challenge and there is a need to seek new therapeutic options for its relief. In this context, natural products stand out, including plant-derived lectins, which are proteins which have shown antinociceptive potential. This study aimed to evaluate the antinociceptive activity of the *Schinus terebinthifolia* leaf lectin (SteLL) through acute experimental models of peripheral and central nociception in mice. SteLL was isolated by chromatography of leaf extract (in 0.15 M NaCl) on chitin column. The animals were intraperitoneally treated with PBS (control), indomethacin (20 mg/kg), morphine (10 mg/kg) or SteLL (1, 5 and 10 mg/kg). To verify the peripheral antinociceptive effect, the writhing number was determined in the acetic acid-induced abdominal writhing test. In the formalin test, the time spent by each animal licking its paw was recorded during the first 5 minutes (first phase: neurogenic pain) and at the interval of 15 to 30 minutes (second phase: inflammatory pain), after the formalin administration. The involvement of lectin carbohydrate recognition domain (CRD) and the participation of opioid receptors in the antinociceptive effect were verified. Tail immersion test was performed to assess central antinociception. The latency period for tail removal was determined at 30, 60, 90 and 120 min after treatments. SteLL reduced acetic acid-induced writhing by 84%-100%. In the first phase of the formalin test, SteLL reduced paw licking time by 49%-51%. In the second phase, SteLL reduced paw licking time by 81%-83%. This antinociceptive effect was reversed by ovalbumin (indicating the possible involvement of CRD) and by naloxone (suggesting a modulation of opioid receptors). In the tail immersion test, SteLL reduced the thermal stimulus perception sensitivity even after two hours. SteLL possess both peripheral and central analgesic actions. The peripheral effect involves the lectin CRD and is probably mediated by opioid receptors.
Keywords: Pain, Antinociception, Brazilian pepper tree
Supported by: FACEPE, CNPq and CAPES

GD.11 - Trypsin inhibitor from Crotalaria spectabilis leaf with anti-Leishmania activity

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Crotalaria is a genus of Fabaceae family that includes about 500 species of herbaceous plants and shrubs found in tropics and subtropics. Crotalaria spectabilis, flowering plant, is native to Indian, China, and Southeast Asia. It is tolerant to drought and diseases, and are used as green manure, forages, preventing soil nematodes and in folk medicine. Thus, the purposes of this work was to isolate and characterize trypsin-like inhibitors from aqueous phosphate extract from fresh leaves of C. spectabilis (CS-P). Protein amount was determined by Bradford method and protein profile was evaluated by SDS-PAGE. Isolate and characterize inhibitors by affinity chromatography using trypsin-Sepharose and LC-MS. The purification was about 6.05-fold yielded 61% of trypsin-like inhibitors obtained from 3,2 mg of protein from CS-P, and this inhibitor was named as CSPI. SDS-PAGE analysis identified three proteins with approximately 32, 37 and 44 kDa. CSPI inhibited the activity of both trypsin and LSPIII, an extracellular serine protease from *Leishmania amazonensis* (LSPIII), about 67 and 75%, respectively, using Nα-p-Tosyl-L-arginine methyl ester as substrate. Trypsin inhibition by CSPI was the highest at 65°C, but after 24 and 48 h of incubation at 65 ºC, the inhibitor activity was completely abolished. CSPI maintained the activity until 55 days, when stored in freezer (-20°C), however, the extract CS-P preserved the protease inhibitory activity about 2 years, stored in similar conditions, possibly because the high content of flavonids. The main flavonoids identified were: quercetin 3-O-neohesperidoside (756.21130), tricin 7-diglucuronoside (682.53998), quercetin 3,4'-diglucoside (626.14832), and quercetin-3-O-alpha-L-rhamnopyranoside (756.21130). Other serine protease inhibitors were obtained from C. paulina and pallida seeds, and they had different biochemical characteristics of CSPI, isolated from leaves. Furthermore, CSPI was the first plant polypeptide protease inhibitor that inhibited LSPIII, and studies from our group demonstrated that LSPIII inhibition induced the death of *L. amazonensis* promastigotes and amastigotes *in vitro*.

Keywords: trypsin inhibitor, C. spectabilis, L.amazonenis
Supported by: CNPq e FIOCRUZ

GD.12 - The extraction method affects the concentration of secundary metabolites present in *Aloysia citriodora* Palau extracts

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A native plant *Aloysia citriodora* is considered a promising species for the development of new drugs, being very widespread in South American folk medicine. In this work, the leaves of *A. citriodora* were subjected to extraction with distilled water, simulating the popularly consumed tea, and hydrocolic, in order to structurally isolate and identify the main chemical constituents present in its leaves in different forms of extraction. With the high molecular mass separated from the low mass, the crude extracts were precipitated with refrigerated ethyl alcohol, resulting in ethanol-soluble fractions, containing the secondary metabolites, named AC-SBH for the hydroalcoholic extract and AC-SBA for the aqueous extract. AC-SBH and AC-SBA were then analyzed by the techniques of high performance liquid chromatography (HPLC) and mass spectrometry (ESI-MS). A total of 38 compounds were found in AC-SBH and 37 in AC-SBA, based on retention time (Rt), standards of mass fragmentation and ultraviolet spectrum, with data search in the current literature. The aqueous and hydroalcoholic extracts differed qualitatively and quantitatively, where it was observed that for AC-SBA the major component of chrysoeriol-7-diglucuronide, while for AC-SBH, the major components were verbascoside and chrysoeriol-7- diglucuronide. It could also be noted that the relationship between the peaks of the other components for both extractions is different, that is, the relative abundance of the compounds is different from each other, demonstrating that the extraction method affects the concentration of metabolites. It was found that most of the secondary metabolites of *A. citriodora* were extracted in greater quantity in the hydroalcoholic extraction (AC-SBH). The data found here are in line with those found in previous research, but also legitimize new research aimed at different extraction methodologies for isolating compounds of interest in the species.

Keywords: biochemistry, bioactive compounds, structural characterization

GD.13 - Effect of *Capsicum annuum* var. annuum leaf extract on the development of *Callosobruchus maculatus*

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The increase in demand for the safe food productivity for humans and the environment has driven the search for new substances with the objective of controlling and preventing agricultural pests. The present work aims to evaluate the effect of fractions rich in peptides isolated from *Capsicum annuum* on the larval development of *Callosobruchus maculatus*. The leaves were extracted using a solution containing 60% methanol and dichloromethane in a 1:1 ratio. The hydromethanolic extract was used in biological tests and purification of peptides in reverse phase chromatography using C18 column and elution with linear gradient of acetonitrile. To test the effect on the development of *C. maculatus* larvae, artificial seeds of *Vigna unguiculata* cotyledon flour containing the *C. annuum* extract were used. Glucose, cholesterol, triglycerides, proteins, lipase and amylase were measured in 20-day-old larvae. The chromatographic profile of the extract showed 3 fractions eluting in 6%, 24.5% and 27% acetonitrile. The extract and the fractions presented only a single band, with a molecular mass around 5 kDa. The addition of the extract in the concentration of 0.5%, 1.0% and 2.0% in artificial seeds reduced the weight of the larvae by up to 65% and reduced the survival rate of the larvae in seeds, but had no effect of repellency for the laying of eggs. Biochemical parameters are indicative of a delay in development when compared to control larvae. The extract and one of the fractions isolated on HPLC showed inhibitory activity of *Tenebrio molitor* α-amylase, which may explain the toxicity of this compound to the insect's larvae. The extract and the fractions did not exhibit porcine trypsin and chymotrypsin inhibitory activity. Other experiments will be carried out for biochemical characterization and elucidation of the mechanism of action of the obtained fractions.
Keywords: *Capsicum annuum*, insecticidal activity, peptides
Supported by: UENF; FAPERJ; CNPq

GD.14 - PROJECT: Profile of Medicinal Plants with Antineoplastic Potential Activity in Santa Catarina Plateau.

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Medicinal plants are popularly known and used, mainly in traditional communities. This knowledge contributes to scientific community, because facilitates identification and classification of species, seeking evidence of their biological and pharmacological activities, for further use in prevention and treatment of diseases, including cancer. Malignant neoplasms are treated by a set of conventional therapies. However, they may be not capable or sufficient, or may lead to undesirable effects. The search for alternative or complementary methods has become increasingly present in oncological treatments, and this, together with popular knowledge about medicinal plants, led to the development of the present study. It aims to identify, first, through questionnaires, which medicinal plants with antineoplastic purposes are used by residents of Santa Catarina Plateau region. From the samples collected in the homes of the interviewees, the botanical and phytochemical composition of the classified plants will be identified. For that, three extracts will be prepared, following the following solvents: distilled water, ethanol and hydroethanolic solution, and through them will be performed phytochemistry analysis, mainly by high performance liquid chromatography (HPLC) assays. The search for a possible antitumor activity will be predicted by *in silico* tools such as the online PASS (Prediction of Activity Spectra for Substances) or Molsoft Drug-Likeness programs. Afterwards, the activity may be determined by *in vitro* and/or in vivo methods, using for example Ehrlich´s tumor cells. Therefore, medicinal regional plants, effective against cancer, will be identified, thus proving the benefits of their use, besides to provide news opportunities to regional further investigations.
Keywords: *in silico*, Medicinal plants, Acca sellowiana

GD.15 - Metabolomic Profile and Biological Properties of Extracts of *Miconia Albicans* from the North Coast of Bahia

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Brazil has one of the biggest biodiversity in the world, with a large collection of medicinal and economic plants that present pharmacological properties and it's used for the treatment of diseases. This use is based on traditional knowledge, such as *Miconia Albicans* (Canela-de-velho) are widely used by the North Coast of Bahia communities. Thus, it's necessary to identify the pharmacological properties and their active principles for better therapeutic applications. In the present study, was to determine the metabolite profiling and evaluate the medicinal potential of *Miconia Albicans* extract for antioxidant and antimicrobial activity. Leaves and stem of Canela-de-velho were collected in the Mata de São João-Bahia. Extracts were prepared by maceration in different organic solvents. The cellular metabolome was evaluated using 1H nuclear magnetic resonance (NMR). The determination of the antioxidant activity was 2,2-azinobis-3-ethyl-benzothiazolin-6-sulfonic acid radical scavenging method and total phenols concentration by the Folin-Ciocalteu. The antimicrobial activity was performed against Gram-positive and Gram-negative bacteria, likewise non-filamentous fungi through broth microdilution susceptibility test. Concerning the antioxidant activity and total phenols the extracts showed potential promising. The total phenols concentration the leaf ethanol extracts had a higher concentration (81.1 mgEGA.g-1) compared to the stem extract (73.7 mgEGA.g-1). The ethanol extracts of M. albicans antimicrobial activity weren't efficient up to the maximum concentration tested against fungi, gram-positive and gram-negative bacteria. As for the metabolites present in the extracts, there is a strong indication of the presence of compounds such as myricitrin, mearnsetin, ursolic acid and oleanoic acid. But the NMR analysis results still in process. M. albicans extracts present good levels of total phenols. However, their antimicrobial activity was not efficient and new concentrations should be tested. The results of the NMR are still being processed for further identification of biocompounds and correlation with the pharmacological properties and traditional use. **Keywords:** Antimicrobial activity, Antioxidant activity, Ethnopharmacology

GD.16 - Metabolite profiling, phenolic compounds and antioxidante activity of ethanol extracts from *Conocarpus erectus* L. Var.

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Phenolic compounds have been related to the antioxidant capacity of vegetable due to different actions in the oxidative and anti-inflammatory processes. *Conocarpus erectus* L. Var. belongs to the combretaceae family, has a large amount of phenolic compounds and it is used by riverside communities to treat skin infections, anemia and headaches. Due to medicinal use, further studies are needed to identify the plant's bioactive compounds and phytotherapeutic properties. In the current study, the objective was to determine phenolic compounds, antioxidant activity and evaluate the metabolomic profile of ethanol extracts from *C. erectus*. Samples of the stem and leaf collected in the Pojuca River Mangrove, North Coast of Bahia, were dried at room temperature and ground before preparing the extracts by maceration in etanol. The extracts were submitted to rotaevaporation to acquire the crude extracts. Total phenols were quantified by the Folin-Ciocalteau method and antioxidant activity by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Metabolomic analyzes were performed using liquid chromatography coupled to a mass spectrometer. The leaf extract showed total phenol content of 66,55 mgEGA.g-1 and high antioxidant activity with EC50 of 18,74 μgmL-1 when compared to stem extract. By metabolomics, secondary metabolites were identified, such as catechin and rutin, which are phenolic compounds and antioxidants. Based on the results, it appears that the analyzed extracts have metabolites with possible phytotherapeutic activity. So, *C. erectus* is a promising species, however further studies are needed to identify the metabolites responsible for the properties that justify its use in traditional medicine.
Keywords: Antioxidant activity, Button mangrove, Metabolomics. **Supported by:** PIBIC

GD.17 - Effects of γ-oryzanol on the attenuation of oxidative stress markers in a chronic unpredictable mild stress model of *Drosophila melanogaster*

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Chronic unpredictable mild stress (CUMS) acts as one of the main factors related to increased oxidative stress, causing changes such as depression, among other neurobiological disorders. In this sense, the investigation of changes resulting from exposure to CUMS becomes important, and for this, the *Drosophila melanogaster* model stands out, which has high reproducibility, low cost, and good management, in addition to a good acceptance of antioxidant compounds capable of reducing the effects from oxidative stress, such as γ-oryzanol (ORY). The objective of this work was to evaluate the protective effect of ORY on oxidative stress markers in *Drosophila melanogaster* submitted to CUMS. The flies were divided into 4 groups: Control, CUMS, ORY (25 µM), and ORY (25 µM) + CUMS. For the CUMS model, flies were exposed to heat, cold, starvation and sleep deprivation, all for 10 days. To analyze the effects of oxidative stress, the head and body of the flies were separated, and the formation of reactive species (RS), lipid peroxidation via malondialdehyde (MDA), cell viability by reducing resazurin, and the activity of the enzyme superoxide dismutase (SOD) were evaluated. It was verified an increase of RS levels in the heads of flies exposed to CUMS, and ORY treatment reduced these levels. There was a significant increase in MDA levels in the heads of flies exposed to CUMS alone compared to the control. Cell viability decreased in the body of flies exposed to CUMS, while ORY treatment prevented this damage. It was also observed a reduction in SOD activity in the heads of flies from the CUMS group, while treatment with ORY prevented this reduction. It is concluded that ORY has antioxidant potential, reducing oxidative markers, mainly in the flies' heads, reducing possible neurobiological disorders derived from CUMS.
Keywords: CUMS, depression, *Drosophila melanogaster.* **Supported by:** FAPERGS, CNPq and CAPES

GD.18 - Flavonoid agathisflavone reprograms microglia towards a neuroprotective inflammatory profile

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In the central nervous system, microglia orchestrate the inflammatory response to diverse insults, including neuroinflammation associated to neurodegenerative diseases (NDD). Microglia recognize damaged cells acquiring a pro-inflammatory cytotoxic profile that can exacerbate brain damage. However, considering microglia plasticity modulation of their inflammatory response to injury may also promote resolution stages of inflammation and tissue regeneration. Agathisflavone, a biflavonoid purified from Poincianella pyramidalis (Tul.) has demonstrated anti-inflammatory and neuroprotective properties in *in vitro* models of NDD. Here, we investigated the effects of agathisflavona directly in microglial cells submitted to inflammatory damage in view to elucidate mechanisms of neuroprotection associated to modulation of inflammatory response. Microglia were isolated from cortical primary cultures of newborn Wistar rats and were exposed to Escherichia choli lipopolysaccharide (LPS,10 ng/mL) and treated or not with agathisflavone (1-10 µM), for 24h. To investigate possible neuroprotective effects of agathisflavona treatment, differentiated PC12 neuronal cells were exposed to the microglia secretoma (MS) derived from cultures in each experimental condition. We observed that the inflammatory stimulus with LPS induced the microglia to assume an activated cellular state with pro-inflammatory profile characteristic (increased CD68), confirmed by phenotypic changes with more rounded or amoeboid cells. However, when treated with agathisflavone, microglia up-regulated expression of CD206 (anti-inflammatory) and down-regulated CD68 expression, as well presented mainly more branched-like phenotype, in addition to a reduction in the expression of inflammatory mediators IL-6, IL1-b, TNF, NRLP3 and chemokines CCL5 and CCL2, characterizing change to an anti-inflammatory state.Moreover, we observe the preservation of neurites and regulation in the expression of β-tubulin III and Caspase-3 in PC12 cells exposed to MS derived from agathisflavona and agathisflavona plus LPS treated cultures. Together, these data reinforce the capacity of the flavonoid in reprograming microglia to neuroprotective anti-inflammatory profile standing out as a promising molecule for the treatment or prevention of neurodegenerative diseases.
Keywords: Flavonoids, Anti-inflammatory, Neuroprotection. **Supported by:** Capes, CNPq e FAPESB

GD.19 – PROJECT: Evaluation of the biological activity of compounds isolated from *Lepidium meyenii* and its synthetic derivatives

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Lepidium meyenii is a plant traditionally found in the Andean region of Peru that has several biological activities, such as immunomodulatory, antioxidant, and antitumor. Several metabolites have already been identified in *L. meyenii* roots and leaves, such as alkaloids, phenolic compounds, glucosinolates, macaenes, and macamides. However, to this date few studies have performed large-scale metabolite profiling of *L. meyenii* extracts and correlating it with its biological activities. To isolate bioactive compounds from the root of *L. meyenii* and test their antioxidant, antibacterial and cytotoxic activities. The compounds will be isolated from ethyl acetate extracts obtained from the plant root using high performance liquid chromatography (HPLC) and identified through nuclear magnetic resonance (NMR) analysis. The antioxidant activity will be determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method. The antimicrobial activity will be evaluated against Gram-positive and Gram-negative bacteria, as well as non-filamentous fungi through the broth microdilution method and the cytotoxicity will be evaluated against the rat glioma cell line (C6) and astrocytes. Also, macamides will be synthesized and their biological activities will be evaluated. To isolate and identify new compounds from *L. meyenii* and evaluate their antioxidant, antibacterial and cytotoxic potential. Therefore, the study could provide empirical support for the medicinal use of this plant, which directly implies benefits for society in the treatment and prevention of pathologies, also contribute to expanding scientific knowledge about the pharmacological potential of *L. meyenii*.

Keywords: Peruvian Maca, Metabolomics, Natural Products

GD.20 - Bioactivity of natural products from *Rhizophora mangle* L.: metabolomic profile, phenolic compounds, and antioxidant capacity.

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Phenolic compounds are secondary metabolites present in plant species and have important antioxidant properties that neutralize free radicals, favoring the cure of several diseases, such as cardiovascular diseases and infections. *Rhizophora mangle*, popularly known as red mangrove, of the family Rhizophoraceae, is an obligatory species of mangroves, widely used by traditional and riverside communities as herbal medicines for the treatment of skin infections, but its chemical composition and pharmacological properties is still little known. This study aimed was to investigate the metabolomic profile of ethanol extracts (CEERm) from the leaf, stem and root of *R. mangle*, as well as their phenolic compounds and *in vitro* antioxidant activities. Samples of *R. mangle* were collected in the Pojuca river mangrove, Mata de São João-Bahia, Brazil. CEERm were obtained by maceration in ethanol at room temperature. After maceration was performed, the ethanol was removed under reduced pressure at 40 °C. Liquid chromatography–mass spectrometry was applied for metabolomic analysis, and data processing and compounds identification were performed by Metlin and MetaboAnalyst (V5.0) web-platforms. Total phenolic compounds were quantified by the Folin-Ciocalteau method and antioxidant capacity was assessed by 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay. The root extract had the highest concentration of phenolic compounds (71.16 mgEGA.g $^{-1}$) while the leaf extract had the lowest concentration (46.35 mgEGA.g $^{-1}$). The IC50 of the CEERm antioxidant activity ranged from 9.05 (stem) to 31.39 µgmL $^{-1}$ (leaf). The innovative approach used allowed the identification of likely candidate bioactive compounds in the extracts, such as tannins, flavonoids and gallic acid that showed a positive correlation with the antioxidant capacity in different botanical parts of *R. mangle*. These results also indicate that the *R. mangle* have antioxidant potential and source of new natural products, probably due to the presence of the phenolic compounds identified in this study.

Keywords: Metabolomics, Secondary metabolites, Rhizophoraceae
Supported by: FAPESB, CNPq, Capes, Prefeitura de Mata de São João, UFBA.

GD.21 - Trypsin Kunitz-type inhibitor from *Cajanus cajan* leaves

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Cajanus cajan is a legume widely consumed in Asia, Africa and Americas due to the high protein content in their seeds. It is employed a medicinal plant for the treatment of different pathologies. Therefore, the aims of this work were isolate and characterizate biochemically and structurally inhibitors of trypsin-like serine proteases obtained from aqueous phosphate extract of *C. cajan* fresh leaves (CC-P). The trypsin-type protease inhibitor was isolated from *C. cajan* leaf extract CC-P by affinity chromatography with immobilized trypsin in agarose matrix. Protein content was determined by Bradford method and protein profile evaluated by SDS-PAGE. This inhibitor was isolated with purification of 3.64-fold yielded 54% of trypsin-like inhibitors and was denominated TIC, that had a molecular weight about 15 kDa by SDS-PAGE analysis. TIC was assayed against trypsin, papain, pepsin and collagenase, and inhibited only the trypsin activity. TIC has higher affinity for trypsin (Ki = 1.617 μM) than chymotrypsin (Ki = 6.46013 μM) and was a competitive inhibitor. The primary structure of TIC was studied by mass spectrometry and showed homology with a kunitz type trypsin inhibitor when used NCBI database. The inhibitory activities of TIC were maintained even after the following experimental situations: 24 h treatment at 70 °C, 1h treatments with buffers of different pH values and increasing concentrations of β-mercaptoethanol. However, the activity of TIC was affected at different levels in the presence of oxidizing agents, such as hydrogen peroxide and dimethylsulfoxide. These results highlight that *C. cajan* leaves are sources of protease inhibitor with important structural stability.
Keywords: C. cajan, trypsin inhibitor, Kunitz-type inhibitor. **Supported by:** Capes and FIOTEC

GD.22 - Potentially cytoprotective compounds in the establishment of diabetes-related pancreatic beta cell functions

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Chronic hyperglycemia is a characteristic of diabetes. It is known that beta cells play a very important role in glucose homeostasis. Protecting and restoring beta cell function can help minimize the damage from *Diabetes mellitus*. The use of chemical compounds that mimic situations found in the pathogenesis of diabetes, such as glyoxal and methylglyoxal, can help to clarify the various deleterious molecular mechanisms that operate in the cell. Methylglyoxal (MG) is a reactive carbonyl species found at high levels in uncontrolled diabetic patients and may influence the production of reactive oxygen species (ROS). Thus, the use of potentially cytoprotective compounds can have a beneficial effect in situations of high MG levels. Glycyrrhiza glabra, popularly known as licorice, has as one of its main components glycyrrhizin, which belongs to the class of saponins, and, among other properties, it has shown an antioxidant and protective effect in different contexts of cytotoxicity. Rutin is a flavonoid with antioxidant and anti-inflammatory properties, found in different fruits and vegetables, and which has the ability to scavenge reactive species such as hydroxyl, superoxide and peroxyl radicals, thus being a potent attenuator of the harmful effects of MG. The aim of this work was to evaluate *in vitro* the cytotoxicity of methylglyoxal in RINm5F insulin-producing cells, and to test the cytoprotective effect of potentially antioxidant compounds. MTT reduction method (cell viability assessment) and intracellular ROS detection by H2-DCFH-DA. Our results indicate that glycyrrhizin partially inhibits MG-induced cytotoxicity and rutin showed a cytoprotective effect, causing an increase in cell viability in cells exposed to MG. The results obtained through the DCFH-DA intracellular ROS detection test showed that MG increases ROS production. Treatments with glycyrrhizin or rutin decrease ROS production when cells were co-incubated with MG.
Keywords: diabetes, ROS, methylglyoxal. **Supported by:** FAPERJ

GD.23 - Genistein attenuates amyloid-beta-induced cognitive impairment in rats by modulation of hippocampal synaptotoxicity and hyperphosphorylation of Tau

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Alzheimer's disease is a progressive neurodegenerative disorder characterized by extracellular accumulation of amyloid-beta (Aβ) peptide, which induces synaptic dysfunction, alteration of intracellular signaling pathways, hyperphosphorylation of the Tau protein, and cognitive impairment. Genistein, one of the major isoflavones present in soy and soy products, has been shown to modulate some of the pathogenic events associated with the neurodegeneration process. However, its underlying mechanisms remain to be clarified. The objectives of the present study were to evaluate the ability of genistein to protect against Aβ1-42-induced cognitive impairment in rats and to elucidate some of the possible mechanisms involved in its neuroprotective effects in the hippocampus. Adult male Wistar rats (aged 90 days) received bilateral intracerebroventricular infusions of Aβ1-42 (2 nmol) and genistein 10 mg/kg orally for 10 days. Behavioral analyses were initiated 24h after completion of drug treatment and performed sequentially on days 11-15. On the day following completion of the behavioral tasks (day 16), the hippocampi were collected for neurochemical analyses. The Aβ-infused animals showed significant impairment of memory, which was accompanied by the following neurochemical alterations in the hippocampus: decreased levels of the synaptic proteins synaptophysin and postsynaptic density protein 95 (PSD-95), hyperphosphorylation of Tau with increased activation of glycogen synthase kinase-3β (GSK-3β) and c-Jun N-terminal kinase (JNK), and inactivation of extracellular signal-regulated kinase 1/2 (ERK). Treatment with genistein improved Aβ-induced cognitive impairment by attenuation of synaptotoxicity, hyperphosphorylation of Tau, and inactivation of ERK. These findings provide further evidence of the neuroprotective effect of genistein in an in vivo model of Aβ toxicity and, importantly, extend the current knowledge concerning the mechanisms associated with the neuroprotective effects of this compound in the hippocampus.
Keywords: Alzheimer's disease, genistein, neuroprotection. **Supported by:** CNPq and INCT (EN 465671/2014-4)/CNPq

GD.24 - Evaluating Iso-Mukaadial Acetate and Ursolic Acid Acetate as Plasmodium falciparum Heat shock protein 70 Inhibitors (PfHsp70): An antimalarial target.

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The present study investigates the connection between *in silico* docking and in vitro experiment of iso-mukaadial acetate and ursolic acid acetate against Plasmodium falciparum and human heat shock protein 70 (Hsp70). Herein, different modelling techniques including molecular docking, binding free energy and molecular dynamics simulations were performed to gain fundamental understanding into the binding mechanisms of selective compounds against Hsp70. The molecular docking results revealed ligands flexibilities, conformations and positions of key amino acid residues and protein-ligand interactions as crucial factors accounting for selective inhibition of Hsp70. The simulation results also suggest protein-ligand van der Waals forces as the driving force to evaluate the selectivity of the studied compounds. This study will offer a vital understanding of the selectivity mechanisms and rational design of new selective compounds targeting Hsp70.
Keywords: Iso-mukaadial acetate, ursolic acid acetate, PfHsp70
Supported by: URC/FRC

GE - Plants and Synthetic Biology

GE.01 - The gene families of Glutathione peroxidase and Glutathione-S-transferase from *Ricinus communis* L.: phylogenetic analysis and *in silico* characterization

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Ricinus communis L. (Castor) uses several defense mechanisms against different types of abiotic stresses. Antioxidant enzymes such as glutathione peroxidase (GPX) and glutathione-S-transferase (GST) are responsible for maintaining normal levels of reactive oxygen species (ROS), reducing and/or preventing the cascade of damage caused by oxidative stress in plants. The gene families of these enzymes have several members that are present in various parts of the cell. The objective of this study was to characterize the glutathione peroxidase (RcGPX) and glutathione-S-transferase (RcGST) gene family of *R. communis* in comparison with other seven species (*Arabidopsis thaliana*, *Oryza sativa*, *Zea mays*, *Populus trichocarpa*, *Solanum lycopersicum*, *Glycine max* and *Sorghum bicolor*). The sequences were retrieved in the Plant Comparative Genomics of the Joint Genome Institute-Phytozome, after searching the conserved domains in Simple Modular Architecture Research Tool (SMART). Alignment was performed using the MUltiple Sequence Comparison by Log-Expectation (MUSCLE) and phylogenetic analysis using the Molecular Evolutionary Genetics Analysis Software (MEGAX). Finally, cell location prediction was done using subCELLular LOcalization predictor (CELLO) and the verification of conserved motifs in Multiple Em for Motif Elicitation (MEME). It was found that GPX and GST have several members in their gene families, varied amino acid sequences, which are very conserved in GPX, but not in GST. RcGPX and RcGST share common ancestors with *Glycine max* and *Arabidopsis thaliana*, respectively. RcGPX and RcGST consist of multiple isoenzymes with distinct subcellular locations that exhibit different tissue-specific expression patterns, with greater predominance in the cytoplasm. RcGPX has several conserved motifs shared with enzymes from other species, the same not occurring with RcGST, even considering the existing phylogenetic relationships. It was concluded that these enzymes are important in regulating the response to different types of environmental stresses in *R. communis* and other plant species.

Keywords: Antioxidant enzymes, Castor, Gene analysis.
Supported by: CNPQ; FAPESB; PMBqBM/UFBA and UFBA.

GE.02 - Responses of different wheat genotypes to *Herbaspirillum seropedicae* inoculation by proteomic analysis

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Inoculation with plant growth-promoting bacteria (PGPB) has proven to be a promising strategy in supplying the plant's nitrogen and other important nutrients. *Herbaspirillum seropedicae* SmR1, a PGPB, promoted different responses in Brazilian wheat (Triticum aestivum var. Lini) cultivars (CD104 and CD120) under *in vitro* and greenhouse conditions. The aim of this work was to investigate the protein profile in wheat leaves (CD104 and CD120) when inoculated with *H. seropedicae* to understand the plant physiology and photosynthetic system. ZADOK 2.1 wheat plants were obtained by growth in MS medium without (SN) and with (CN) nitrogen source, without sucrose. After 24h, some tubes of SN medium received bacterial inoculum (SNI). The tubes were kept in a culture room at 24 °C and 14h light /dark for 20 days. Leaves were harvested to obtain protein extract for proteomics based on protein fractionation by SDS-PAGE followed by LC-MS/MS - Q-TOF type. Intact plants were used to obtain nitrogen index (NBI) and chlorophyll (CHL), flavonoids and anthocyanins, chlorophyll fluorescence. As results, CD120 in SNI medium promoted increase in NBI and CHL, reduced anthocyanins, chlorophyll fluorescence in photochemical and non-photochemical extinction. Only one protein was uniquely found in SNI condition, being related to protection of photosystem II. CD 104 in SNI condition presented 5 photosynthesis proteins and others related to hypersensitivity and diseases resistance. Both cultivars in SN presented antioxidant proteins, although chlorophyll fluorescence was not severely affected. The plant-bacteria interaction is genotype dependent and *H. seropedicae* was able to partially meet the N requirement for the CD120 genotype and promote defense and tolerance responses for the CD104 genotype.

Keywords: Wheat, proteomic analysis, photosynthetic system
Supported by: FAPPR and CAPES

GE.03 – PROJECT: Secondary metabolites from *Zea mays L. corn* silk extracts

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Corn silk is a part of the female flower of *Zea mays* L., which is well-known in the traditional Chinese medicine for its properties. Several reports have described its pharmacological activities, but few studies have performed a global chemical profiling of the extracts, especially correlating it with the activities. Thus, corn silk extracts may be of great interest as a potential source of bioactive compounds. The objective of this project is to isolate bioactive compounds from the corn silk ethyl acetate extract. The antioxidant activity will be performed by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assat, whereas antimicrobial activity will be evaluated by the broth microdilution method against Gram-positive and Gram-negative bacteria, and against non-filamentous fungi. The antitumor potential of the extracts will be evaluated against the murine glioma (C6) cell line. Extracts fractionation will be performed using several chromatographic techniques, such as column chromatography, preparative thin layer chromatography and High-efficiency liquid chromatography (HPLC-UV). The isolation of new bioactive metabolites will provide an important leads into the understanding of the corn silk medicinal properties in terms of its secondary metabolites. In addition, it will be possible to correlate the metabolites of extracts with antioxidant, cytotoxic and antimicrobial activities. Therefore, providing important clues for the discovery of new active compounds and possibly for the development of new pharmaceutical products. In addition, it will contribute to the sustainable use of Brazilian biodiversity, since the use of medicinal plants with well-established protocols and quality control procedures can expand the range of therapeutic options and thus contribute to health improvement.

Keywords: Corn silk, Metabolomics, Murine glioma C6
Supported by: FAPESB

GE.04 - Differential proteomic analysis of wheat root *(Triticum aestivum var. lini)* cv. CD104 in the absence of nitrogen and the presence of the bacteria *Herbaspirillum seropedicae Smr1.*

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Herbaspirillum seropedicae is a diazotrophic, endophytic bacterium that has shown good results in the interaction with wheat plants *(Triticum aestivum L.).* This study aimed to investigate proteins present in the roots of wheat cultivar CD104 when inoculated with the bacterium in the absence of nitrogen and carbon source to understand the plant-bacteria interaction. Wherefore, wheat seeds were sterilized, pre-germinated, and transferred to glass test tubes containing liquid MS culture media without sucrose to obtain *in vitro* cultivation under treatments: with nitrogen source (CN), without nitrogen (SN), and without nitrogen with the bacterium inoculum (SNI) added to SN tubes after 24 hours of seed transfer to tubes of SN and CN All tubes were kept in a culture room at 25 °C and a photoperiod of 14 h of light for 20 days. The roots were taken to obtain protein extracts for shotgun proteomic analysis. Proteins, 1069, were identified and other information on metabolic pathways was obtained from identified proteins in each treatment. The RUBISCO protein and others as beta-1,3-glucanase and chitinases observed in SNI suggest the activity of carbon fixation, hypersensitivity responses, and resistance to diseases in plants promoted by the bacteria. A total of 211 exclusive proteins of *H. seropedicae* were identified in SNI treatment. Proteins were related to synthesis activity, energy and carbohydrate metabolism, and biological nitrogen fixation activity. Proteins related to antioxidant metabolism, as glutathione S-transferase, were verified in the roots of the SN treatment. The results suggest that wheat roots in the absence of nitrogen were responded to this condition and the presence, decreased responses to stress in the absence of nitrogen, and some pieces of evidence of biological nitrogen fixation in the root.
Keywords: Shotgun proteomics, wheat roots, plant growth promoting bacteria
Supported by: FAPPR

GE.05 - Investigation of antiglioma activity from *Aloysia virgata* (Ruíz & Pavón) Juss

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Aloysia virgata is a medicinal plant belonging to the Verbenaceae family, widespread in South America, and its tea is used to treat symptoms of diseases related to the digestive system. In this work, the leaves of A. virgata were submitted to two types of extraction, aqueous (AQ), simulating the popularly consumed tea, and hydroalcoholic (ET), which generally extracts the maximum amount of compounds a plant. Both extracts obtained were fractionated by liquid-liquid partition, with the following solvents in increasing order of polarity, chloroform, ethyl acetate and butanol for ET; and ethyl acetate and butanol for AQ. The partition process then yielded 4 fractions for ET, named CE (chloroform ethanolic fraction), ACE (ethyl acetate ethanolic fraction), BE (butanolic ethanolic fraction) and AE (aqueous ethanolic fraction); and 3 fractions for AQ, ACA (aqueous ethyl acetate fraction), BA (aqueous butanolic fraction) and AA (aqueous aqueous fraction). The extracts and fractions were then used in the treatment of glioblastoma cells, initially at three concentrations: 0.01, 0.05 and 0.1%. All treatments were performed in triplicate, for 24, 48 and 72 hours in a 96-well plate, with 5,000 cells per well, using two glioma strains, C6 and U251. Cell viability was measured using the MTT test. The results obtained so far demonstrate that both strains showed sensitivity to treatments with CE, ACA and ACE, at concentrations of 0.05 and 0.1%, in 48 and 72 hours, reducing cell viability by at least 50%, compared to the control. The other fractions, as well as AQ and ET, did not show a satisfactory decrease. Although these are preliminary results, they are unprecedented when using the plant in glioma cells. Thus, more studies are being conducted to prove the effectiveness of A. virgata compounds in reducing the growth of this type of cells.
Keywords: Anti-tumor activity, bioactive compounds, glioblastoma

GE.06 - Integrated production of high-value aromatic alcohols directly from lignocellulosic biomass

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Sustainable production of fine chemicals from renewable plant biomass offers an excellent alternative to the continued use of finite geological oil reserves for fine chemistry purposes. However, in order to compete with current petrochemical refinery processes, alternative biorefinery processes must overcome significant costs and productivity barriers. The production of high-value aromatic alcohols directly from lignocellulosic biomass is an attractive alternative to add value in biorefineries worldwide. Herein, we demonstrate the biocatalytic production of the versatile chemical building block, coniferol, directly from lignocellulosic biomass. Following the biocatalytic treatment of lignocellulose to release and convert ferulic acid with feruloyl esterase (XynZ), carboxylic acid reductase (CAR) and aldo- keto reductase (AKR). This whole-cell catalytic cascade not only achieved the equivalent release of ferulic acid from lignocellulose compared to alkaline hydrolysis but also displayed efficient conversion of ferulic acid to coniferol. This system represents a consolidated biodegradation-biotransformation strategy for the production of high-value fine chemicals from waste plant biomass, offering the potential to minimize environmental waste and add value to agro-industrial residues.

Keywords: Lignocellulose, biocatalysis, Coniferol

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GE.07 - Identification and quantification of flavonoids in CD150 wheat inoculated with *Azospirillum brasilense*

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Several plant organs produce flavonoids that are secondary water-soluble phenolic compounds, whose presence is related to external stimuli, with antioxidant and regulatory functions for plant processes. A bias in the study of flavonoids refers to the role of biocommunication between bacteria and plants such as wheat. The objective of this work was to determine the flavonoids produced in wheat roots inoculated with native rhizobacteria of biotechnological potential, to identify flavonoids that could increases nitrogen fixation through plant-bacteria communication. The *in vitro* experiment consisted of four treatments and five replications of CD 150 wheat plant, inoculated with *Azospirillum brasilense*, fed with solutions with or without nitrogen addition. After twenty-one days, the weight and length of roots and leaves were measured. The roots and stems were evaluated, by scanning electron microscopy, to verify the conducting vessels of wheat inoculated and not inoculated with the *Azospirillum brasilense* bacterium. The extracts were subjected to flavonoid identification by high performance liquid chromatography. Preliminary data demonstrated a higher concentration of total flavonoids in treatments performed with nitrogen in the presence of *Azospirillum brasilense*. The extracts showed the presence of three main types of flavonoids (isoflavone, quercetin and kaempferol). The role of these flavonoids in rhizosphere communication, strengthening the idea of chemotaxis between plant and microorganism and their biological nitrogen fixation ability.

Keywords: Bacteria, Flavonoids, Wheat

GE.08 - Biomass and essential oil content of basil (*Ocimum basilicum* L.) cultivated in semiarid region.
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Basil is an herbaceous species of Asian origin, used as a condiment and medicinal plant. The production of this species has been the subject of much research around the world given the production of essential oil that arouses commercial interest and has pharmacological properties. The production of the species to obtain essential oils in semiarid regions is challenging for family farming. Therefore, the objective was to study the production of essential oil of Ocimum basilicum L. under protected cultivation and in full sun. The plants were cultivated at UESB, in the city of Itapetinga-BA, in 15cm high containers in a greenhouse and in the field for 75 days, individually watered. After harvest, they were weighed on an analytical balance to determine fresh matter, stored in kraft paper, and conducted for hydrodistillation for two hours. The essential oil was collected and weighed on an analytical balance. The material resulting from the extraction was carried to an oven at 60°C to determine the dry matter. Essential oil content was calculated based on dry matter and data expressed as an average. The two cultivation systems showed differences in biomass. Plants grown in a greenhouse recorded 129 g of fresh mass and 29 g of dry matter. Plants grown in full sun obtained 107g of fresh biomass and 23g of dry matter, respectively. Consequently, the essential oil content was also higher in protected cultivation, with 0.6% in the greenhouse and 0.4% in the field. The greater production of biomass and essential oil in protected cultivation can be explained by the protection of individuals against herbivory as well as better absorption of water and nutrients by reducing evapotranspiration. However, to obtain biomass and essential oils from basil in semiarid regions, cultivation under protection from photosynthetically active irradiation at 50% is suggested.
Keywords: basil, biomass, essential oil. S**upported by:** FAPESP, CNPq and CAPES

GE.09 - *In silico* characterization of catalase gene family from *Ricinus communis* L.
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Ricinus communis (Castor) is an oilseed with industrial and social importance in Brazil and in different parts of the world. Environmental factores can lead to an increase in reatives oxygen species (ROS) and affects metabolic pathways that determined the germination process of it seeds, promoting the oxidative stress. The catalase reaction involves the decomposition of two molecules of hydrogen peroxide (H_2O_2) to water (H_2O) and oxygen (O_2).The objective of this study was to characterize the catalase genes from *Ricinus communis* L. (RcCAT) compared to genes of 10 other plant species. CAT sequences were retrieved from the Plant Comparative Genomics do Joint Genome Institute (Phytozome) after searching for conserved domains in Simple Modular Architecture Research Tool (SMART). The alignment was performed by MUltiple Sequence Comparison by Log-Expectation (MUSCLE) and the phylogenetic analysis were made in Software Molecular Evolutionary Genetics Analysis (MEGAX). Lastly, cell sublocalization prediction was done using web server for protein Subcellular Localization Predictive System (CELLO). The CAT protein was show to have well conserved residues (LA(E)F) in comparison with the other species analyzed. All RcCAT contained a catalase core domain as well as an immune response catalase domain. According to phylogenetic analyses, these CAT genes were grouped in clades that suggest the integration of the CAT enzyme subfamilies. Phylogenetic relationships showed that there is a common ancestor between RcCAT and MeCAT (CAT of *Manihot esculenta*).The prediction of subcellular localization indicated that the candidate sequences they mostly owned peroxisomal localization. It was concluded that the CAT is a multigenic enzyme and has a variation in its number of genes according to each species. They are isoenzymes involved in various functions such as growth, development and stress tolerance with a fundamental role in the ROS response in plants.
Keywords: Antioxidant enzymes, Castor bean, Phylogeny.**Supported by:** CNPq; FAPESB and UFBA.

GE.10 - A minimal pathway for the regeneration of redox cofactors

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Metabolism is an intricate network of biochemical pathways that make a cell out-of-equilibrium, but only a limited group of fundamental molecules have been conserved under evolutionary pressure to carry out specific functions.1The nicotinamide adenine dinucleotides NAD(H) and NADP(H) belong to this category of hub metabolites,2involved mostly in the transfer of reducing equivalents between reactants. An ambitious challenge of building a synthetic cell via bottom-up methodologies requires a metabolic component, thus ensuring the constant availability of nicotinamide cofactors.Here, we constructed a synthetic pathway in which both the redox cofactors involved in cell catabolism and anabolism, respectively NAD+ and NADP+, are continuously reduced and oxidized. This is mediated by the catalysis of the purified enzymes formate dehydrogenase and soluble transhydrogenase. Regulation between the two oxidative states is given by the concentrations of enzymes and substrates required by the specific reactions. The action of formate dehydrogenase leads to NADH accumulation. This allows the reduction of NADP+ over time by soluble transhydrogenase, which simultaneously restores the required NAD+ for the first reaction. Consequently, the formed NADPH becomes available for further redox enzymes that could drive NADP+ regeneration. The coupled reactions are triggered by a membrane permeable redox donor, formic acid. This makes the cofactor regeneration feasible confined *in vitro* in phospholipid vesicle compartments, as well as in solution. We also investigated the behaviour of our coupled redox system across different compartment scales, using LUVs and GUVs. Developing such coupled redox reactions will provide the necessary energy in terms of reducing equivalents in order to increase the achievable metabolic complexity inside the synthetic cell-like systems. [1] Schmidt, S., et al., Trends Biochem. Sci. 28, 336–341 (2003). [2] Pfeiffer, T., et al., PLoS Biol. 3, 1269–1275 (2005). Acknowledgments: We thank Yi Yang for the provision of the template vector pRDNA3.1-hygro-cyto-iNap1 and Marco Fraaije for assistance and discussion on SthA properties. M.P. and D.J.S. are supported by NWO Gravitation program (Building a Synthetic Cell) grant 024.003.019. B.P. is supported by ERC Advanced Grant (ABCvolume) grant #670578. E.J.E and D.J.S. are supported by Dutch Ministry for Education, Culture and Science (OCW), Bonus Incentive Scheme.
Keywords: Redox cofactors, metabolic pathways, bottom-up approach

HA - Molecular Mechanisms of Diseases

HA.01 - PROJECT: Evaluation of the functional role of mirna in signaling pathway modulation associated with the development and degree of severity of *Diabetes mellitus* type ii: *in vivo* and *in silico* study.

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Diabetes mellitus (DM) is a chronic disease resulting from defects in insulin production, secretion, or signaling. DM affects about 400 million individuals worldwide. It is estimated that the diagnosis is given around 7 years after the onset of the disease and usually with associated comorbidities. In a previous study, miRNA Let-7a, Let-7b, 106b, 93, and 17 had greater sensitivity and specificity for prediabetes and were differentially modulated in the analyzed transcriptome. Hypothesis: The miRNAs Let-7a, Let-7b, 106b, 93, and 17 interfere with glucose metabolism, being involved in several intracellular pathways related to insulin resistance (IR) and the destruction of pancreatic β-cells. To investigate the functional role of Let-7a, Let-7b, 106b, 93, and 17 miRNAs in glucose metabolism by validating the expression pattern of miRNAs in a cohort of healthy, pre-diabetic, and diabetic individuals. A prospective cohort study will be carried out with healthy, pre-diabetic, and diabetic individuals, followed by CEDEBA between 2021 to 2025. The patients' blood will be collected for extraction of total RNA and miRNA microarray and Elisa for cytokines. The validation of miRNA targets will proceed *in vitro* with HSkMC cells transfected with miRNA targets and Western Blotting will be executed for evaluation of the transcription of genes involved in the insulin signaling pathway. It is expected to detect the miR and target genes responsible for DM.

Keywords: Predictive Analysis, Transcriptome, Insulin Resistance
Supported by: FAPESB

HA.02 - Search for new therapeutic targets for Chagas disease: functional prediction and analysis of metabolic pathways

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Chagas disease is a social and economic burden even for specialized clinical centers and in 2019, 9,490 deaths were reported worldwide. The treatment of the acute phase is recommended benznidazole (Bnz) and nifurtimox, both highly toxic, and treatment interruption is common. It is necessary to find new targets for the formulation of new drugs in order to improve therapeutic adherence. The objective is, through the use of different programs, to perform the functional prediction of proteins with unknown function described in the *Trypanosoma cruzi* proteome (CL Brener strain), in order to find new protein targets. For functional prediction, the following programs and databases were used: Pfam, String, Psort, Smart, CDD, Prosite, TritryDB, HMMER, Uniprot, BlastP (NR) and MHOLline (comparative modeling with Modeller vs 10.0). From the data obtained in the MHOLline program, we used the SCOP and SCOPe databases. For the analysis of metabolic pathways we consulted the MetaCyc, Trypanocyc, KEGG and Reactome databases. From the proteome deposited at the NCBI (GCA_000209065.1) proteins with unknown function were separated, totaling 11,171. These sequences were submitted to MHOLline, which built a 3D model for 1,421 proteins, from which 43 models were selected according to the identity and coverage value (>35% and >70% respectively). Based on the use of the aforementioned programs for functional prediction, the function was predicted for 17 proteins, among the functions are: Adenylate Kinase, Ankyrin repeat, Uridyltransferase, Putative RNA-binding, AcylCoa binding, Ubiqutin-fold, O-acetyl-ADP -ribose, Tetracopeptide, Transcription factor IIB C-Terminal, Mitochondrial membrane anchored protein and RAS-related protein. Among the analyzed metabolic pathways, we saw that the protein Adenylate Kinase, if inhibited, could lead to impairment of the adenosine ribonucleotide pathways of new biosynthesis and purine and pyrimidine metabolism, with direct impacts on the production of the ADP molecule, used as an energy currency within of the cells.

Keywords: *Trypanosoma cruzi*, treatment, functional prediction
Supported by: Capes and CNPq

HA.03 - Modification of PPARy-coregulator's recruitment by Ser273 phosphorylation

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The nuclear receptor PPARg is the master regulator of adipogenesis, lipid, and glucose metabolism. The phosphorylation of adipose tissue subtype of PPARy at Ser273 has been linked to the dysregulation of a subset of specific genes that induce insulin resistance. However, the molecular mechanisms that drive this change in gene expression are unclear. In this work, we aim to investigate how this phosphorylation may disturb the interaction between PPARg and some coregulator proteins as a new mechanism that leads to insulin re-sistance. Through cellular and *in vitro* assays, we show that PPARg phosphor-ylation inhibition increased the activation of the receptor. Thus, this blocking increases the recruitment of PGC1-a and TIF2 coactivators, whilst decreases the interaction with SMRT and NCoR corepressors. Moreover, we investigate whether the CDK5, responsible for Ser273 phosphorylation could also disturb the PPARg-corregulator's balance. Finding that CDK5 presence decreases the PPARg interaction with PGC1-a, TIF2, and NCoR, while increases the cou-pling of SMRT. Together these results indicate that the insulin resistance as-sociated with PPARg phosphorylation is linked to a differential coregulators recruitment, which may promote dysregulation in gene expression.

Keywords: PPARg, protein-protein interaction, phosphorylation

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HA.04 - Peptides reduce cell viability of glioblastoma cells

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Glioblastoma multiforme (GBM) is a spontaneously occurring central nervous system tumor that is highly aggressive and invasive. Two proteins seem to be important for the development of glioblastomas, the Focal Adhesion Kinase (FAK) and the αB-crystallin (CryAB). Previous work has shown that the interaction between FAK/CryAB in cardiomyocytes cells is essential for cell survival and the reduction of this interaction promotes apoptosis. Through a series of biochemical approaches, the peptides involved in this interaction were identified. propose that the association of FAK with CryAB contributes to the maintenance of cell viability in glioblastoma cells. This study was performed in glioblastoma cells (U87-MG). We performed western blotting assays to confirm the expression of both proteins in these tumor cells. We realized co-immunoprecipitation assays to verify the interaction of the proteins FAK/CryAB. We performed MTT assays to verify the viability of the cells after treatment with the peptides previously identified. These peptides were synthesized by company AminoTech Research and Development. We validated the expression of both proteins in the glioblastoma cells and we showed that these proteins also interact in these tumor cells. The treatment of the cells with the peptide FP01 (25μM), derived from the α-Crystalline domain (CryAB85-94) reduced in around 35% of cell viability and the treatment with the peptide FP02 (25μM), derived from the FAT domain (FAK921-930) reduced in around 30% of cell viabilty. Futhermore, we performed immunoprecipitation assays after the treatment of cells with the peptides, in order to verify the decrease in the interaction among the proteins. We also extract proteins from the cell lysate to perform western blotting assays and verify proteins important to the apoptosis process. Both experiments are in progress. These previous results propose that the association of FAK with CryAB contributes to the maintenance of cell viability in glioblastoma cells.

Keywords: FAK, αB-Crystalline, glioblastoma cells

Supported by: CNPq

HA.05 - Investigation of anti-apoptotic modulators in the course of infection of macrophages by *L. (L.) amazonensis.*

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Tropical infections such as leishmaniasis are caused by intracellular protozoa of the genus Leishmania, and American Tegumentary Leishmaniasis (ATL) has *L. (L) amazonenis* as its main etiological agent in the New World. In a pathological state, programmed cell death (apoptosis) pathways should be activated in infected cells, either autocrine or mediated by cytotoxic immune cells, playing a key role in resolving pathogen-induced infections. However, previous results from the group and the literature demonstrate that trypanosomatids can activate pathways promoting a pro-survival state by also inhibiting apoptosis of infected cells. Leishmania spp. and *T. cruzi* are able to subvert apoptosis, keeping cells alive long enough for infection and intracellular proliferation of new parasites, in a safe niche. The objective of this project is to investigate in cells infected *by L. (L) amazonensis* the expression/activation of anti-apoptotic genes related to proliferation and cell cycle progression, mainly IAPs (Inhibitors of Apoptosis Proteins) such as XIAP and Survivin. As a tool for the functional assays of host-parasite interaction in infection, the Raw 264.7 strain of murine macrophages and human macrophages differentiated from the monocyte lineage THP-1 will be used. Infected strains will be investigated by flow cytometry to determine changes in mitochondrial membrane potential and an evaluation of protein complex formation by coimmunoprecipitation and mass spectrometry will occur. Previous studies by the group through RNAseq and other functional approaches revealed an active participation of these proteins in apoptosis resistance events during infection. It is predicted that a greater knowledge about the survival strategies that Leishmania (L) amazonensis uses to protect itself and circumvent the attempts of elimination made by the host should contribute to a better understanding of the mechanisms of subversion of cell death in the pathophysiology of leishmaniasis.

Keywords: Leishmania, apoptosis, parasite-host interaction. **Supported by:** FAPERJ

HA.06 - Molecular aspects of the SARS-CoV-2 variants and their virulence

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The SARS-CoV-2 betacoronavirus has emerged as a new threat to global health, demanding its molecular mechanisms to be fully elucidated. The natural evolutive surge of new variants is imposing additional challenges. A molecular understanding is fundamental to the design of efficient diagnostic tools, scientifically verified therapeutical and prevention options. To quantify the stability of spike trimeric structures and their binding affinities with the human cell receptor for different viral strains at several pH regimes. By means of biophysical computational methods (Constant-pH Monte Carlo simulations), the stability of several spike (S) trimeric structures at different conformational states and pH conditions was investigated, as well as the free energy of interactions between the receptor binding domain (RBD) and the Angiotensin Converting Enzyme 2 (ACE2) for the most common variants of concern (Alfa, Beta, Gamma, Kappa, Epsilon, and Iota). Furthermore, the electrostatic epitopes were mapped by the PROCEEDpKa method. Analyses of the results allowed the observation of the conformational state's influence on the stability of the S protein. The stability is directly dependent on the viral S protein sequence for each strain. For instance, SARS-CoV-1 S protein has a probability of ca. 67% for the open state. Conversely, the wildtype SARS-CoV-2 S protein has a smaller probability, which correlates with a lower infection rate. In contrast, the Beta variant has virtually 100% of probability for the S protein to be at the open state. This indicates a higher possibility of infection. The binding affinities of some variants, including Gamma, show an increased attraction to human cells. SARS-CoV-2 has developed a mechanism that favors the open state, combining more stability with more affinity for ACE2. These results contribute to a better understanding of molecular physiopathology and viral evolution, which consequently helps in the fight against the disease.

Keywords: binding affinities, conformational states, mutations
Supported by: FAPESP; CNPq

HA.07 - Project association of long non-coding RNA MALAT1 polymorphism and clinical course of patients with Chagas disease

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Chagas Disease (CD) is a disease caused by *Trypanosoma cruzi* and presents variable clinical manifestations. Most patients will not develop the clinically significant disease, but about 30-40% of those infected may develop the defined clinical forms (cardiac, digestive, or cardiodigestive), usually 10-30 years after the initial infection. Several factors are associated with this variability, and among these are the genetic characteristics of the host. Some studies have shown that lncRNAs are involved in the development of cardiovascular disease, MALAT1 stands out. However, to date, no study has evaluated the role of polymorphisms in the gene of this long non-coding RNA with the clinical evolution of patients with CD. Therefore, this project aims to analyze the association between lncRNA MALAT1 polymorphisms with clinical forms, death and stroke risk scores, and other clinical, electrophysiological, and echographic features of patients with chronic CD. A cross-sectional study will be conducted in which peripheral blood samples will be collected from patients with CD to extract DNA, then genotyping of MALAT1 polymorphism by restriction enzyme digestion technique will be performed. Then, the clinical forms of the patients will be determined, and imaging, echocardiogram, and electrocardiographic data will be evaluated by consulting and analyzing the patients' medical records. Finally, the data will be tabulated and evaluated by appropriate statistical analysis. We expect that the inheritance of certain alleles of the MALAT1 gene is associated with a higher frequency of the clinical cardiac form and higher risk of death and stroke, as well as with echographic and electrophysiological changes in CD patients. This project aims to identify new genetic biomarkers for the prognosis of patients by better understanding the pathophysiological mechanisms that act in the clinical evolution of patients with DC.
Keywords: Chagas Disease, Polymorphism, Long non-coding RNA. **Supported by:** CAPES

HA.08 - The rs2910164 polymorphism in miR-146A is not associated with clinical and echocardiographic manifestations and scores in chronic Chagas disease

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Functional genetic polymorphisms involved in the immune response may modulate the clinical variability in Chagas disease (CD). Recent studies showed that miR-146A is regulated differentially in multiple conditions, including CD, in which it appears upregulated. The C allele in rs2910164 single nucleotide polymorphism (SNP) in miR-146A gene seems to reduce its production and efficiency and is associated with clinical outcomes in multiple patient populations. However, this association has not been studied in CD patients yet. This study aimed to evaluate the association between rs2910164 SNP and clinical and echocardiographic manifestations and scores in CD patients. We conducted a cross-sectional study with 186 chronic CD patients, of which 93 were male and 93 females, with a mean age of 47,78 ± 11,57 years. They were genotyped by the polymerase chain reaction-restriction fragment length polymorphism method. The clinical forms, the risk of sudden death (Rassi score), the score of cardioembolic ischemic stroke, and echocardiographic parameters were obtained by accessing the patients' medical records. We observed that 104 patients presented GG genotype, 69 GC, and 13 CC. The expected and observed genotype frequencies obeyed Hardy-Weinberg equilibrium. The statistical analyses conducted showed no significant associations between the allelic and genotypic frequencies and the clinical forms of chronic CD (cardiac, digestive, or cardiodigestive), the risk of sudden death, the score of cardioembolic ischemic stroke nor echocardiographic parameters (such as left atrial and diastolic, systolic left ventricular diameters; and left ventricular ejection function). We conclude that there is no association between rs2910164 polymorphism in miR-146A and clinical and echocardiographic manifestations and scores in CD patients. Therefore, it seems to be more valuable to investigate the influence of other polymorphisms in miR-146A or other molecules in regards to CD, although these results could be reinforced by studies with bigger population samples and more robust methodologies.
Keywords: Chagas Disease, MicroRNAs, Single Nucleotide Polymorphism. **Supported by:** CAPES; CNPq

HA.09 - PROJECT: Nanoparticle activity on modulation of *in vitro* aggregation and neurotoxicity of alpha-synuclein protein

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Parkinson's disease is the second most common neurodegenerative disorder in people over 60 years of age. Among its main symptoms are: bradykinesia, tremor, rigidity and postural instability, in addition to non-motor clinical events such as depression, anxiety, memory loss and olfactory defects. Its main pathological characteristics are the death of dopaminergic neurons in the substantia nigra and the presence of protein inclusions called Lewy bodies, whose main constituent is the alpha-synuclein protein. By appearing in this region, aggregates of the protein in question, and certain species of this aggregation are toxic to neuronal cells, Parkinson's disease is considered an amyloidosis. In this way, there is a need to find molecules and compounds that are capable of acting as neuroprotectors, inhibiting the toxicity of such species, and thus preventing cell death and maintenance of its morphology and viability. In this work, the effect of iron oxide and cobalt ferrite nanoparticles isolated and coated with organic matter will be evaluated, and their roles as neuroprotectors in human dopaminergic neuroblastoma cells in the presence of alpha-synuclein protein. Biophysical and biochemical analyses, cytotoxicity assays, flow cytometry and evaluation of cell morphology. In addition to analyzing the role of these nanoparticles in modulating the aggregation kinetics of the alpha-synuclein protein, and its ability to break up previously formed aggregates. Define the minimum nanoparticle concentration necessary to inhibit aggregation, evaluate the role of the nanoparticle produced by this protocol in inhibiting the toxicity of oligomers and amyloid fibers formed by alpha synuclein, observe whether there is a nuroprotective role of nanoparticles when pre-treating the neuroblastoma cells.

Keywords: Alpha synuclein, Nanoparticle, Parkinson

HA.10 - Genetic diversity of the structural region of hepatitis C virus genotypes 1 and 3 in patients with chronic hepatitis C and its clinical and laboratory implications

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Around 71 million people are infected with hepatitis C virus (HCV) worldwide and chronic infection is associated with complications such as cirrhosis and hepatocellular carcinoma. HCV has a single-stranded RNA genome of approximately 9.6 kb, which is translated into structural and non-structural proteins. So far, 8 HCV genotypes and 90 subtypes have been described, with genotypes 1 and 3 being the most prevalent in Brazil. In addition, each infected individual has a unique viral set (quasispecies). Due to high genetic diversity, there is still no vaccine for HCV. Therefore, the structural region has been studied as a target in the development of vaccines considering its importance in the viral infectious cycle. The aim of this work is to comparatively analyze the genetic diversity of the structural region (core, E1 and E2) of HCV genotypes 1a, 1b and 3a in patients with chronic hepatitis C with different clinical and laboratory characteristics. From a cohort of twenty patients with chronic hepatitis C, HCV genome was sequenced using a customized panel (Ampliseq On-Demand) (ThermoFisher). After preparing libraries using barcodes, massively parallel sequencing was performed to generate reads on the Ion Proton platform (ThermoFisher). Reads were imported and analyzed using CLC Genomics Workbench v.8.5 (Qiagen) software. Reads were mapped with their respective HCV genome sequence references and the differences were evaluated. For genotype 3a we observed that the E2 region had the highest percentage of amino acid changes (18.73%) and when there was a change of amino acids in the E2-HVR1 region, there was no change in E2-HVR2, and vice versa. Same analysis are being performed with HCV-1a and -1b. Studying the structural region of HCV is essential to better comprehension of genotypes differences and to assist in the development of effective vaccines.

Keywords: bioinformatics, biotechnology, hepatitis C
Supported by: IFRJ, CNPq, FAPERJ, CAPES

HA.11 - PROJECT - Evaluation of the wound healing activity of a combination of Artemisia vulgaris and *Persea cordata*, using the moxatherapy technique,in mice

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Moxatherapy is a Chinese traditional medicine technique where Artemisia vulgaris burning is used to relieve pain and assist healing process. Data using animal models report that this technique may increase cell proliferation rate, speeding healing, stimulating growth of collagen fibers, possibly mediated by the induction of TGF-β, an important molecule during tissue repair. Medicinal plants may be used during wound healing. One example is *Persea cordata*, which has been commonly used by the population of Santa Catarina Plateau in the treatment of wounds. This species has its significant wound healing effect possibly due the presence of molecules such as N-Hexane, dichloromethane, ethyl acetate and butanol. This study aims to associate *Persea cordata* during moxibustion, to evaluate its healing activity, applied into experimental wounds in mice. The experimental protocol will be submitted to local CEUA. Animals will be acquired and kept in standard conditions and are going to be submitted to a cutaneous excisions method. Mice will be divided in experimental groups: 1) No treatment, 2) Moxa (Artemisia) 3) Moxa (Artemisia plus Persea); being treated during fourteen days. Important parameters of tissue repair investigation will be investigated, such as histological analysis, tissues level of hydroxyproline and leucocyte migration (myeloperoxidase and N-acetyl-glucosaminidase enzymatic activities). The moxibustion therapy using Artemisia vulgaris burning plus *Persea cordata*, may be effective to increase the rate of wound healing of cutaneous wound in mice, being an excellent new therapeutic option. These results, thus may provide development and innovation using regional medicinal plants.

Keywords: Moxatherapy, wound healing, *Persea cordata*

HA.12 - Effect of *Uncaria tomentosa* aqueous extract on the response to lipotoxicity- induced oxidative stress in cultured skeletal muscle cells

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Type 2 *Diabetes mellitus* (T2DM) is a chronic non-communicable disease with an increasing number of cases in recent years worldwide. It is characterized by chronic hyperglycemia, associated with dyslipidemia, which corresponds to the increase in the concentrations of triglycerides and fatty acids, in tissues such as skeletal muscle. The accumulation of intramuscular fatty acids is related to cell death, redox imbalance and oxidative stress. The use of herbal medicines such as *Uncaria tomentosa* (Ut) has been proposed as an auxiliary treatment for patients with T2DM, considering the possibility of overlapping conventional therapy. Based on this, the aim of this work was to evaluate the effect of the Ut aqueous extract on these events, induced by the free fatty acid (FFA) palmitate or palmitic acid (PA), in skeletal myoblasts of C2C12 lineage. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal bovine serum (FBS), at 37 ° C humidified atmosphere and 5% CO_2. The treatments were performed by the incubation of cells with PA, in different concentrations, in the presence or absence of 250 µg/ml Ut aqueous extract, for 2, 6 or 24 h. Which resulted in an increase of, at least, 50% in cell viability compared to control. After these periods, oxidative stress was evaluated by fluorescence spectroscopy, using the fluorescent marker DCFDA. The treatment of cells with Ut aqueous extract, for 6 h, followed by exposure to 500 µM PA, resulted in decrease of 38% in the ROS formation, in relation to those incubated with PA only. In summary, the Ut aqueous extract promoted a raise in cell viability, reduced cell death and attenuated ROS formation in cultures incubated with 500 µM PA.

Keywords: Uncaria tomentosa, palmitic acid, oxidative stress
Supported by: FAPESP

HA.13 - Chrysin restores memory deficit in hypothyroidism mice through the neurotrophinergic system

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Hypothyroidism is associated with neuropsychiatric disorders, causing a memory deficit, this cognitive decline is closely related to Alzheimer's disease. The present study evaluated the effects of flavonoid chrysin, a natural compound that demonstrates several beneficial neuronal effects. In this context, this study investigated the protective effect of chrysin in cognitive impairment in hypothyroid female mice by exploring neuroplasticity. Hypothyroidism was induced by continuous exposure to 0.1% methimazole (MTZ) in drinking water for 31 days. Exposure to MTZ was associated with low plasma levels of thyroid hormones T3 and T4 compared with the control group. Subsequently, euthyroid and MTZ-induced hypothyroid mice were intragastrically administered vehicle or chrysin (20 mg/kg) once a day for 28 consecutive days. After treatments, the following behavioral assessments were performed: open-field test (OFT) and morris water maze (MWM). Then, the levels of neurotrophins (BDNF and NGF) in the hippocampus and prefrontal cortex were measured and also tested the affinity of chrysin with neurotrophinergic receptors through molecular docking. Hypothyroid mice showed a deficit of spatial memory and chrysin treatment reversed this deficit. It also reduced the levels of neurotrophins in both cerebral structures in the hypothyroid mice, meanwhile, the chrysin treatment was able to increase the levels of BDNF in hipocamppus and NGF in both structures. Additionally, molecular docking analysis showed that chrysin potentially binds to the active site of the TrkA, TrkB, and p75NTR receptors. Together, these findings provide a comprehensive effect of chrysin that was able to reverse the behavioral and neurochemical changes associated with memory deficit induced by hypothyroidism, by modulating synaptic plasticity in the neurotrophinergic system.
Keywords: Flavonoid, Memory loss, Neurotrophins. **Supported by:** FAPERGS, CNPq and CAPES

HA.14 - Can aging increase the peripheral neuropathy and comorbidities associated with paclitaxel treatment?

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The pace of population aging around the world is increasing dramatically. Due to cell aging and lack of hormonal protection characteristic of old age, this group is more susceptible to developing cancer. Chemotherapy drugs are widely used anti-cancer treatments. However, chemotherapeutic agents such as paclitaxel (PTX) can cause peripheral neuropathy and promote emotional and cognitive impairment in cancer survivors. A longer life brings with it opportunities, but the extent of these opportunities and contributions depends heavily on one factor: health. Thus, elucidating the role of aging in cancer pain and associated comorbidities becomes increasingly relevant. The present study aimed to investigate the impact of aging on PTX-induced peripheral neuropathy and associated comorbidities in BALB/c mice. Male mice were divided into four groups: YOUNG (2 months); YOUNG+PTX; OLD (20 months); OLD+PTX. PTX was administered intraperitoneally (i.p.), at a dose of 2 mg/kg, once a day, for 3 consecutive days, or 0.9% saline solution (10 mL/kg, i.p.). Nociceptive response was evaluated on days 4, 8, 11, and 14 of the experimental protocol. To investigate the effects of PTX/aging on comorbidities associated with peripheral neuropathy, the cognitive impairment (on days 6-7, and 12-13), and the anxiety-like behavior (on days 5 and 15) were evaluated. Our results demonstrated that PTX treatment caused mechanical and thermal hypersensitivity in both young and old mice. Furthermore, both young and old mice that received PTX also demonstrated emotional and cognitive impairments. Importantly, we revealed that older animals had greater mechanical and thermal hypersensitivity and greater cognitive impairment than animals of the YOUNG+PTX group. Based on this evidence, it can be inferred that aging contributes to the exacerbation of neuropathic pain and comorbidities related to PTX treatment. These results demonstrate the importance of searching for new therapeutic approaches that consider the specificities of these patients.
Keywords: paclitaxel, peripheral neuropathy, aging
Supported by: CNPq, CAPES and L'ORÉAL-UNESCO-ABC for Women in Science

HA.15 - Recognition of *Trypanosoma cruzi* epitopes by IgG of benznidazole treated Chagas disease patients

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Chagas disease, caused by the protozoan *Trypanosoma cruzi*, affects millions of people worldwide. Treatment is challenging due to lack of effective drugs and assays to monitor parasite persistence. Here, we use our recent develop gPhage platform to analyze and compare antibody response in Chagas patients, before and after benznidazole treatment, to identify epitopes that could be used as molecular marker for disease status. Then, we searched for antigens/epitopes recognized preferentially by patients treated with benznidazole that are responders (PCR-negative for *T. cruzi*) or non-responders (that remain PCR-positive). We used IgG purified from sera of patients (N=20) collected before and after treated with benznidazole. Patients were then classified on responders (N=10) and non-responders (N=10) based on their PCR-status. In order to enrich for antigens recognized by IgG from patients before and after treatment, we devised a two-tier biopanning procedure. One of the IgG samples of the same patient (before or after treatment) was first used to pre-clear the *T. cruzi* genomic phage library (gPhage) before performing the positive selection on the remaining IgG samples. After 4 rounds of selection, phage bound to IgGs were characterized by Next Generation Sequencing. Sequences were assembled and epitopes identified by alignment with *T. cruzi* genome and clustering. We observed a significant and inverse correlation between the number of phage display *T. cruzi*-antigens recovered by IgG from patients and their PCR status. There was a significant enrichment in *T. cruzi*-specific antigens bound to IgG from non-responders compared to responders. The surface antigen 2 (B13) was the most prevalent antigen recovered, although we also identified other molecular marker of interest. Our work corroborates previous studies indicating the *T. cruzi* antibody response is a potential marker for cure. Several antigens, including the well-known B13, are potential molecular markers of disease status.
Keywords: Chagas disease, benznidazole, epitopes. **Supported by:** CNPq, FAPESP, CAPES

HA.16 - Study of the effects of palmitic acid and pregnancy steroids on the survival of insulin-producing cells

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Gestational diabetes (GD) attacks 3-17% of pregnant women, four-times prevalent between obese women, correlating adiposity and GD. Adiposity interferes in adipokines amount, reflecting adipose tissue alterations what could contribute to metabolic diseases. Study pregnancy steroids and fatty acids effects on viability and DNA fragmentation of insulin-producing-cell line. INS-1E were cultivated in RPMI with 5% fbs, bicarbonate, sodium pyruvate, 2-mercaptoethanol and 5% CO2 in humidified atmosphere. Cells were treated with 50, 100, 200 and 300μM palmitic acid (PA) , for 24h or 48h, alone or combined with mixes of progesterone (P4) and estradiol (E2): mix1 (M1: concentrations of P4 and E2 in healthy pregnant women); mix-2 (M2: P4 and E2 in GD pregnant women). Was performed the growth curve for INS-1E (n = 2) and toxicity of PA (n=4) and steroid hormones (n=5) curves. After incubations, membrane integrity and DNA-fragmentation were evaluated by cytometry. A 3-days folding time for INS-1E was consistent with literature. Treatment with PA caused a slight loss of membrane integrity after 24h (200μM, 8% and 300μM, 10%, regarding to control) and 48h (200μM, 12% and 300μM, 16%, regarding to control). Percentage of DNA-fragmented cells was pronounced: 24h (200μM, 73% and 300μM, 90%, regarding to control) and 48h (200μM, 77% and 300μM, 91%, regarding to control). The toxic effect of PA was dose-dependent without any significant influence of incubation time. Membrane integrity, after treatment with 25μM PA and hormones did not show significant difference in all groups regarding control. Was observed DNA fragmentation in all groups compared to the control (PA: 44%, PA+M1: 44%, PA+M2: 50%, M1: 67% and M2: 68%). Growth curve allowed to establish parameters for experiments with the strain; PA toxicity was dose-dependent, but not time-dependent; only pregnant hormones combinations were more toxic to cells than those with fatty acid.
Keywords: Gestational Diabetes, sex hormones, INS-1E
Supported by: FAOESO

HA.17 - A conformation sensitive fragment antibody-based assay allows isolation of a neurotoxic oligomeric assembly of the β-amyloid peptide under non-denaturing condition

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Soluble oligomers formed by β-amyloid peptide (AβOs) are currently implicated in the pathogenesis cascade of Alzheimer's disease (AD). The biochemical identity of those oligomers is yet debated, including their molecular mass and conformation. Importantly, the neurotoxicity of AβOs has been shown to be neutralized by the scFv fragment antibody NUsc1, which preferentially targets a subpopulation of AβOs > 50 kDa. NUsc1 antibody is a promising candidate to assist in the isolation and subsequent biochemical characterization of AD- relevant toxic AβOs. In this work, we describe a method for the isolation of toxic AβOs under non-denaturing conditions based on the use of the conformation-sensitive single-chain antibody fragment NUsc1 as capturing antibody. Cobalt-coated magnetic beads were functionalized with His-tagged NUsc1, and used in the isolation of either synthetic or AD-mice brain-derived AβOs. After pull down, the complex NUsc1-AβO was successfully released from the beads using imidazole, as followed by Western Blot (WB). NUsc1-targeted AβO species were stabilized by cross-linking (XL) and molecular mass was analyzed by WB. Alternately, NUsc1-AβOs complex was passed through 50 kDa cutoff filters and retained fraction was also crosslinked and analyzed by WB. WB analysis of eluates from magnetic beads confirmed that the complex NUsc1-AβO was successfully isolated under non-denaturing conditions, both from synthetic and AD-mice brain-derived AβOs. Interestingly, the majority of cross-linked AβO species targeted by NUsc1 were larger than 100 kDa, in line with the results obtained from NUsc1-AβO cross-linked after complex isolation. Taken together, our findings indicate that NUsc1 preferentially targets AβO species larger than 100 kDa either prepared *in vitro* or extracted from tissue. The determination of the molecular mass of NUsc1-reactive AβOs by size-exclusion chromatography (SEC) is ongoing, as well as investigations on the molecular shape of these species.
Keywords: AβOs, Alzheimer's Disease, scFv

HA.18 - Hsp70 inhibition effects in an ubiquitinated GFP transfected cell line

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Chaperones are specialized in protein folding and cooperate with the ubiquitin/proteasome system during protein quality control in eukaryotic cells. Human heat shock proteins 70 (Hsp70 or HSPA) comprise a highly conserved chaperone family that feature a pivotal role in proteasome system. Studies have been shown that HSPAs inhibitors increase proteotoxicity and suppress proliferation, indicating that these chaperones directly influence cell survival probably through activation of a proteostasis network such as ubiquitin-proteasome degradation system. Based on that, we hypothesized that the HSPA inhibitor MKT-077 causes proteasome breakdown. To test this hypothesis we used transfected HEK293T cells capable of expressing ubiquitin fused GFP (uGFP) that was directed to proteasome system right after expressed. Once HSPA is inhibited, u-GFP delivering to proteasome is harmed and it leads to GFP accumulation that can be measured by fluorescence. Therefore, HEK-uGFP cells were treated with 0.5 to 24 μM of MKT-077 (HSPA ligand) during 5 h and then GFP fluorescence signal was evaluated by flow cytometry and/or plate reader. Besides, cytotoxicity was also evaluated by MTT assay using MKT-077 at 10 to 800 μM during 5 h. Results demonstrated that the IC50 for MKT-077 was around 200 μM after 5 h of treatment in HEK-uGFP cells and the uGFP accumulation raised with the increased MKT-077 concentrations. These results confirm that HSPA inhibition causes uGFP accumulation since its delivery to proteasome is harmed. We believe that this report model can help to identify interfering features in the intracellular balance between protein folding and protein degradation. It can be a potential strategy to treat diseases involving chaperonepaties such as cancer and neudegenerative diseases. Funding: FAPESP, CNPq and CAPES
Keywords: HSPA, proteasome, co-chaperones. **Supported by:** FAPESP

HA.19 - Albumin overload impairs albumin endocytosis in proximal tubule cells through increased o-glcnacylation

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Renal disease is strictly associated with urinary protein loss, proteinuria, a condition that reflects protein overload at the proximal tubule epithelial cells, PTECs. It is well known that albumin overload promotes renal disease progression. Identifying the molecular mechanisms mediating this process is essential for the development of new treatments. Our group has previously demonstrated an association among essential hypertension and tubular proteinuria with development of tubule-interstitial injury. The molecular mechanism involved hyper-O-GlcNAcylation in PTECs, but the trigger is unknown. We aimed to study the possible role of PT albumin overload as a trigger for dysregulated O-GlcNAcylation and its impact in PT albumin endocytosis. LLC-PK1 cells, a well characterized PTECs, were incubated overnight with 20 mg/mL albumin mimicking PT albumin overload. LLC-PK1 cells were transfected with minimegalin constructs (mMeg-HA) to study the traffic and expression of megalin, a receptor involved in PT albumin endocytosis. Albumin endocytosis was assessed by albumin-FITC. Surface megalin expression was determined by confocal microscopy. O-GlcNAcylation was also evaluated in 2 different animal models: 1) subclinical acute kidney injury, subAKI; 2) Adriamycin-induced nephropathy (CEUA-045/17). We observed that the incubation of the cells with albumin induced: 1) an increase in O-GlcNAcylation; 2) a reduction in albumin binding and endocytosis; 3) a reduction of surface mMeg-HA expression. Thiamet G (5 µM), an O-GlcNAcylation enhancer, mimicked these effects. On the other hand, OSM-1, an inhibitor of O-GlcNAcylation, blocked the albumin effect on O-GlcNAcylation, albumin endocytosis and mMeg-HA expression. Importantly, subAKI and adriamycin-induced nephropathy mice models showed increased renal cortex O-GlcNAcylation correlated with decreased proximal tubule albumin endocytosis and reduced megalin expression. Our results indicate that albumin overload impairs PT protein reabsorption by a mechanism involving an increase in O-GlcNAcylation, which decreased megalin surface expression. We propose that this mechanism promotes the development of progressive renal dysfunction in proteinuric conditions.

Keywords: Albumin overload, Megalin, O-GlcNAc
Supported by: FAPERJ, CAPES and CNPq

HA.20 - Interaction of proteins from plasma kallikrein-kinin and plaminogen activator systems with breast cancer cells.

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The proteolytic plasminogen activator system (PAS) plays important role in cancer invasion and metastasis. PAS includes urokinase plasminogen activator (uPA), its membrane-linked receptor (uPAR), plasminogen/plasmin and inhibitors (PAI). The plasma kallikrein-kinin system (KKS) links inflammation and fibrinolysis because plasma kallikrein (PKa) releases bradykinin (BK) from high molecular weight kininogen (HK) and is an activator of pro-uPA. The aim of this work is to study in breast cancer the interaction of KKS and PAS through HK assembly, BK endocytosis and interaction of PKa/uPA or uPA/syndecans. The cell lineages studied were MCF-10A (non-metastatic), MCF-7 (less-metastatic) and MDA-MB-231 (highly-metastatic). The techniques employed were confocal microscopy and RT-PCR. The antibodies used were anti-BK (MBK3 clone), anti-PKa (U691.10), anti-uPA (TC31014, TC21063), anti-syndecan-1 (sc390791) and anti-syndecan-4 (CS12236S). HK-Dylight-650 bound to cell surface in MCF-10A (1,947.0 ± 0.003 pixels/cell), MCF-7 (648.0 ± 0.002 pixels/cell) and MDA-MB-231 (1,251.00 ± 0.002 pixels/cell). In endocytic vesicles, HK-Dylight-650 and LT-green colocalized in MCF-10A (974.0 ± 0.002 pixels/cell), MCF-7 (61.0 ± 0.001 pixels/cell) and MDA-MB-231 (385.0 ± 0.001 pixels/cell). In comparison the HK binding and endocytosis was MCF10A>MDAMB-231 > MCF-7. BK was detected in endocytic vesicles colocalized with LT-red in MCF-10A (19.7%), MCF-7 (40.9%) and MDA-MB-231 (53%). BK in endocytic vesicles of MDA-MB-231 allows its proliferative effect in metastatic cells. In MDA-MB-231 plasma prekallikrein (PK) mRNA was determined and PKa was present in both non-permeabilized and permeabilized cells. In highly-metastatic cells PKa and uPA colocalized in both conditions non-permeabilized (58.9%) and permeabilized (65.5%) without difference (p=0.1705); syn-4 and uPA colocalization in non-permeabilized (55.3%) and permeabilized (66.8%) was different (p=0.0265); syn-1 and uPA colocalized in non-permeabilized (33.7%) and permeabilized (41.9%) without difference (p=0.2208). Our data suggest that in highly-metastatic breast cancer cells PKa function as pericellular and endogenous protease on HK and pro-uPA that may assemble to membrane by syndecans.
Keywords: cell biology, enzymes, proteoglycans . **Supported by:** FAPESP, CAPES and CNPq

HA.21 - Comparison of tumor progression model secretome to molecular subtypes of high-grade serous ovarian cancer

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High-grade serous ovarian cancer is the most common and lethal among ovarian cancers, representing around 70% of cases, it has a high mortality rate, mainly due to late diagnosis of severe forms of the disease. Diagnosis, correct classification and treatment can represent improvement of survival rate in 95% of cases, thus generating motivation for understanding the molecular characteristics of each type and discovering biomarkers. To compare our metastasis model of ovarian cancer cells with analyzes of large groups of patient samples performed by Clinical Proteomic Tumor Analysis Consortium (CPTAC). We used the induction of the Epithelial to Mesenchymal Transition (EMT) process in Caov-3 cell line of ovarian cancer by treatment with the epidermal growth factor (EGF) and obtained the culture supernatant on which an analysis was performed with the shotgun proteomic approach and then we compared these results with analysis done by the CPTAC using only the most abundantly expressed proteins of each molecular subtype. The treatment of Caov-3 cells with EGF showed characteristic markers. The analysis of differentially expressed proteins in the supernatant returned the result of 99 targets with the arbitrary cutoff. While comparing our results with the comprehensive analysis disclosed by the CPTAC study, characteristic profiles of each subtype was provided, highlighting the mesenchymal and proliferative subtype with greater coincidence and structural proteins linked to the location and transport process. The treatment of Caov-3 cells with EGF enabled the occurrence of EMT according to the analysis of characteristic markers of this process. The Caov-3 cell secretome, despite having identified proteins of all subtypes, resembled the mesenchymal subtype, confirming the characteristics required in our model and providing important molecular entities for the study and monitoring of metastasis processes.
Keywords: Ovarian cancer, Epithelial to Mesenchymal transition, Proteomics
Supported by: FAPESP, CNPq and CAPES

HA.22 - Project - Investigation of neuroinflammatory parameters related to Parkinson's disease

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Parkinson's Disease (PD) is considered an age-related multifactorial disease, comprising environmental and genetic factors. Genetic forms include mutations at 23 *loci*, and several of these genes are associated with mechanisms linked to immunity. The alpha-synuclein protein is referred to as the center of the immune response that occurs in PD, and its aggregates trigger microglia activation, initiating inflammatory responses in the brain. The present study aims to evaluate the contribution of different molecular factors related to PD neuroinflammation, by estimating the expression of *SNCA*, *PRKN*, *DJ-1* and *LRRK2* genes and dosage of their products, as well as the quantification of cytokines, chemokines and circulating growth factors. Patients will be divided into groups, which will contain 20 patients, according to the severity of the disease. 5 ml of blood will be collected, which will be separated into cells and plasma. The cells will be subjected to total RNA extraction, which will be used to estimate gene expression by RT-qPCR. While plasma will be used for measuring the products of these genes through ELISA, as well as for quantification of cytokines, chemokines and growth factors through cytometry. Immunophenotyping will be evaluated in FlowJo software (v.10) and descriptive and statistical analyzes in GraphPad Prism software (v.8.0.2). It is expected that the expression of genes and plasma concentrations of the evaluated proteins increase with the severity of the disease, due to the intensification of the neuroinflammatory response. Knowing these factors underlying the neuroinflammatory process associated with PD may contribute to a better understanding of the pathophysiology of the disease and also to the search for therapeutic strategies in order to improve the patient's quality of life. Furthermore, the inference of these correlations may provide support for the definition of predictor biomarkers of disease worsening.

Keywords: Parkinson's disease, neuroinflammation, gene expression
Supported by: FAPEAM

HA.23 - PROJECT- Functional analysis of HBZ and Tax in the expression of antioxidant response components in ATLL and LTR in the suppression of HTLV-1 infection in T cell lines

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ATLL is a lymphoproliferative disease triggered by the HTLV-1 virus. There is no cure for ATLL. The development and maintenance of ATLL require contributions from HBZ and Tax viral proteins involved in the modulation of antioxidant response components. HBZ is the only protein that maintains its expression in all ATLL cases. HBZ activates the HMOX-1 expression, an enzyme that acts in the detoxification of free heme and has been described in cell lines transformed by HTLV-1. Tax stimulates viral genes expression, through its interaction with cellular factors and with the non-coding ends, LTRs, of the proviral genome. Expression of Tax has been reported to induce reactive oxygen species, with DNA damage, in primary human CD4+ T lymphocytes. Emerging evidence from several studies indicates that the antioxidant enzyme SOD1, which catalyzes superoxide radical dismutation, is overexpressed in cancers. Thus, its activity may be essential to cells fail to undergo apoptosis. Preliminary studies from our research group demonstrated that the NFE2L2 factor, related to the antioxidant response, is a positive regulator of the most expressed genes in ATLL. Therefore, our aim is to evaluate the importance of viral proteins HBZ and Tax, and LTRs, for cell proliferation and viral persistence in HTLV-1 infected cell lines. The CRISPR/Cas9 gene-editing system will be used to knockout the pX viral region, that encodes HBZ and Tax proteins, and for the cleavage of the LTRs regions. As a result, we expect to understand the redox balance in HTLV-1 infected cell lines, as well as its importance for the maintenance of leukemogenesis, and promote the removal of the proviral genome to suppress the HTLV-1 infection. Genome editing technology could be a promising therapeutic approach for ATLL.

Keywords: HTLV-1, ATLL, CRISPR/Cas9

HA.24 - Heparanase and syndecan-1 expression in different breast cancer subtypes

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The heparanase-1 (HPSE) and the heparan sulfate proteoglycan syndecan-1 (SDC1) participate in molecular mechanisms involved in microenvironment tumor communication, carcinogenesis, and tumor metastasis. Targeted therapies and precision medicine demonstrate the importance of identifying specific molecule profiles for each breast cancer subtype besides the current classification defined in luminal tumors A and B, HER2 positive, and triple-negative to obtain a better diagnosis, prognosis, and responses to different therapies. We decided to investigate HPSE and SDC1 in circulating lymphocytes from patients with different breast cancer subtypes and cell lines representing each molecular subtype. Blood samples were obtained from 50 patients with breast cancer and 50 women not affected by cancer (control group). Lymphocytes were obtained following a protocol using the Ficoll Hypaque® method. We also evaluated HPSE and SDC1 in different cell lines and the non-tumor lineage (MCF-10A). Total RNA extraction was performed using the TRIzol® method. Reverse transcriptase SuperScriptIII™ was used to obtain cDNA. The qPCR technique was applied to analyze the relative expression of HPSE and SDC1 using the PowerSybrGreen®. The results were represented by the geometric mean based on endogenous reference genes RPL13a and GAPDH. ethics committee approval protocol number 2.753.436 (CEP/FMABC). The results showed that lymphocytes from patients with different breast cancer subtypes and different tumor lineages showed higher expression of HPSE and SDC1 compared to control groups. ROC curve analyzes showed a positive predictive value for the luminal B and triple-negative breast cancer subtypes (AUC greater than 0.7). We conclude that HPSE and SDC1 may represent additional molecular markers for breast cancer. Triple-negative tumors are tumors with a worse prognosis and less responsive to conventional therapies. Therefore, HPSE and SDC1 may represent potential target molecules as new treatment alternatives and represent an additional diagnostic factor given the great molecular diversity of triple-negative breast tumors.

Keywords: Heparanase, Syndecan-1, Breast Cancer Subtypes.

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HA.25 - Peptide inhibitor for FAK and SRC protein Interaction decreases JAK2 V617F positive cells viability.

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Peptides for protein-protein interaction inhibition has been investigated due to their higher specificity. The tyrosine kinase FAK participate in several cellular process as adhesion, survival, migration and apoptosis and has been described upregulated in many neoplasias. When FAK is activated, it interacts with other proteins, including the tyrosine kinase SRC. The BCR-ABL negative Myeloproliferative Neoplasms (MPN) are hematological neoplasms characterized by excess of proliferation and resistance to apoptosis. The description of genetic alterations as JAK2 V617F mutation contributed to the MPN pathophysiology knowledge, but there is still no curative drug treatment, justifying further studies. The present work had investigated the effect of a peptide to disrupt FAK and SRC interaction in the viability and apoptosis of HEL 92.1.7, a JAK2 V617F positive cell line. For this purpose, it was performed FAK immunoprecipitation (IP) and SRC detection by Western Blot (WB), the peptide sequence was selected using literature data and online proteins databank and the peptide ETDDpYAEIIDEED was synthetized linked to TAT translocator (-YGRKKRRQRRR) by a commercial supplier. HEL cells were treated with the peptide at 1uM, 5uM and 10uM for 48h; 10uM and 25uM for 12h and 25uM for 24h. TAT peptide was used as negative control and doxorubicin was used as positive control. After treating, MTT assay was performed to determine the cell viability and cleaved PARP detected by WB to investigate apoptosis. It was possible to demonstrated FAK/SRC interaction in HEL 92.1.7 cells and after testing the different concentrations and time of treatment, the lowest cell viability was verified when cells were treated with the peptide at 25uM for 24h (73% of viability compared to control). However, it was not detected cleaved PARP after this treatment. Therefore, the peptide reduced the cell viability, but more studies are necessary to investigate the mechanisms involved.

Keywords: FAK, Peptides, Myeloproliferative Disorders

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HA.26 - Clinical, epidemiological, and laboratory profile of individuals with diagnostic suspicion of leprosy treated at the CREDEN-PES.

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Leprosy caused by *Mycobacterium leprae* persists as a public health problem in Brazil. It is an infectious disease of slow and progressive evolution that, if left untreated, can cause the development of permanent physical disabilities. In this context, the fight against leprosy is a priority for the Ministry of Health. The main action strategies are the early detection of cases and examining contacts to promote the breaking of the transmission chain. This study aims to characterize the clinical-epidemiological and laboratory profile of individuals with diagnostic suspicion of leprosy treated at the CREDEN-PES-SMS/GV Center for Endemic Diseases and Special Programs from 2017 to 2021. This is a cross-sectional epidemiological study that is being carried out from the survey of secondary data obtained through CREDEN-PES medical records and the application of questionnaires. After signing the consent form, volunteers are submitted to collecting auricular dermal scraping samples for bacilloscopic examination and investigation of strains resistant to Multi-Drug-Therapy (MDT) by the qPCR method. Samples were collected from 260 individuals, 46.53% female and 53.47% male. About 22.7% of the participants were diagnosed with leprosy, 38 being multibacillary and 21 paucibacillary. Of the cases with leprosy, 64.4% had a bacilloscopic index (BI) ranging from 0.25 to 5, with a mean of 2.26. The age ranged from 9 to 88 years, and 02 cases were registered in children under 18 years of age with positive bacilloscopic index (BI). The vast majority of participants declared skin color brown. Tests to assess resistance to MDT are ongoing. The data presented reinforce the importance of actively searching for new cases and evaluating their contacts due to the active transmission of the disease, especially among children under 18 years of age.

Keywords: leprosy, multi-drug resistance, diagnosis
Supported by: FAPEMIG, NIH/CNPq (Emory University), ILSL, UFJF/GV

HA.27 - Unraveling the neurotropic potential of the emergent viruses Oropouche and SARS-CoV-2 using adult human brain slice cultures

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Neurotropic viruses can cause central nervous system (CNS) diseases. Indeed, about 30% of confirmed encephalitis cases are attributed to virus infections. Cellular and molecular mechanisms on CNS viral infections have been obtained using rodent models. Despite these advances, significant biochemical and functional differences between rodent and human brains limit their application as disease models in translational neuroscience. Here we have used slice cultures from adult human brains to investigate whether Oropouche (OROV) and SARS-CoV-2 viruses, reported to cause neurological symptoms in some infected individuals, are capable of infecting human neural cells in a context of preserved brain cytoarchitecture and neural connections. Brain tissue was obtained from patients undergoing temporal lobectomy for the treatment of refractory epilepsy (Ethics Committee approval HCRP 17578/15). Cortical fragments were collected at the surgical room and immediately transported to the laboratory, where the tissue was carefully sliced using a vibratome and cultured in 24-well plates. At days *in vitro* 1-2, brain slices were infected by OROV or SARS-CoV-2 for 2h. Infected slices were cultivated for 24-48 h post-infection. Our results indicate that both OROV and SARS-CoV-2 infect human neural cells and that these cells support virus replication. Interestingly, while OROV infects mainly microglia, SARS CoV-2 was seen to preferentially infect astrocytes. Both viruses also infected neurons to a lesser extent. OROV infection led to tissue damage and the release of the pro-inflammatory cytokine TNF-α. SARS-CoV-2 infection increased the RNA-expression of pro-inflammatory cytokines such as CCL2, IL-8, and IL-6 by brain slices. We are currently driving efforts to unravel the ultrastructural consequences of OROV and SARS-CoV-2 infections in adult human brain slices using transmission electron microscopy. Given the uncertainties on both acute and long-lasting neurological consequences of neural infection by OROV and SARS-CoV-2, our present work helps to raise awareness about the potential impact of these viruses on the human brain.

Keywords: Human Brain Slice, Oropouche, SARS-CoV-2

HA.28 - RAGE-associated serum markers along with motor and cognitive clinical parameters as predictors of Parkinson's Disease

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Parkinson's disease (PD) affects nearly 10 million people globally. The course of disease is highly variable and there are no established biomarkers with diagnostic value or predictive models. The receptor for advanced glycation end products (RAGE) is crucial in the propagation of inflammatory events, exerting a major role in neuroinflammation and dopaminergic denervation. We asked if inflammatory parameters and circulating RAGE agonists in serum of patients with Parkinson's disease correlate to clinical assessments. Also, we question how these parameters behave over time and /if they correlate. If so, what are the potential predictors of Parkinson's disease. Clinical interview, cognitive and motor tests and blood samples were collected from PD patients and controls ("analysis"). Serum parameters were measured by Multiplex and ELISA. We evaluated the correlation of inflammatory cytokines with RAGE agonists in serum, in parallel with cognitive (MoCA) and motor/non-motor (UPDRS) clinical parameters o'f PD. The patients were re-evaluated for the same parameters at a later period of approximately 1 year (reanalysis"). In analysis, increase in HMGB1 in PD is correlated with other RAGE agonists, such as nitrotirosine, 4-HNE, CML along with S100B, but when analyzed together they do not predict the outcome. Although α-synuclein does not differ between control and PD, it is positively correlated to TNF-α; in PD, and together they are factors that predict the disease. The reanalysis regarding the side dish of patients are being processed and correlated to analysis. Our preliminary results suggest that more parameters should be developed in order to discover RAGE-associated potential molecular markers that may aid in clinical diagnostic. The reanalysis should inform us how are inflammation and RAGE status and how they correlate with disease progression.

Keywords: Parkinson's Disease, RAGE, biomarkers

Supported by: CNPq, CAPES

HA.29 - Effects of NT157 on tyrosine kinase signaling pathways in BCR-ABL1 T315I cells

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Chronic myeloid leukemia (CML) is characterized by the presence of oncoprotein BCR-ABL1, which constitutively activates the tyrosine kinase activity triggering hematopoietic stem cells neoplastic transformation. Although, some reports using tyrosine kinase inhibitors (TKI) have represented an advance in CML treatment with up to 20% of patients being resistant to those inhibitors. Studies aiming proteins that bind indirectly to the BCR-ALB1 have identified insulin receptor substrates (IRS) as potential targets, representing new therapeutic strategies for CML. Evaluation of proteomic alterations in primary samples from patients carrying T315I mutation undergoing treatment with an IGF1R-IRS1/2 inhibitor, NT157. Peripheral blood mononuclear cells (PBMCs) from a CML patient with a BCR-ABL1 T315I phenotype were treated with 6.4 µM NT157 for 48 hours and submitted to analysis of cell viability and apoptotic molecular markers. To determine the changes in protein abundances of the cells, we performed a global proteomics analysis. The proteomic data was acquired using a Q-Exactive-HF LC-MS/MS system and processed using Label-Free-Quantification (LFQ) approach with MaxQuant software. The 6.4 µM NT157 treatment for 48 hours significantly decreased cell viability and increased apoptosis in PBMCs. All 3244 proteins were confidently identified with FDR < 1%. LFQ analysis highlighted a list of 116 upregulated and 85 downregulated proteins differentially detected among cells not treated and cells treated with NT157. In particular, BCR protein, which is directly involved in CML pathogenesis is decreased by the NT157 treatment. Among the processes found altered we can highlight several metabolic pathways involved in TCA cycle, angiogenesis and immune response. NT157 showed antineoplastic effects in primary cells from patients CML BCR-ABL1 T315I including reduced cell viability and increased apoptosis. Inhibition of IGF1R/IRS signaling has revealed a potential therapeutic approach and maybe an alternative to TKI in the context of patient's inhibitor resistance.

Keywords: Proteomics, NT157, Chronic myeloid leukemia

Supported by: Cnpq

HA.30 - PROJECT: Molecular and cellular evaluation of the expression of heme oxygenase-1 and peroxisome proliferator-activated receptors and their differential contributions on pathophysiology and clinical complications of sickle cell anemia

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Sickle cell anemia (SCA), the most severe form of sickle cell disease (SCD), is a hereditary hemoglobinopathy with vast distribution worldwide. In SCA, the initial pathological phenomenon consists of the polymerization of S hemoglobin chains, triggering other pathological phenomena such as intravascular hemolysis, production of molecules related to oxidative stress and inflammation. Heme oxygenase-1 (HO-1), acts, in this process, decreasing the availability of free heme in the blood which, in turn, results in cytoprotective, anti-inflammatory and antioxidant effects. The expression of HO-1 is regulated by nuclear factor erythroid 2-related factor 2 (Nrf2), the latter being regulated by receptors activated by proliferation of peroxisomes (PPARs). PPARs are associated with the reduction of inflammation and the control of oxidative stress, and are involved in the regulation of a series of genes associated with those mechanisms. The objective is investigate differential cellular expression of HO-1 and PPARs in innate immune cells and variants of these genes correlate with intravascular hemolysis, oxidative and inflammatory stress in SCA. Therefore, it is intended to evaluate a sample of participants with SCA between six and fifty years old enrolled at the Regional Blood Center of Governador Valadares in a retrospective cohort. Blood samples will be used in flow cytometry and qPCR analysis in order to assess the relation of clinical and laboratory manifestations of severity and hemolysis with the differential expression of HO-1 and PPARs. As results of these analysis it is expected to find possible mollecular explanations for the different phenotypes of SCA, demostrating the role of HO-1 and PPARs. The clarification of those mechanisms may open field to new prognostic tools, constituting possible predictive markers of severity, and therapeutical approachs, aiming at reducing the clinical complications of the disease.
Keywords: sickle cell anemia, HO-1, PPAR
Supported by: CNPq, CAPES, FAPEMIG, UFJF

HA.31 - Featuring ACE2 binding SARS-CoV and SARS-CoV-2 through a conserved evolutionary pattern of amino acid residues

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The importance of the RBM is further explored here in relation to its structural topology. Thus, instead of only analysing specific residues that make contacts with ACE2 after binding, we go a step further and track the molecular origin that drives the viral attachment to this cell receptor. This investigation has revealed a highly conserved amino acid residue sequence Tyr-Gly-Phe in coronavirus variants that employ this receptor. Consequently, we hypothesize that the short sequence-YGF is vital for RBD-ACE2 interaction because of the formation of a hydrophobic pocket proper to the receptor specificity. Here, we investigate the occurrence and importance of the specific amino acid residue sequence YGF for SARS-CoV and SARS-CoV-2 strains able to use ACE2 proteins as receptors. Mutations may affect the spike receptor-binding complexed with hACE2 either leading to higher, lower or even neutral binding affinity. Thus, we apply the fast and accurate MutaBind2 method to estimate the binding free-energy change upon mutation to predict its functional effects. We investigate the relevance of the hydrophobic pocket driven by the YGFY and YGFQ sequences in promoting the stability of SARS-CoV and SARS-CoV-2 S RBD complexed with hACE2, respectively. YGF-based mechanism can act as a protein signature to distinguish CoVs able to use ACE2 as a cell entry receptor whenever this residue sequence is located at the CoV RBM region. It must be accentuated that the occurrence of other XGF sequences, mainly with X being a hydrophobic residue, in the RBM, or even in the RBD region, can disrupt the proposed topological mechanism for ACE2 binding.
Keywords: SARS-CoV, SARS-CoV-2, ACE2

HA.32 - Genetic markers in the diagnosis of cholestatic liver diseases in a group of Brazilian patients

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Cholestatic liver diseases are pathophysiological changes characterized by reduced or absent bile flow in the duodenum. Cholestasis can be extra or intrahepatic or due to a functional change in the hepatocyte. It presents clinically by the triad: jaundice, dark urine and pale stool. This group of diseases can have different origins and several subdivisions, three of them well-known: Primary biliary cholangitis (PBC), Primary sclerosing cholangitis (PEC) and Hereditary cholestasis (HC). Because of these different forms, the diagnosis is complicated. Nowadays, diagnosis is mainly made by the set of liver enzymes, imaging tests and histopathological exams. However, a large number of cases do not have a conclusive diagnosis. Thus, it's very important finding biomarkers to characterize these diseases. The aim of this project is to evaluate the molecular variants of the ABCB4, ABCB11 and ATP8B1 genes from patients with different forms of cholestatic liver diseases in the search for biomarkers. For materials and methods, we will use genotyping by sanger sequencing or real-time PCR TaqMan assay, with subsequent association between genotype and clinical aspects and laboratory tests. We are recruiting patients with different forms of cholestatic liver diseases in the Ambulatório de Doenças Auto-imunes belonging to the Hospital Universitário Clementino Fraga Filho (HUCFF) of the Universidade Federal do Rio de Janeiro (UFRJ). Clinical and laboratorial characteristics are being evaluated. With this association, we intend to improve the patient's diagnosis and their life quality, reducing their comorbidities and mortality rate. In addition to cholestatic liver diseases this study can intervene in the clinical improvement of patients in other organ systems potentially damaged by this group of diseases.
Keywords: Cholestasis, Diagnosis, Biomarker
Supported by: FAPERJ, IFRJ

HA.33 - Metabolic alterations in COVID-19 observed by 1H-NMR

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Blood serum metabolites reflect the body's physiological state and change with the age, disease, or response of the body to any external effect, thus may add-in to a better understanding of viral infections. Our knowledge about the metabolic changes specifically occurring upon SARS-CoV-2 infection is still limited and expected to point to nucleotide, carbohydrate, lipid, and amino acid metabolism alterations. This work aimed to define serum metabolites that may differentiate patients that tested positive vs. negative for SARS-CoV-2 among two cohorts of patients - in patients with grave symptoms and ones with moderate symptoms by NMR-metabolomics. The informed consent was obtained from all participants (n = 350), and the present study was approved by the Research Ethics Committees. The sera were obtained from 5 mL of peripheral blood collected in a dry tube after peripheral venipuncture, realized for the routine hematological procedure. Just 200 µL of samples after dilution with deuterium oxide (250 µL) were used to record the high-resolution 1H-NMR NOESY1D 1D (noesy1dgppr1d), CPMG (cpmgpr1d), and edited by diffusion (stebpgp1s191d) spectra. The spectra were acquired on the Bruker AVANCE III 600 MHz spectrometer using the inverse triple-core probe (TBI) at 25 °C, processed, and analyzed through the MetaboAnalyst platform. At least fifty serum metabolites were found as strongly affected by the severity of the symptoms, and 60% of those were increased. High concentrations of glucose and lipids were seen, but, cholesterol, lysophosphocholines, and proteins were decreased. Serum metabolite variations in patients with moderate symptoms were also seen in serum lipids, although VLDL and LDL were somewhat less altered when compared to those measured in intensive care patients. So, there must be a connection between glucose excess, high VLDL, and low serum proteins with the disease severity, and NMR could be successfully used to map those.
Keywords: metabolites, infection, 1H-NMR
Supported by: CNPq, and FAEPEX-PRP.

HA.34 - PROJECT: Analysis of the neuroprotective effects of linseed oil and/or alpha-lipoic acid supplementation of rats with haloperidol-induced orofacial dyskinesia

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Tardive dyskinesia (TD) is manifested by atypical involuntary movements that are associated by the long-term use of antipsychotic medications, such as haloperidol, that cause the pharmacological blockade of D_2 dopaminergic receptors. Although the origin mechanism of this disorder is not yet fully understood it is pointed a role for oxidative stress, inflammation, and neurotrophic factors levels, which are potential therapeutic intervention tools. Previous studies showed that the flaxseed oil (*Linum usitatissimum* L.), rich in the polyunsaturated essential fatty acid α-linolenic acid, and the α-lipoic acid are both antioxidants, have anti-inflammatory properties and can influence Brain Derived Neurotrophic Factor (BDNF) levels. This work aims to evaluate the influence of linseed oil and/or α-lipoic acid supplementation on neuroprotection against the development of orofacial dyskinesia induced by sub-chronic haloperidol administration in rats. 108 males Wistar Rattus norvegicus will be used, arranged in 6 study groups: I) control: no supplemental diet or haloperidol; II) haloperidol administration; III) haloperidol with lipoic acid administration; IV) haloperidol with linseed oil administration; V) haloperidol with lipoic acid and flaxseed oil administration; VI) control: clozapine administration. After the previous interventions the animals will go under validation tests for the tardive dyskinesia experimental model, euthanasia, antioxidant assessment (TBARS test, SOD activity and GSH levels), molecular (Western Blotting) and immunohistochemical evaluations for TH, BDNF, GFAP, DRD3 markers on the prefrontal cortex, midbrain, and striatum regions. It is possible to say that both substances may reduce the extrapyramidal effects produced by subchronic use of haloperidol, improving motor function, relieving oxidative stress and restoring BDNF levels. This could advance neuroprotective methods for patients on haloperidol and for other drugs with neurological side effects.

Keywords: haloperidol, linseed oil, thioctic acid. **Supported by:** CAPES

HA.35 - Challenges of stable silencing of β3 integrin subunit of triple negative breast adenocarcinoma cell lines (MDA-MB-231) *in vitro*.

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Breast cancer is the major disease that affects women, especially the triple negative phenotype, which has a metastatic potential leading to a poor prognosis. Metastasis is the main cause of death of cancer patients; however, a better understanding on its underlying mechanisms is essential in the search for more efficient therapies. During metastasis development, the tumor microenvironment and the extracellular matrix (ECM) play important roles, providing the tumor cells a surrounding environment that supports its development and spreading. In addition, the expression profile of integrins changes, including the β3 integrin subunit, which is often overexpressed in tumors. The objective of this study was to analyze the effects of β3 subunit silencing in tumor cells of triple negative breast adenocarcinoma cell lines (MDA-MB-231) *in vitro*. For that, we produced lentivirus shRNA ITGB3 using third generation system (Plasmids: VSV-G envelope; pLP1 and pLP2 packaging; ITGB3 3'-UTR-shRNA and ITGB3 CDS-shRNA transfection) in human kidney embryonic cell lines (293FT). Viral particles were used for transduction of target cells. The success of silencing was confirmed by qPCR and western blotting. Although we have confirmed 84% silencing of β3 subunit by RT-qPCR, immunofluorescence assays of cells plated in fibronectin coating suggested recovering of integrin expression but in a different distribution pattern, apparently in the cell border. This intriguing result lead us to develop the hypothesis that the presence of specific ECM components may be able to overcome the silencing mechanism. Considering the complexity of microenvironment within the breast and all the changes that occur during tumor progression, this finding could be a clue for anti-integrin therapies failure. However, new experiments will be done to understand the consequences of silencing β3 subunit in different ECM proteins and tumor progression.

Keywords: β3 integrin, breast cancer, silencing. **Supported by:** FAPESP, CNPq, CAPES

HA.36 - Microbial metabolites reduce α-synuclein aggregation in a *Saccharomyces cerevisiae* model

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Parkinson's disease (PD) is a progressive neurodegenerative disease associated mainly with aging. The current understanding of the pathophysiology of PD suggests a central role in the accumulation of the protein α-synuclein and several evidences have been directing that the initial site of this process would be the enteric nervous system. It is known that the intake of phenolic substances contributes to the redox balance of the organism, however its bioactivities are highly impacted by microbial biotransformation that occur in the intestinal lumen. The objective of this work was to evaluate the influence of phenolic compounds and probiotic microorganisms on the aggregation of α-synuclein protein expressed in the yeast *Saccharomyces cerevisiae*. A pre-inoculum was prepared in SC-GLU at 160rpm/30°C, and after 24 h of growth, cells were transferred to SC-GAL medium supplemented with caffeic acid phenethyl ester (CAPE) or mangiferin; CAPE and mangiferin fermented by probiotic blend; medium fermented by probiotic blend; and control with only SC-GAL. CAPE and mangiferin concentrations were 0.1 mM and cell suspensions were incubated at 160rpm/30°C for 35h. Yeast growth was kinetically monitored by measuring the OD600 and also submitted to fluorescence microscopy, spot plating and detection of metabolites by HPLC-Q-TOF-MS. Results showed that CAPE and mangiferin without fermentation did not inhibit protein aggregation, but fermentation was able to reduce this aggregation by about 50%. Inhibition of α-synuclein aggregation was correlated with the presence of fermented metabolites. The detection of 3-hydroxyphenylpropionic acid (3-HPPA), a microbial metabolite associated with the reduction of α-sin toxicity converges with recent theories that the microbiota influences the etiology of PD. Therefore, our studies suggest that interactions between the microbiome and certain dietary factors may support new therapeutic strategies to modulate the onset and/or progression of synucleinopathies.
Keywords: Parkinson's disease, phenolic compounds, probiotics. **Supported by:** FAPERJ, CNPq and CAPES

HA.37 - An integrated study of transcriptome, lipidome and proteome in search of new therapeutic targets for pathological angiogenesis

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Angiogenesis, the formation of new blood vessels from pre-existing ones, is essential in physiology and pathology. Cancer and retinopathy are examples of diseases for which anti-angiogenic drugs are already available. Despite its success, we need to better understand the molecular mechanisms driving angiogenesis in order to develop a new generation of anti-angiogenesis drugs for these patients. Recently, using RNA-seq from an angiogenesis *in vivo* animal model (OIR, oxygen-induced retinopathy), our group has shown that differentially expressed genes in the retina of these animals could be used as a prognostic tool for a human angiogenesis dependent disease. We will expand these studies by characterizing the lipidome and proteome of mice retinas under pathological angiogenesis to integrate with the transcriptome data. Samples were collected at different postnatal days (P12, P12.5, P15, and P17) from mouse pups under pathological (OIR model from day 7 to day 12) and physiological development. Total lipid extracts were analyzed through non-targeted lipidomics by HPLC coupled to high-resolution mass spectrometry. For proteome, samples were collected in the same conditions and are being prepared for analysis. We identified and quantified 301 lipid species. PCA analysis revealed alterations in retinal lipidome mainly according to time, but also to the condition, physiological or pathological angiogenesis. The most significantly altered lipids in pathological angiogenesis correspond to storage lipids (CE and TAG) and membrane lipids (phospholipids). A preliminary integration of these results with transcriptome shows a cholesterol metabolism enzyme as a possible new marker for pathological angiogenesis. Lipidomic analysis suggests that pathological angiogenesis leads to intense remodeling of membrane and storage lipids. Proteomic analysis is ongoing and will provide, along with transcriptome and lipidome data, a better understanding of the different pathways associated with pathological angiogenesis.
Keywords: Angiogenesis, Retinopathy, Oxygen-Induced Retinopathy. **Supported by:** CAPES, FAPESP and CNPq

HA.38 - Evaluation of the effect of superparamagnetic iron oxide nanoparticles functionalized with Wedelolactona in modulating the aggregation of alpha-synuclein protein and neurotoxicity of aggregates formed.

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Parkinson's disease (PD) has as main pathological characteristics the death of dopaminergic neurons in the substantia nigra and the presence of Lewy Bodies, who have as main constituent the aggregates of alpha-synuclein protein (α-syn), which classifies DP as an amyloidosis. There is thus, a need to find compounds that are of acting as modulators of amyloid aggregation inhibiting the formation of toxic species and thus preventing death and maintaining morphology and cell viability. Previous projects of our group identified two possible compounds with the ability to inhibit α-syn aggregation *in vitro* and to undo mature fibers, namely, Wedelolactone (WED) and superparamagnetic iron oxide nanoparticles (SPIONs), respectively. WED, studied during my master's degree, has shown to reduce in approximately 90% thioflavin-T-binding, which is a marker for amyloid formation. While SPION, was able to “break” pre-formed aggregates through treatment with hyperthermia, in addition to modulate aggregation demonstrated. In this way, we will functionalize the SPIONs with Wedelolactone compound, forming the SPIONs@wed and evaluate the role of these in the formation of alpha-synuclein protein aggregates, and in the neurotoxicity of the species formed in their presence. The green synthesis of SPIONs was carried out by the sol-gel method, using coconut water as a framework for the reaction. We will start now, the functionalization phase of these with WED, initially only by joining the two molecules in an attempt to make hydrogen bonds between them and in this way we obtain the functionalization. After confirming the SPIONs@WED formation through characterization by several physicochemical methods, experiments will be carried out in order to evaluate their effect on the aggregation of α-syn protein. We aim with functionalization to increase the effectiveness of aggregation modulation and design a compound with the possibility of being targeted and tracked in the dopaminergic cell.

Keywords: alpha synuclein, amyloidosis, Parkinson's disease
Supported by: FAPERJ

HA.39 - PROJECT: The hsa-miR-1 expression profile in the evolution of Chagas disease by oral transmission

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Chagas disease is an emerging infectious disease considered a public health problem, characterized by a short acute phase, followed by a long asymptomatic chronic phase known as the chronic indeterminate form, which can last a lifetime or progress to cardiac and /or digestive form. Oral transmission has epidemiological importance, related to a greater manifestation of symptoms, as well as an increase in mortality, mainly associated with myocarditis. In the course of infection, association pathways were predicted between target molecules and miRNAs, so that in the acute phase the gene for cyclin D1 was shown to be potentially affected by the expression of some miRNAs and, in the chronic cardiac phase, this same gene was specifically regulated by hsa-miR-1, in which decreased expression of this miRNA results in a marked translation of cyclin D1 in cardiac tissue. This research project aims to evaluate the expression profile of hsa-miR-1 in the evolution of patients with Chagas disease after acute infection by oral transmission. The study group will consist of eight patients, corresponding to the Chagas disease outbreak diagnosed after ingestion of sugarcane juice in the town of Marcelino Vieira in October 2015. This trial will be conducted in five stages (1, 2, 3, 4 and 5), referring to the acute phase of the infection in October 2015 (time 1) and the chronic phase after one and a half year (time 2), two and a half years (time 3), three and a half years (time 4) and six years (time 5) after acute infection, by collecting peripheral blood for quantitative analysis of hsa-miR-1 using real-time PCR. As a result, a correlation between hsa-miR-1 expression levels and the evolution of Chagas disease is expected.

Keywords: Chagas Disease, hsa-miR-1, Oral Transmission

HA.40 - The role of histone 3 trimethylations in the pathophysiology of major depression in rats subjected to chronic unpredictable mild stress model

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The pathophysiology of Major Depressive Disorder (MDD) is not fully understood, but it is known to be a multifactorial disorder. For preclinical studies, the chronic unpredictable mild stress model (CUMS) is considered a model with high translational potential for the study of TDM. Furthermore, studies show that environmental factors such as stress can cause epigenetic changes. In this context, considering that histones are subject to complex epigenetic modifications, such as methylation, this phenomenon can modify the nucleus-histone interaction. Although many studies have been performed, no study reports methylation of lysine residues (K) 4, 9, 27, 36, and 79 of histone 3 (H3) with the heritability of depression. However, first, the objective was to investigate possible DNA damage and alterations in the methylation pattern of H3 residues in Wistar rats submitted to CUMS. For this, 29 rats were used (8 controls and 21 submitted to CUMS for 42 days). During CUMS, sucrose consumption was performed, followed by open field and object recognition. Finally, blood and bone marrow samples from the femur were collected to perform repair kinetics and micronucleus, while the hypothalamus and hippocampus were used to verify possible epigenetic modifications. Regarding DNA damage, we found that repair of the damage occurred in a shorter time in control and resilient animals than in depressive-type animals. Finally, evaluating the trimethylations pattern of H3, we observed H3K27 hypermethylation while H3K9 was hypomethylated in the hypothalamus of animals with a depressive-type phenotype. In the hippocampus, we observed hypermethylation of H3K4 and H3K36 in resilient animals and hypermethylation of H3K9 in animals submitted to CUMS. From our results, we elucidate that there are different patterns of trimethylation between depressed and resilient animals. These patterns may be related to the development of the phenotype, as the offspring inherited behaviors similar to those of their parents.**Keywords:** Depression, Epigenetics, Histone methylation

HA.41 - The effect of hypoxia in the motility of MDA-MB-231 breast tumor cells and endothelial cells *in vitro*

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Solid tumors, such as breast cancer, may present hypoxic areas that induces angiogenesis and cell migration contributing to metastasis. Integrins are cell surface receptors that play a key role in cell motility. Inhibition of cell migration by blocking such receptors might impair metastasis. Desintegrins are integrin inhibitors, such as Dis*Ba*-01, a RGD disintegrin from *Bothrops altenatus* with high affinity to $\alpha_V\beta_3$ integrin. Dis*Ba*-01 was demonstrated to inhibit cell migration and angiogenesis in different *in vitro* models in normoxia. This inhibitory effect, however, has never been investigated in low oxygen conditions as usually found in solid tumors. The goal of this work was to understand the migratory behavior of cells under $\alpha_V\beta_3$ integrin blocking in hypoxia using two migration models, the Boyden chamber and the wound healing assays. MDA-MB-231 cells and HUVEC were treated with Dis*Ba*-01 for 30 minutes in room temperature, transferred to 24-well inserts for transwell migration assays in hypoxia (1% O_2, 5% CO_2, and 94% N_2) and normoxia. For wound healing assay, cells were treated with Dis*Ba*-01 for 24 hours in the two conditions. For cell morphology analysis, cells were exposed to Dis*Ba*-01 for 4 hours in normoxia and hypoxia, and stained with phalloidin. MDA-MB-231 cell migration was inhibited by Dis*Ba*-01 with IC_{50} values were 9.8 nM and 16.62 nM in normoxia and in hypoxia, respectively, after 16h. Dis*Ba*-01 inhibited HUVEC migration in hypoxia after for 24h, without effect in normoxia. In the wound healing assays in hypoxia, Dis*Ba*-01 was effective only at its highest concentration. DisBa-01 changed the morphology of MDA-MB-231 cells to a more circular form similarly in both oxygen conditions. We concluded that larger Dis*Ba*-01 concentrations are needed to inhibit $\alpha_V\beta_3$ integrin under hypoxic conditions and therefore to inhibit cell motility.
Keywords: Cell migration, integrin αVβ3, hypoxia. **Supported by:** FAPESP, CNPq and CAPES

HA.42 - Exosomes from MDA-MB-231 cell line induce apoptosis gene expression, and phenotype and functional changes in Dendritic Cells: Possible role of Caspase-9 gene and pro-apoptotic miRNAs

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Over the recent years, it has been demonstrated that dendritic cells (DCs) from cancer patients have phenotype altered becoming cells with reduced ability to activate immune system. Tumor derived exosomes (TEX) are critical components of intercellular information network between tumor and host, emerging, in recent decades, as important modulators immune response in the cancer development and establishment context. To evaluate if TEXs isolated from MDA-231 tumor cell line culture supernatant can induce phenotypic alterations functional changes and evaluate caspase 9 and blc-2 gene expression in monocyte-derived DC (Mo-DC) *in vitro* differentiation. To quantify pro-apoptotic miRNAs (29b, 34a, 155 and 146) TEX expression. Exosomes from MDA-231 culture were obtained through ExoQuick-TCTM kit. Structural and morphological exosomes characterization were performed by Western blot, Nanosight and Transmission Electron Microscopy. Mo-DCs were obtained from healthy donors and maintained in culture in presence or absence of TEXs (30µg/mL). Mo-DC phenotypic characterization, cell viability, apoptosis and co-culture T cell proliferation were analyzed by cytometry flow. mRNAs apoptosis genes and miRs expression were identified for qPCR. Exosomes was successfully isolated and characterized. It was observed TEXs from MDA-231 induced phenotypic Mo-DCs alterations, exhibited by CD80 downregulation ($p < 0,05$) which corroborated with functional tests that demonstrated reduced ability to stimulate proliferation of CD4 and CD8 cells and induction of tolerogenic T cells differentiation ($p < 0,05$). Mo-DCs treated with TEX showed 42% death, 37.% in apoptosis process, caspase-9 gene overexpression ($p < 0,05$) and bcl-2 gene downexpression ($p < 0,05$). Finally, all pro-apoptotic miRs evaluated presented overexpression in TEX. These results suggest TEX action on the DC functional immunostaining in order to induce a tolerogenic state that suppress an adaptive antitumor immune response favoring tumor evasion. Since casp9, bcl-2 and proapoptotic, miRNAs are altered, it is possible that their overexpression is involved in the apoptosis of Mo-DC.

Keywords: breast cancer, exosome, Dendritic Cells

Supported by: FAPEMIG, CAPES, CNPq

HA.43 - PROJECT: Analysis of the neuroprotective effects of supplementation with flaxseed oil and/or α-lipoic acid in the hippocampus of rats in haloperidol-induced orofacial dyskinesia model

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Typical antipsychotics are a class of medications associated with motor disorders when used chronically, such as haloperidol. Tardive dyskinesia (TD) is a motor syndrome that has as its main pharmacological cause the blockade of dopaminergic D2 receptors. Its exact pathophysiology is not defined and theories point to the involvement of inflammatory causes, oxidative stress, poorly adaptive plasticity and neurochemical imbalance. In this context, research on substances with antioxidant action has intensified, such as the use of flaxseed oil (*Linum usitatissimum L.*) and α-lipoic acid, highlighted by neuroprotective action, in important neuroplastic regions associated with memories, such as the hippocampus. This study seeks to evaluate the influence of supplementation with flaxseed oil and/or α-lipoic acid on neuroprotection on the development of orofacial dyskinesia induced by sub-chronic administration of haloperidol in the hippocampus of rats. Ninety-six Wistar, males, three months, weighing 250-320 g, arranged in 6 groups (n=16) will be divided between the saline control groups; haloperidol administration; submitted to haloperidol administration with supplemental lipoic acid diet; administration of haloperidol with supplemental flaxseed oil diet, haloperidol administration with supplemental diet of lipoic acid and flaxseed oil and submitted to clozapine administration. Evaluations of the behavioral profile of animals will be performed under aspects of motor activity, induction of orofacial dyskinesia and acquisition of memory, anxiety and learning, evaluation of dopamine receptor D3 expression levels, BDNF (brain-derived neurotrophic factor) and GFAP (glial fibrillar acid protein) by quantification by Western Blott, comparison of morphological and morphometric parameters by immunohistochemistry and also evaluation by biochemical assays to measure the activity of antioxidant enzymes from lipoperoxidation assays, evaluation of catalase concentration, enzymatic activity of superoxide dismutase and reduced glutathione. With this, we aim to promote neuroprotective, anti-inflammatory, neuroplastic, behavioral and antioxidant actions, the neurological damage associated with the administration of neuroleptics in animal models.

Keywords: Oxidative Stress, Antioxidant, Tardive Dyskinesia
Supported by: CAPES

HA.44 - The flavonoid Agathisflavone improve tissue repair in acute spinal cord injury in the rat

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Spinal cord injury (SCI) is a serious disease that lacks effective therapy. Among therapeutic alternatives, flavonoids as well as mesenchymal cells have been widely studied in the literature. Agathisflavone is a flavonoid capable of inducing neurogenesis and presenting anti-inflammatory properties. We hypothesize that, the treatment with agathisflavone compared to untreated rMSCs, agathisflavone exposed rMSCs infused after acute spinal cord injury (SCI) increase production of neurotrophic factors and modulate inflammatory damage favoring tissue regeneration. rMSCs were obtained from adult Wistar rats and cultured in supplemented DMEM. Cells were exposed to agathisflavone (1µM) and toxicity analyzed by MTT test after 24h and 72h; For in vivo experiments adult male Wistar rats (n=6/group) underwent acute SCI with an F2 fogarty catheter and after 4h were treated with a single application (via caudal vein) of 1x106 control rMSCs or pretreated with agathisflavone (1µM, every 2 days, for 21 days *in vitro*). Alternatively, animals (n=6/group) were treated with a single dose of methylprednisolone (MP, 60 mg/kg ip), or treated daily with agathisflavone (10 mg/kg ip). After 1, 3 and 6 days treatment BBB scale was used to evaluate the motor functions of the animals; after 7 days of the treatment the SCI area was analyzed after H&E staining, and RT-qPCR was performed to analyze expression neurotrophins and arginase. Treatment of animals with of 21days agathisflavone-treated rMSCs was able to protect the injured spinal cord tissue, associated with the increase in expression of NGF,GDNF and arginase, and reduction on the macrophage infiltrate, as well as with the improve of the motor functions (with the highest BBB score). Moreover, treatment of animals with agathisflavone alone was also able to protect injured spinal cord tissue, to increase expression of neurotrophins and modulate the inflammatory response Agathisflavone modulates rMSCs metabolic profile and neuroinflammatory response during the spinal cord injury.

Keywords: Agathisflavone, Acute Spinal Cord Injury, Flavonoid
Supported by: FAPESB, CAPES, CNPq

HA.45 - Project: Effect of rutin on neurogenesis and neuroprotection: study of the pharmacological action of flavonoids in glial biology and neuronal population at different stages of life and in a study model for Parkinson's disease.

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Parkinson's Disease (PD) is characterized by a complex pathogenesis that mainly involves mitochondrial damage, oxidative stress, α-synuclein accumulation and neuroinflammation. Such mechanisms responsible for the loss of dopaminergic neurons in the disease are still discussed, however aging is described as the main risk factor for the development of PD. Currently, it is known that the cellular changes inherent to the disease cover several brain regions, including the nigrostriatal system and the cerebellum. The current therapeutic application is only aimed at reducing symptoms and the search for a treatment that reduces the rate of neurodegeneration or that interrupts the development of the disease is continuous. In this sense, flavonoids represent a large class of compounds with neuroprotective, antioxidant and anti-inflammatory potential. More specifically, the flavonoid rutin has the ability to regulate the microglial response and to protect neurons against damage induced by glutamate, as evidenced in previous studies developed by the Laboratório de Neuroquímica e Biologia Celular research group. Thus, this project aims to investigate the effect of rutin on glial biology and neuronal population at different stages of life and its implications for PD. For this, young (3 months) and old (24 months) wistar rats will be treated orally with the flavonoid rutin (10 mg/kg) for 21 days, and on the 14th day the animals will be subjected to behavioural tests. Immunohistochemistry and qPCR will be adopted to assess molecular markers of glial response and neuronal population under conditions of exposure or not to the neurotoxic agent aminochrome a 6 nmol. It is expected with the development of this project to characterize more broadly the therapeutic potential of rutin for PD, showing its neuroprotective effects against aminochrome toxicity in different regions of the brain, besides to preventing possible changes associated with aging.

Keywords: Neuroprotection, flavonoids, aging

Supported by: FAPESP, CNPQ

HA.46 - The conformational scFv antibody NUsc1 protects differentiated human neuroblastoma cells against Alzheimer's-associated Aß oligomers toxicity

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Soluble oligomers of the Aβ peptide (AβOs) are neurotoxins linked to synaptotoxicity and neurodegeneration in Alzheimer's disease (AD). AβOs are heterogeneous in conformation and toxicity and the extent to which different Aβ species contribute to the pathophysiology of AD remains uncertain. In previous work, we selected a scFv fragment antibody, named NUsc1, that distinguishes AβOs from both monomeric and fibrillar Aβ. Different from other conformational anti-Aβ antibodies, NUsc1 preferentially targets a subset of AβOs larger than 50kDa present in AD brain tissue. Here we aimed to investigate whether NUsc1 is capable of preventing the dysfunction and degeneration induced by AβOs in a human neuronal-like cell model. SH-SY5Y neuroblastoma cells were differentiated into mature neurons and challenged with AβOs, either alone or in combination with NUsc1, for 24h. AβO binding to SH-SY5Y cells was assessed by immunofluorescence. Viability was evaluated by both MTT and Live/ Dead assays. AβO binding to differentiated SH-SY5Y cells revealed a punctate binding pattern. Treatment with AβOs induced a decrease in cell viability as followed with the MTT assay, although not leading to cell death. Importantly, MTT reduction capacity was not affected when the cells were co-treated with AβOs and NUsc1. Additionally, we have compared the neuroprotection conferred by NUsc1 with the protection by the pan anti-Aβ 6E10 antibody, which recognizes multiple forms of Aβ and is known to reduce Aβ-induced toxicity in AD models. We have demonstrated that the conformation-sensitive scFv antibody NUsc1 binds to and neutralizes the neurotoxicity of AβOs on differentiated SH-SY5Y cells. These results support the use of this cell lineage as an *in vitro* neuronal-like model to study early molecular and cellular events underlying the neurotoxicity triggered by AβOs, and the potential of NUsc1 as a target-selective therapeutic candidate for the treatment of AD.
Keywords: Alzheimer's Disease, ß-amyloid oligomers, SH-SY5Y neuroblastoma cells
Supported by: FAPESP, CNPq, CAPES, FAEPA

HA.47 – PROJECT: Assessment of the FAK/SRC pathway role in the proliferation and apoptosis of JAK2 V617F positive cell line.

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The Chronic Myeloproliferative Neoplasms (MPN) BCR-ABL negative are hematopoietic disease that affect the myeloid lineage cells. These include Polycythemia vera (PV), Essential Thrombocythemia (ET) and Primary Myelofibrosis (MF). The identification of the acquired mutation *JAK2*V617F in patients with PV, TE and MF has led to a better understanding of the pathogenesis of these diseases since the constitutive activation of the JAK2 plays an important role in cell proliferation increases and apoptosis resistance. However, there is still no pharmacological treatment that leads all patients to molecular remission or cure and so, the study of new molecular targets is justified. In turn, the Focal Adhesion Kinase has an important role in cell proliferation, migration and survival and, as it is overexpressed in several neoplasms, it becomes a promising target in the development of cancer drugs. Thus, the present study proposes to investigate the role of the FAK/SRC signaling pathway in the proliferation and apoptosis of a *JAK2*V617F positive cell line. For this purpose, the following assays will be performed: (1) immunoprecipitation, in order to confirm FAK and SRC interaction in the cell line; (2) FAK inhibition and JAK inhibition assays, alone and in combination, followed by cell viability determination, the detection of the FAK/SRC and JAK/STAT pathways inhibition and apoptosis proteins by Western Blotting. Considering the role of FAK protein in other neoplasms, it is expected that FAK inhibition will decrease cell viability/proliferation and will induce apoptosis, which will be evidenced by the presence of cleaved PARP and Caspase 3. Moreover, it will be possible infer whether the FAK inhibition interferes with the activation of JAK/STAT pathway. The identification of new molecular targets may contribute to better comprehension of pathophysiology mechanisms and, in the future, to the development of new therapies.
Keywords: Myeloproliferative Neoplasms, FAK, Apoptosis. **Supported by:** FAPEMIG

HA.48 - Association of serum levels of IGF-1 with vaso-occlusive crisis in children with sickle cell anemia

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Sickle cell anemia (SCA) progresses with dysfunction of multiple organs and episodes of acute pain that are caused by vaso-occlusive crises (VOC). Previous studies suggest that insulin-like growth factor I (IGF-1) can act on afferent neurons increasing pain sensitivity. Assess whether serum IGF-1 levels may be associated with episodes of VOC. A cross-sectional population-based study evaluated 39 individuals with SCA, 51,3% (20/39) male, mean age 8.2±2.2 years (range = 3.7-11.9 years). The population was grouped according to the VOC report in the last year. Clinical data and serum levels of IGF-1 were evaluated in each group. At least one VOC was reported in 59% (23/39) of children. Both groups had similar age (p = 0.66), sex (p = 0.20), body mass index (BMI) SDS (p = 0.52) and height SDS (p = 0.53). The IGF-1 SDS was correlated with the BMI SDS (p = 0.03, r = 0.34), but it did not show any correlation with other anthropometric data evaluated in the research. IGF-1 is produced primarily in the liver under the stimulation of the pituitary growth hormone and is important for height growth, especially for intrauterine growth. Although our study did not show a correlation between the serum level of IGF-1 and birth length (BL), both variables had the same direction in the groups evaluated in the research. Therefore, comparing the groups involved in the study, BL (46.5 cm vs 49.8 cm) and IGF-1 SDS (-1.25 vs -0.62) were significantly (p = 0.02 and 0.03, respectively) higher in the group with episodes of VOC than in the group that did not report any event in the last year. These results suggest that serum level of IGF-1, known to be associated with intrauterine growth, may have an impact on the VOC of children with SCA. **Keywords:** IGF-1, vaso-occlusive crisis, sickle cell anemia
Supported by: FAPEMIG/PPSUS MCT, Finep/CT-Infra and PROQUALI-UFJF and CAPES

HA.49 - Guanosine increases mitochondrial activity via glutamate transport coupling and prevents hippocampal slices damage following oxygen/glucose deprivation

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Stroke is a major cause of death and incapacity worldwide and ischemic stroke represents 70% of all strokes globally. The rupture of brain energy metabolism due to reduced oxygen and glucose supply, disruption in ATP synthesis and ROS production, besides glutamate excitotoxicity are hallmarks in the pathogenesis of brain ischemia. Oxygen and glucose deprivation (OGD) in brain tissue preparations reproduces pathological features induced by stroke providing a valuable ex vivo protocol for studying the mechanism of action of neuroprotective agents. We previously showed that the nucleoside guanosine promotes the recovery of ATP, lactate levels and cellular viability in hippocampal slices subjected to 15 min of OGD and followed by 3 hours of reoxygenation (OGD/R). We here investigated the underlying mechanisms triggered by guanosine on cellular energetic metabolism recovery in an *in vitro* ischemic model. We assessed cellular viability by tackling lactate and glutamate transport and the involvement of mitochondrial oxidative phosphorylation (OXPHOS), through high-resolution respirometry. OGD/R caused a severe mitochondrial dysfunction through reduction of basal, phosphorylating state and maximal oxygen consumption rates (OCR). Guanosine (100 μM) addition in the reoxygenation period recovered mitochondrial dysfunction, reduction in ATP levels and glutamate uptake into hippocampal slices evoked by ischemia. To understand the neuroprotective mechanism of guanosine, we used α-cyano-4-hydroxycinnamate (4-CIN, 500 μM) a monocarboxylate transporters (MCTs) inhibitor, and DL-threo-β-benzyloxyaspartic acid (TBOA, 10 μM) a non-substrate glutamate transporters inhibitor. 4-CIN prevented guanosine effects on ATP levels and glutamate and lactate transport, but it did not abolished guanosine effects on mitochondrial OCR. On the contrary, TBOA abolished all guanosine effects, indicating a dependence on glutamate transport on the mitochondrial OXPHOS activity. Guanosine treatment prevents mitochondrial dysfunction after OGD/R, pointing to a promising effect of improving brain mitochondria bioenergetics. Therefore, guanosine could act as an adjuvant in battling the sequelae after ischemia, preventing negative outcomes. **Keywords:** guanosine, brain ischemia, mitochondria
Supported by: CNPq, INCT-EN, CAPES

JA - Signaling, Gene Regulation and Proliferation

JA.01 - Evaluation of the role of allantoin in drug resistance in leukemia

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Leukemia is a hematological cancer characterized by the exacerbated proliferation of cells of hematopoietic tissue. In the beginning of chemotherapy treatment, there may be the development of Tumor Lysis Syndrome (TLS) due to the large amount of intracellular content from lysis of tumor cells. For this, the treatment of TLS, carried out in parallel to the chemotherapy treatment, uses a recombinant urate oxidase enzyme. The enzyme is responsible for converting high serum uric acid levels into allantoin, which is more easily eliminated in the urine. However, there are no clinical studies that show the action of allantoin during chemotherapy treatment until its complete elimination by urine. Our objective was to investigate if allantoin interferes with the action of cisplatin, *in vitro*, in sensitive chronic myeloid leukemia cells. Chronic myeloid leukemia cells (K562) were cultured in RPMI 1640 culture medium supplemented with 10% fetal bovine serum, 1% antibiotic, and maintained in a stove at 37°C and 5% CO2. The cells were treated with cisplatin 10 µg/ml and allantoin in concentrations of 25 µg/ml, 50 µg/ml, 100 µg/ml and 200 µg/ml. The cell viability assay was performed using the MTT assay, flow cytometry was used to analyze the induction of DNA fragmentation and the loss of mitochondrial membrane potential was observed by fluorescence microscopy. Our results show that allantoin does not induce cell death and cisplatin leads to decreased viability, in K562 cells. However, we observed that in the presence of allantoin there is a reduction in death caused by cisplatin, allowing us to deduce that allantoin enhanced the survival of K562 cells, preventing the efficient action of cisplatin. Since our results show an alteration in the viability of a sensitive leukemia cell, continuing this study, we will simultaneously investigate the effects of allantoin in sensitive and resistant leukemia cells.
Keywords: allantoin, cisplatin, leukemia
Supported by: FAPERJ

JA.02 - Biochemical analysis of the activation of apoptotic pathays in the hippocampus of rats fed cafeteria diet and treated with atorvastatin

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The accumulation of adipose tissue causes a series of metabolic disturbances. This accumulation can occur due to excessive consumption of food and nutrients and can cause increase in circulating cholesterol levels and in reactive oxygen species, leading to cell death by apoptosis, which can be mediated by Caspase 3. One drug option used to control hypercholesterolemia is atorvastatin. The aim of this study was to evaluate serum cholesterol levels, adipocyte accumulation, and to assess caspase 3 mediated apoptotic pathway signaling in the hippocampus in rats fed a hyperlipidic, hyperglycemic cafeteria-type diet and treated with atorvastatin. Male Wistar rats were divided into four groups: commercial diet - saline (CoSal), commercial diet - atorvastatin (CoArt), cafeteria diet - saline (CafSal), and cafeteria diet - atorvastatin (CafArt). Atorvastatin was administered at doses of 10 mg per rat kg per day and saline at a dose of 0.9%, both by gavage, and the cafeteria groups received water with an addition of 20% of sucrose. Rats were submitted to daily weighing and on the 25th day they were euthanized. Blood was collected for cholesterol quantification and retroperitoneal and epididymal fats were removed for weighing. In addition, hippocampi were collected to evaluate the expression of caspase 3. All groups gained equivalent weight during the assessed time. However, for the CafSal group, the percentage of fats and serum cholesterol were higher, and atorvastatin (CafArt) was able to return to values equivalent to the control group. In addition, the CoArt group presented values equivalent to the control in all analyzed parameters and the expression of hippocampal caspase 3 did not show differences between the studied groups. Obesity did not alter the total weight but the percentage of fat and circulating cholesterol and atorvastatin reversed this effect. None of the variables affected the expression of caspase 3.
Keywords: Obesity, Neuroprotection, Apoptosis
Supported by: FAPEMIG e UFSJ

JA.03 - UDESC-CAV/LACEN: Validation of a laboratory for COVID-19 diagnostic support network in Serra Catarinense

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Due to the fight against the COVID-19 pandemic, caused by SARS-CoV-2 infection, there is an urgent need to increase the number of exams performed for the diagnosis of this disease. Despite several efforts in creating large-scale diagnostic methods, real-time quantitative reverse transcription polymerase chain reaction PCR (RT-qPCR) is still the gold standard for establishing a COVID-19 diagnosis. We performed a SARS-CoV-2 RNA RT-qPCR assay methodology using multiple detection temperature (MuDT™) technology, validated by Centers for Disease Control and Prevention (CDC, USA) developed by Charité Virology Institute, from Berlin University, Germany. Using all the biosafety level 2 (BSL-2) guidelines for research and clinical diagnosis, a commercial SARS-CoV-2 RNA multiple RT-qPCR kit for the E, N and RdRP genes was employed on two Real-Time PCR Detection Systems. Molecular SARS-CoV-2 detection involved the steps of RNA viral extraction from oropharyngeal swab (OPS), SARS-CoV-2 reaction mix preparation and RT-qPCR performance. In both RT-PCR detection systems it was possible to validate the results of positive and negative virus detection by Laboratório Central de Saúde Pública of Santa Catarina State (LACEN/SC). The MuDT™ technology can perform a simultaneous detection and identification of 3 target genes specific for COVID-19, in a one-step reaction workflow. The assay is designed to detect E gene for all Sarbecovirus including SARS-CoV-2 and N and RdRP genes specific for SARS-CoV-2, enabling a performance with high sensitivity and specificity. The kit Internal Control (IC) verify the whole process validation from extraction to PCR whole process control. The validation of a laboratory inside the university with skilled labor using a convenient and reliable methodology can contribute to facing COVID-19 pandemic in Serra Catarinense. This approach allows performing a greater number of exams in a faster way, reinforcing the importance of research work from universities in strategic selection of patients' management with SARS-CoV-2 infection.
Keywords: COVID-19 pandemic, RT-qPCR, SARS-CoV-2
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JA.04 - Effect of insulator sequence on twist deformation of a single DNA molecule

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Insulator is a DNA element that regulate gene expressions. Insulators have been identified in several organisms, showing enhancer-blocking activity or barrier activity that protect transgenes. Ars Insulator (ArsIns) identified in sea urchin has AT-rich region which is sensitive to nucleotide base modification, suggesting a non-B-DNA structure. This AT-rich region alone exhibits the insulator activity, but specific binding proteins have not been detected, suggesting that local DNA sequence could affect the large deformation necessary for regulation of gene expressions. In this study, we twisted single DNA molecules with or without ArsIns sequence using opto-magnetic tweezers, and examined the effect of the local sequence on the large deformation of DNA. DNA with ArsIns (Ins-DNA, 7058 bp) contained ArsIns (578 bp) approximately in the center of the DNA, and DNA without ArsIns (No-Ins-DNA, 7058 bp) contained a part of lambda-phage DNA instead of ArsIns at the same position. One end of the DNA was attached to glass surface and the other end was attached to a magnetic bead. The DNA was stretched by radiation force of a focused laser to the bead, and twisted by rotating magnetic field created by two pairs of Helmholtz type coil. We obtained relationship between the bead position in height and number of the twist, which is referred as twist curve. The decrease of the bead position during twisting means the formation of plectoneme (super-helical structure with DNA crossing). Ins- and No-Ins- DNA showed clearly different shapes in the twist curves. Moreover, while only one type of twist curve appeared for No-Ins-DNA, two types of twist curve appeared for Ins-DNA; one of the curve was equivalent to that observed for No-Ins-DNA. These results suggest that large deformation of DNA is sensitive to local DNA sequence, and ArsIns can stochastically switch the large structure of DNA. **Keywords:** DNA, Insulator, twist deformation

JA.05 - Influence of supplementation with polyunsaturated fats of different sources on cell proliferation

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Nutraceutics are nutritional supplements from different sources and are claimed to improve health with no or little side effects. Therefore, omega-3 polyunsaturated fats consumption has been associated to longevity. As many cellular events depends on cell membrane lipids characteristics and polyunsaturated fats supplementation is able to modify cell membrane composition. It is important to understand cell behavior in the presence of nutraceutics, such as polyunsaturated fats. Here in, it is presented the influence of polyunsaturated fats supplementation upon cell proliferation. Cell medium was supplemented with fish oil (FO), or shark liver oil (SLO), or oro inca oil (OIO). Each oil was complexed with BSA 1% overnight and They were diluted in cell medium (1:100, 1:200, 1:400). Cell lines with different proliferation ratio and origins were chosen. N2A, HeLa and 3T3 proliferation ratio was tested using Alamar blue die. 3T3 proliferation ratio was tested up to 100h. N2A and HeLa was test up to 180h. OIO (1:100) was able to decrease N2A, HeLa and 3T3 proliferation. OIO (1:200 and 1:400) decreased 3T3 and increased N2A and HeLa cell proliferation. FO (1:100 and 1:200) had no influence in 3T3 and N2A and increase HeLa proliferation. FO (1:400) decreased 3T3 and increased N2A and HeLa proliferation. SLO (1:100) decreased 3T3 and increased N2A and HeLa proliferation. SLO (1:200 and 1:400) lead to no augment in 3T3 proliferation and increased N2A and HeLa proliferation. The fats tested had different sources omega-3 and composition. OIO is a plant derivative oil rich in ALA which can be converted in EPA and DHA in small amounts. DHA and EPA are present in OF. And SLO has alkylglycerols in composition. Despite the modification in cell lines proliferation influenced by the different oils, cell lines characteristics seems to be determinate to influence proliferation in the presence of polyunsaturated oils.
Keywords: fish oil, oro inca oil, shark liver oil,
Supported by: Fundação Araucária, CNPq

JA.06 - Biochemical characterization of the interaction between Fbxo7 and androgen (AR) and estrogen (ER) receptors

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Ubiquitously eXpressed Transcript isoform 2 (UXT-V2) also known as Androgen Receptor Trapped clone-27 (ART-27) is a prefoldin-like protein involved in NF-kB signaling, apoptosis, androgen and estrogen response. UXT-V2 regulates the androgen and estrogen signaling pathways in prostate and breast cancer, acting as a transcriptional coactivator that binds to the N terminus of the androgen/estrogen receptors (AR/ER). Fbxo7 is a component of E3 ubiquitin ligase that interacts and mediates the proteasomal degradation of UXT-V2. The aim of this study was to evaluate the interaction of Fbxo7 with AR and ER receptors. We also evaluate if the receptors are substrates of SCF(Fbxo7). The interaction of Fbxo7 and AR/ER was evaluated by co-immunoprecipitation in HEK293T cells transfected with Fbxo7 and the receptors plasmids in the presence or not of UXT-V2 plasmid. The *in vivo* ubiquitination assays were also carried out in HEK293T cells transfected with Fbxo7, ubiquitin-myc and the receptors followed by immunoprecipitation. Our results suggest that Fbxo7 interacts with AR and that the UXT-V2 protein has a negative effect on this interaction. Furthermore, we also verified a potential interaction between Fbxo7 and the ER receptor. These results suggest a possible interaction of AR and ER receptors with Fbxo7 and the functional consequences of this interaction in the androgen/estrogen signaling pathways are being investigated.
Keywords: Fbxo7, AR/ER receptors, UXT-V2
Supported by: CNPq, CAPES and FAPESP

JA.07 - Identification of miRNAs in mice and their correlation with intracellular pathways associated with the healing process of sickle cell ulcers.

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Sickle cell ulcers (UF) are among the most serious complications of sickle cell anemia. Treatment is limited to removal of necrotic tissue and infection control. Understanding the mechanisms involved in the pathogenesis of UF is relevant for the development of therapies and the prognosis of patients. In this context, microRNAs represent a promising tool, as they can influence the modulation of genes and growth factors essential for UF healing. To study the expression profile of miRNA and its correlation with the intracellular pathways associated with the development of UF. Public transcriptome data [access code GEO - GSE121996] were analyzed. Samples were examined using bioinformatics tools on the Galaxy platform. A filtering was performed based on the expression level ratio (fold-change) and the adjusted p value < 0.05, to identify microRNAs differentially expressed in different periods after the induction of the lesion. Differently expressed miRNAs were identified in comparisons of time 0h vs 6h, 0h vs 24h and 0h vs 5 days. Mir7-2 and Mir511 were underexpressed in 0h vs 24hrs and 0h vs 5 days comparisons. Mirlet7c-2 was underexpressed in 0h vs 6h, 0h vs 24h and 0h vs 5 days comparisons. Mir26a-1 was overexpressed in the 0h vs 24hr and 0h vs 5 day comparisons. Preliminary results show an increase in differentially expressed microRNAs throughout healing. Underexpressed microRNAs are related to cell differentiation and proliferation while Mir26a-1 is involved in synaptic plasticity. The future perspective is to establish a correlation between these microRNAs and their target genes during wound healing from sickle cell ulcers, in addition to validating them in in vivo models.
Keywords: sickle cell ulcers, transcriptome, bioinformatics
Supported by: FAPESB

JA.08 – PROJECT: Analysis of neurodevelopmental alterations in wistar rats subjected to antidepressant treatment during the prenatal period

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During pregnancy, women are subject to the development of depression. Selective Serotonin Reuptake Inhibitors (SSRIs), like fluoxetine, are usually the first-line treatment for this disease. However, fluoxetine is able to overcome the transplacental barrier and affect the fetus, causing changes in serotonin levels early in life and in the long term can cause damage to brain circuits that control cognitive and emotional behavior as a result of early exposure fluoxetine during neurodevelopment. In this study, we will address the question of how exposure to fluoxetine (25mg/kg/day) from the thirteenth day (GD13) to the twenty-first day of pregnancy (GD21) can lead to behavioral changes in men and women offspring at 90 days (PN90). For this, we will analyze the performance of individuals in behavioral tests: splash test, open field, discriminative avoidance of the maze in cross and context-based fear, of which they are responsible for assessing behaviors similar to anxiety and depression and learning / memory processing. In addition, we will perform neurochemical experiments to understand the dynamics of serotonergic circuits in neurodevelopment, using neuropeptides as specific markers. Our study seeks to find out whether there are sexual differences modulated by serotonin during neonatal development in offspring subjected to perinatal exposure to fluoxetine (GD13-GD21) and whether it affects behavioral patterns of anxiety, depression and memory.
Keywords: Neurodevelopment, Depression, Fluoxetine

JB - Immune System

JB.01 - Association of immune biomarkers and oral biofilm in the induction of dental papilla growth by suction technique.

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Gingival recession is highly prevalent among the low-income population and is one of the main causes of pain, tooth loss, and esthetic deficiency. There are still no reports of non-surgical techniques to repair this damage. Due to this gingival area's small vascularization, alternative treatments have presented low success rates. From observations made in several denture wearers, it was confirmed important growth of palate epithelial tissue into the suction chambers in patients with this prosthetic device. Studies show that this epithelium has no capacity for malignant transformation. We propose an alternative and innovative approach of low-cost and non-invasive treatment, using the suction plate technique, for gingival neoformation and resolution of recession situations. To evaluate 30 individuals separated into group 01 (control) and group 02 (patients with gingival retraction). The patients will be molded with alginate, and then suction plates will be made, which should be used for 28 days. Crevicular fluid samples will be collected on days 0, 1, 7, 14, 21, 28 and stored at -80°C until used. Cytokines, chemokines, growth factors, gene expression of antioxidant enzymes, and the local microbiota will be analyzed at each collection time. A pilot study has already been conducted. Case follow-up of a 38-year-old patient, leucoderma, no history of morbidities, with the gingival recession in the upper incisor region, where he was wearing dental implants, complaining of functional and aesthetic problems was submitted to the use of suction plate for 28 days. During the entire papilla growth induction process by suction, we observed reddish areas, edema, and sometimes bleeding. This confirms the viability of the technique. Preliminary results provide positive subsidies for study designs that will underpin public health policies, aiming to establish more effective non-invasive measures for the control and clinical management of patients with gingival recession. **Keywords:** gingival recession, biomarkers, suction technique. **Supported by:** FAPEMIG, CPqRR/FIOCRUZ-MINAS

JB.02 - Effect of gene therapy with the factor derived from pigmented epithelium in a murine silicosis model.

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Silicosis is an occupational disease related to the exposure of workers to silica particles. Inhalation of silica leads to chronic inflammation and fibrosis of the lung parenchyma. So far, there is no efficient treatment for this disease. In this context, gene therapy emerges as a favourable treatment for inserting genes with therapeutic potentials for silicosis. The associated adenovirus 8 (AAV8) has emerged as a promising vector since it presents a natural tropism for airway epithelial cells and is poorly immunogenic. The factor derived from pigmented epithelium (PEDF) has anti-inflammatory, antifibrotic, and antioxidant activity. Thus, the present study investigated the hypothesis that the gene encoding PEDF, delivered by the vector AAV8, reduces inflammation and pulmonary remodeling in a murine silicosis model. For this purpose, female C57BL/6 mice were randomly divided into two groups: control (C) and silica (SIL). All experimental groups were subdivided into two subgroups: saline subgroups (C-SAL and SIL-SAL) and PEDF subgroups (C-PEDF and SIL-PEDF). We performed three experimental models: treatment 15 days after sílica instillation and treatment 1 and 28 days before sílica instillation. The relative amount of PEDF mRNA, respiratory mechanics, and the fraction of granuloma area were investigated. Instillation of silica caused histological changes in the lung parenchyma, such as the presence of granulomas, in addition to causing a high mortality rate. In all models, there were no improvements in pulmonary function and histology, in addition to the low expression of the PEDF gene in the SIL-PEDF subgroup. The treatment was ineffective in improving lung function and granuloma fraction in any of the adopted therapeutic strategies. Besides, a decrease in the efficiency of PEDF expression could be observed in the treated sick animals. Further analysis is necessary for a better understanding of the anti-fibrogenic and anti-inflammatory action of AAV8-PEDF *in silico*sis. **Keywords:** Silicosis, Gene therapy, PEDF. **Supported by:** FAPERJ, CNPQ e CAPES

JB.03 - Ursolic acid derivatives reduces carrageenan-induced paw edema

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The ursolic acid (UA) already is the target of studies that investigate its anti-inflammatory potential and, therefore, structural modifications can enhance its biological activities. The aim of this study was to evaluate the immunomodulatory effect of the ursolic acid derivatives (UAD) in macrophage response and in carrageenan-induced paw edema model. RAW264.7 was cultured in 96-well plates at 2×105 cells·mL-1 in supplemented RPMI-1640. Cells were maintained for 3h and 48h in the presence or absence of UA or UAD (1-19). Nitric oxide (NO) and NF-κB were measured in RAW264.7 cells stimulated with LPS (1 µg·mL-1) and IFN-γ (0.9 ng·m-1). Cellular viability was measured in non-stimulated cell cultures. The paw edema model was induced by carrageenan (2.5%) injection (20 µL) into the left footpad, and 20 µL of PBS into the right footpad, of all groups. The left and the right paws were measured at 1, 2, 3 and 4 h after the injection of carrageenan and the difference were calculated. Dexamethasone was used as control treatment. 30 minutes before the paw edema induction, the UA, UAD 1 and UAD2 (200 mg/Kg) were administered intraperitoneally (100 µL). The derivatives UAD2, UAD3, UAD4, UAD7, UAD9, UAD10, UAD11 and UAD14 were able to reduce the NO production in relation to the control ($p < 0.05$; IC50 > 90 µM). The NF-κB expression was evaluated after 3h of culture. The compounds UA, UAD1, UAD2, UAD3, UAD4, UAD5, UAD6, UAD7, UAD8, UAD9, UAD11, UAD12, UAD13, UAD14, UAD15 and UAD18 showed reduction of the NF-κB expression. The UA, UAD1 and UAD2 were able to reduce edema, as well as dexamethasone. The UA and derivatives were able to reduce inflammatory mediators and paw edema. Further studies are necessary to determine the molecular mechanisms of action of these derivatives.

Keywords: inflammation, RAW264.7, ursolic acid

Supported by: FAPEMIG, CNPq and CAPES

JB.04 - High levels of circulating immune complexes are observed in VL patients with acute kidney injury

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In Brazil, visceral leishmaniasis (VL) is caused by *Leishmania infantum*. Active phase of VL can vary from asymptomatic form to severe disease and death. Clinically is observed fever, splenomegaly, hepatomegaly, asthenia, anorexia and acute kidney injury (AKI). Depletion of CD4 $^{+}$ T lymphocytes, polyclonal activation of T and B cells, microbial translocation, cytokine storm and high levels of anti-*Leishmania* Igs are also involved in the VL immunopathogenesis. Considering that AKI is an important feature of VL, we hypothesized that this clinical condition could be related to high levels of Igs and mediated by immune complex (IC) deposition. Our aim was to evaluate the circulating IC (CIC) levels in VL patients and whether there is a relationship with markers of severity and renal failure. Fourteen VL patients recruited from Hospital Eduardo de Menezes (BH-MG) were evaluated since the active phase until 12 months post-treatment (mpt). Ten healthy controls (HC) were included. CIC and TNF levels were measured by ELISA and correlated with anti-*Leishmania* Igs levels and AKI markers. Twelve VL patients met the criteria for AKI according KDIGO2012 guidelines. Interestingly, the anti-*Leishmania* IgG1 levels were correlated with serum creatinine in post-treatment ($r=0.673;p=0.014$) and with the variation of glomerular filtration rate (ΔGFR) between the active and post-treatment phases ($r=-0.900;p < 0.001$). These data support the hypothesis of Igs involvement in renal damage. Indeed, not only IgG1-containing CIC but also IgA-, IgM-, IgG- and IgG3-CIC were higher in VL patients than HC until 6mpt ($p < 0,05$). Interestingly, the ΔGFR was negatively correlated with IgG1-containing CIC ($r=-0.682;p=0.012$). Concomitantly, the post-treatment levels of TNF-α, cytokine involved in IC-mediated renal injury, was positively correlated with anti-*Leishmania* IgG1 levels ($r=0.671;p=0.020$) and negatively correlated with ΔGFR ($r=-0.665;p=0.028$). Our results suggest that the AKI in active phase of VL was associated with high levels of IgG1, probably driven by IC deposition and TNF-α expression. **Keywords:** Visceral leishmaniasis, renal failure, immune complexes. **Supported by:** FAPERJ, CNPq, IOC/FIOCRUZ, IFRJ

JB.05 - Cytokine profile in hpv positive patients with cervical lesions

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Persistent Human papillomavirus (HPV) infection is the main cause of the development of neoplastic lesions and cervical cancer in women. This condition is influenced by several factors, including the immune response profile and HR-HPV (high-risk oncogenic HPV) infection. The aim of this study was to determine the cytokine profile in patients positive for HR-HPV and with cytological alterations such as cervicitis, cervical lesions, and carcinoma. The research carried out was descriptive, observational, cross-sectional involving 85 patients attended at Hospital de Amor, Campo Grande - MS unit, during 2019 and 2020. Patients positive for HR-HPV were selected for the study, using the Cobas® method HPV and underwent cervical biopsy, with 40% of the patients diagnosed with High Grade Intraepithelial Lesions (HSIL), 40% with cervicitis, 14% with Low Grade Intraepithelial Lesions (LSIL) and other less significant changes and 6% with carcinoma. Cytokine dosage was performed by flow cytometry, using the CBA Th1/Th2/Th17 BD™ detection system. This project was approved by CEP UFMS (No. 2,685.400 of 05/30/2018). The average patient's age was 41.66, and 41% had completed high school. The beginning of sexual life after 16 years was reported by 67% of women. As for the use of condoms, 71% of patients said they did not use condoms. The dosage of Il-17 was a higher in young adults (from 25 to 35 years old), being more frequent also in patients with cervicitis and HSIL. Higher frequency of IL-6 was observed in patients with carcinoma. IL-17 together with IL-6 were more frequent in patients positive for HPV 16 only or associated with other HR-HPV. Statistical analyzes will be developed to verify the meaning of such findings. Higher levels of IL-17 and IL-6 may be associated with an important participation of these mediators in the inflammatory response and in the progression of lesions. **Keywords:** immune response, cytokines, HSIL. **Supported by:** Fundect Called FUNDECT No. 06/2017 - UNIVERSAL-MS and UFMS

JB.06 - Saturated cardiolipins and cationic lipids activate TLRs: mechanistic basis for immune response in diseases, gene therapy and vaccine formulation

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Toll-like Receptors (TLRs) are the main protagonists of the innate immune system. Among them, TLR2 and TLR4/MD2 recognize lipids located in bacterial membrane and induce pro-inflammatory reactions. We found that TLR recognition is not limited to bacterial molecules. TLR4 and TLR2 are able to recognize also synthetic cationic lipids, used as nucleic acid nanocarriers, and the mitochondrial lipid cardiolipin. *In silico* analysis coupled with *in vitro* and in vivo experiments brought us to understand the molecular parameters for which a lipid would be inert, an agonist or an antagonist of TLRs: short saturated di-acyl cationic lipids lipopolyamines activate TLR2 and 4, longer(≥C18) saturated cationic lipids activate only TLR2, and ≥C18 unsaturated lipopolyamines do not induce TLR activation(Lonez et al., 2015; Pizzuto et al., 2016, 2018); moreover, saturated tetra-acyl lipids like cardiolipins(CLs), activate TLR4, whereas unsaturated CLs are TLR4 antagonists(Pizzuto et al., 2019). Molecular docking show that all CLs fit into the hydrophobic cavity of MD2, however, our analysis failed to predict the pharmacology of CLs, revealing the limitations of such an approach with this family of molecules. By contrast, docking of lipopolyamines in TLR2 suggested potential TLR2 binding modes reminiscent of natural TLR2 agonist and was able to predict their activity. According to our *in silico* analysis and *in vitro* data, lipopolyamines with both unsaturated chains do not fit into the hydrophobic pocket and do not activate TLR2.(Pizzuto et al., 2017 J Control Release). We therefore recommend the use of unsaturated C18 chains for the synthesis of inert transfection agents or TLR4 antagonists to be used as therapeutics for TLR4-related diseases such as sepsis, while we recommend saturated lipopolyamines in vaccine formulation as their immunostimulatory activity coupled to their carrier properties conferred good adjuvant properties in vivo(Pizzuto et al., 2018 J Control Release). The ability of saturated CL to activate TLR4 may boost the development of vaccine adjuvants, the understanding of the immune response to bacteria rich in saturated CLs as well as of the chronic inflammation in disease characterized by the presence of saturated CLs.
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Keywords: Toll-like receptor, Cardiolipin, Cationic lipid

JB.07 - Project morphological and quantitative analysis of peripheral blood neutrophils from patients with Chagas disease

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Chagas disease, caused by the protozoan *Trypanosoma cruzi* affects about 7 million individuals worldwide, it has two phases of infection, acute (high parasitemia, symptomatic or asymptomatic) and chronic (indeterminate, cardiac, digestive or cardio-digestive). Neutrophils are one of the immune system cells type that act in innate defense, but also modulate the response to chronic inflammation. Characterized by being polymorphonuclear cells, they can also present distinct morphology, with ring-shaped nucleus, in patients affected by diverse pathologies, but there are few reports of this event in individuals with trypanosomiasis. The Neutrophil/Lymphocyte Ratio (NLR) can indicate inflammation, being used as a clinical biomarker and aiding in the prognosis and determination of the severity of several clinical conditions. However, there are no reports in the literature about NLR and trypanosome infections. Evaluate the morphology of the neutrophil nucleus and the neutrophil/lymphocyte ratio in the peripheral blood of patients with the different clinical forms of Chagas disease. Patients with Chagas disease, who previously accept to participate in the research and signed an informed consent form, will have blood collected through venipuncture. The material will undergo an automatic total leukocyte count. Then, hematological smears, stained with Leishman dye, will pass through total and differential leukocytes count by optical microscopy. Polymorphonuclear cell's nucleus will be counted and evaluated. Resulting data will be correlated to medical records parameters of different clinical forms of trypanosomiasis using the Graphpad Prism software. This study aims to investigate the presence of ring-shaped cells in peripheral blood of patients with Chagas disease, as well as to evaluate and correlate the NLR in these patients with the different chronic forms of the disease and to understand the participation and presence of these cells during the development of this pathology.

Keywords: Neutrophils, Trypanosoma, Neutrophil lymphocyte ratio
Supported by: Capes

JB.08 - *In silico* methods applied to identification of potential biomarkers for the development of diagnostic kits for arboviruses

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The Brazilian population is exposed to infections caused by arboviruses widely distributed on the national territory and associated with humans. Cross-epidemics of different arboviruses are frequently and diagnostic methods for patients suspected by Dengue, Zika and Chikungunya virus are limited in many ways. The development of accessible tools becomes essential, because molecular methods are inaccessible for public health. The epitope mapping is widely used in biotechnological applications and constitutes a fundamental portion of the immune system. Experimental methods for epitope mapping are expensive and time consuming. Advances in epitope mapping by computational prediction have molecular insights into the antigen-antibody complex. The objective was the b-cell epitope prediction of homologous targets NS3 and NSP2, found respectively in the Flaviviridae and Togaviridae families. The identification of differential epitopes in each virus is the first step in developing diagnostic methods based on epitopes. The BepiPred-2.0 is used linear in b-cell epitope prediction. Residues above the 0.5 limit were considered epitopes. The statistical cutoff point is defined based on a training set of the server, which used physicochemical parameters of epitope sequences, elucidated experimentally. The ABCpred server was selected for results validation, because is the first server developed based on a recurrent neural network for b-cell epitope prediction. The statistical cut-off was the standard of 0.5 in length with 16 residues. To filter the results, the location in the secondary structure and also Hydrophobicity (Eisenberg) are observed. The peptides have different characteristics in the NS3 and NSP2 targets for each viral type. The results were satisfactory and compared to other viruses of the family. It is intended to use the results in molecular docking simulations, and make the t-cell epitope prediction. The lead epitopes will be validated experimentally using ELISA.

Keywords: epitope prediction, diagnostic methods, arboviruses
Supported by: PRONAMETRO (INMETRO) and CAPES Biocomputacional

JB.09 - E-NTPDase-1 and Ecto5'NTase activities in lymphocytes and platelets

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The purinergic system is a signalling system, where the purine nucleotides, ATP (Adenosine 5'-triphosphate) and ADP (Adenosine diphosphate), and the nucleoside, adenosine, act as extracellular messengers. CD39 (ecto-nucleoside triphosphate diphosphohydrolase 1, E-NTPDase1) converts ATP or ADP into AMP, and then CD73 (ecto-5'-nucleotidase, Ecto5'NTase) dephosphorylates AMP into adenosine. CD39 and CD73 regulate the function of several immune cell types, including lymphocytes. Furthermore, there is evidence that changes in CD39 expression and activity affects the potential thrombogenic of a tissue. We evaluated comparatively CD39 and CD73 activities in men and women platelet and lymphocytes. It has been realized the collection of peripheral blood mononuclear cells (PBMC) rich in lymphocytes and platelet-rich plasma. ATPase, ADPase and AMPase activities were determined by measuring the concentrations of inorganic phosphate (Pi) with the Malachite Green assay. Platelet from woman showed increased ATPase and ADPase activity compared to enzymatic activity on platelets from man; however, the AMPase activity was equal to women and men. Lymphocyte from both women and men showed the same ADPase activity but the ATPase activity was higher to man. Our results suggest that the activity of CD39 and CD73 is different between men and women.
Keywords: CD39, CD73, purinergic system

JB.10 - Mesenchymal stem cell therapy in Ulcerative colitis, a neuroinflammatory perspective

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Ulcerative colitis (UC), trigger a chronic inflammatory process associated with an increase in the permeability of intestinal mucosa, which may lead to the leaky gut phenomenon. This phenomenon causes entrance of molecules from intestinal lumen in the bloodstream, such as proinflammatory mediators that can promote inflammation in central nervous system. However, little is known about the neuroinflammatory process in ulcerative colitis. Mesenchymal stem cell (MSC) therapy is a promising treatment for UC. Induced colitis animal models treated with MSCs show a reduction in inflammatory cell infiltrates in the intestine, in addition to histological and clinical improvement. MSCs membrane particles (MPs) alone also present anti-inflammatory effects in UC model, without the thromboembolic problems observed in MSCs therapy. We induced UC with Dextran Sodium Sulfate (2%) for 7 days in the drinking water of adult wistar rats. Animals were divided in two groups, receiving tap water or 2% DSS and further divided in 6 groups, treated with Saline MSCs or MPs intravenously at treatment day 5. Animals were euthanized and perfunded for immunohistochemical assays. There was no statistical difference between groups in weight gain. However, we can observe that the only group that presented weight loss at the end of the treatment was DSS alone group. Two proinflammatory cytokines were quantified in the serum. MSCs and MPs reversed the proinflammatory state caused by DSS, returning to the control level in rats' serum. MSCs play a central role in tissue regeneration, coordinating the repair mechanism through the secretion of healing factors and promoting an anti-inflammatory action in damaged areas. Enteric glia is known to play a key role in the intestinal immune response, similar to neural glia. Immunohistochemical analyzes of proinflammatory markers are currently being carried out. We expect that DSS caused neuroinflammation which may be attenuated by MSCs or MPs treatment
Keywords: Ulcerative Colitis, Neuroinflammation, Mesenchymal Stem Cells
Supported by: CNPq

JB.11 - Brillouin and Raman microspectroscopy: a new tool for the chemo-mechanical investigation of human bone and cartilage tissue diseases

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Human bone and cartilage are biological tissues characterized by a sophisticated architecture with a strict relationship between their structure, chemical composition, and mechanical performance. As a consequence, an impairment in even one of the sub-constituents at the micrometre level can lead to loss of function of the entire organ, causing the eruption of severe orthopaedic diseases. Osteoarthritis (OA) is a degenerative disease characterized by the progressive erosion of articular cartilage, which covers the surfaces of the long bones in the joints. It is caused by the establishment of inflammatory processes that affects all the joint constituents and subchondral bone, causing in the patient acute pain. Brillouin and Raman micro-spectroscopy is a correlative technique contact-less and not destructive, that allows the simultaneous investigation of both the mechanical and the chemical properties of samples, thanks to endogenous mechanisms, namely the propagation of thermally activated acoustic waves and the macromolecular vibrations. In recent years, this technique has approached applications in the biomedical field, successfully analysing the properties of single cells, complex biomaterials, and tissues affected by oncological and neurodegenerative diseases. Here, we present the results of mapping and imaging performed on both the cortical and trabecular tissue obtained from resections of a human femoral head and diaphysis. The results of the analysis of tissues in healthy conditions will be used to demonstrate the ability of the technique to recognize the major manifestations of osteoarthritic pathology on the cartilage surface and subchondral bone. This proof-of-concept not only constitutes a first step towards the application of the technique in the diagnosis of osteoarthritis but provides an approach that can be extended to other important issues in biomedical research on bone, such as bone infections and cancer.
Keywords: Brillouin spectroscopy, Raman spectroscopy, bone disease

JB-12. *In vivo* metabolic imaging and micromanipulation of individual filamentous fungus cells using different nonlinear laser scanning microscopy modalities

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Nonlinear laser scanning microscopy (NLSM), is an advanced optical technique that utilizes ultrashort laser pulses for structural and functional imaging, as well as laser manipulation of live organisms and cells. Two modalities of NLSM, two photon excitation fluorescence (TPEF) and third harmonic generation (THG) were applied for in vivo and label-free study of oxidative and lipid metabolism of individual cells of filamentous fungus Phycomyces blakesleeanus. Cell membranes and lipid droplets (LDs) are major sources of THG signal. TPEF allows us to determine the redox ratio (reflecting metabolic activity of cells) of the metabolic cofactors FAD and NAD(P)H autofluorescence. In addition, slight modifications of the experimental setup, mostly on software, enabled utilization of femtosecond laser pulses for precise cell microsurgery of hyphal cell wall. The optimized microsurgery procedure we than utilized to obtain protoplasts suitable for patch-clamp electrophysiological recording. Cell surgery of filamentous fungus Phycomyces blakesleeanus, were performed by ultrafast Ti:Sa laser (160 fs pulses). The same laser was used for in vivo autoTPEF imaging of NAD(P)H and FAD at different wavelengths. For in vivo THG imaging of label-free hyphae, we used 1040 nm, 200 fs pulses from Yb KGW laser. In vivo and label-free application of THG imaging enabled, accurately and reliably, detection of changes in distribution, total number, and size of LDs in control and treatment group of cells. Two-photon microscopy made it possible to obtain a redox ratio using autofluorescences of NAD(P)H and FAD in the same regions of live hyphae. The cell microsurgery procedure has been optimized and developed, which enabled the subsequent registration of currents on otherwise unaccessible membrane.
Keywords: nonlinear imaging, cell surgery, cell surgery. **Supported by:** Project HEMMAGINERO, No. 6066079 / Program PROMIS, Science Fund of the Republic of Serbia

JB.13 - MICROFLUIDIC IMPEDANCE CYTOMETRY: A NEW TOOL TO STUDY ANTIMICROBIALS PEPTIDES AT THE SINGLE- CELL LEVEL

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Antimicrobial peptides (AMPs) represent a promising class of compounds to fight resistant infections. In most cases, they kill bacteria by making their membrane permeable [1, 2]. However, in view of their clinical application, the absence of significant toxicity is almost as important as a good activity. Unfortunately, the interaction of AMPs with bacteria and human cells simultaneously is still not fully understood [3], also due to the need to deal with heterogeneous cell populations in microbiological studies [4]. In addition, standard assays for determining the activity of antimicrobials are based on the analysis of the growth kinetics of a bacterial population and therefore require many hours. With the aim of understanding the activity and selectivity of AMPs, we are currently developing microfluidic impedance cytometry as a new tool to study the effects of AMPs at the single cell level. This technique involves the measurement of the electric field screening of individual cells flowing over patterned electrodes integrated in a microchannel, as accomplished by electric current variation under an applied AC voltage. The measured frequency-dependent impedance depends on cell features, i.e., volume and dielectric properties [5] and can be analyzed with appropriate signal processing for the characterization of cellular electrophysiology [6]. In our experiments, we analyzed both bacteria and human cells, incubated with different concentrations of the DNS-PMAP23 AMP, using a coplanar-electrode microfluidic impedance chip [6]. For these studies, we selected human erythrocytes (obtained from healthy donors) and Bacillus megaterium Bm11 cells, because their relatively large dimensions are compatible with the size of the microfluidic channels. Our preliminary data indicate that microfluidic impedance cytometry can sense peptide-induced pores that are smaller than those detected by traditional microbiological approaches. Overall, electrical impedance spectroscopy is a very promising approach for the development of next- generation fast and sensitive antimicrobial activity assays.

Keywords: biophysics, antimicrobial peptides, microfluidic impedance cytometry

KA - Computational Biophysics and Biochemistry

KA.01 - Conservation and evolution of splice sites

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Introns are sequences that interrupt coding regions of eukaryotic genes. They are removed from the pre-mRNA in a process termed splicing, by the action of the spliceosome. For the correct splicing of introns, the spliceosome must recognize two sites present on the intron's boundaries: the 5' (5'ss) and 3' (3'ss) splice sites. However, those recognitions are not simple tasks for the spliceosome, which must differentiate pseudo sites from the real ones, that are degenerated. Much of what is known about these sites was gathered from analysis of introns in plants, fungi, and animals. However, there are still open questions about their evolution and fundamental features, which require studies of broader phylogenetic branches of eukaryotes. In this work, the 5'ss and 3'ss sites of the introns of 29 species of metazoan, fungal, and protozoan clades were studied via the analysis of information content of biological signals and frequency of consensus motifs. The results show a strong linear negative correlation between the information content of the 5'ss site and the percentage of pre-mRNAs with introns in the genome. In addition, it is observed high conservation among species of both the consensus sequences of the sites and the most frequent motifs found in them, showing that the conservation of recognition mechanisms reaches not simply the global and total pattern of recognition of splice junctions, but also the precise and local patterns of recognition. Finally, it was also possible to identify how the splice sites adapt to structural factors of the introns in which they reside, such as length and GC content. These results, together with the literature, point to a very malleable evolution of splicing sites throughout the evolutionary history of eukaryotes, driven, among other factors, by the number of introns in organisms, the characteristics of these introns, and mutations in the spliceosome.
Keywords: molecular evolution, introns, splice sites

KA.02 - Flavonoids from Siparuna cristata inhibitors against SARS-Cov-2 3Clpro? An *in silico* investigation

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The covid−19 is a clinical condition of acute respiratory syndrome caused by SARS−CoV−2, which has claimed millions of victims worldwide from 2019 to the present day. Considering that there is still no specific treatment for this disease, our work proposes the analysis by computational methodology of Flavonoids from *Siparuna cristata*, substances belonging to Brazilian biodiversity, as inhibitors of the SARS−CoV−2 protease $3CL_{pro}$. We evaluated the interaction of $3CL_{pro}$ with the following substances: quercetin (**1**), retusin (**2**), and kumatakenin (**3**) and controls: lopinavir (**4**), ritonavir (**5**) and chloroquine (**6**). The prediction of pKa values of the receptor protein was performed using the PDB2PQR at pH 7.4; the probable protonation states of the ionizable residues were adjusted using the GROMACS computational module. From the ligand Narlaprevir, crystallized with $3CL_{pro}$ (PDBid: 6XQT), residues at 5 Å were selected, as well as the catalytic dyad His41/Cys145 and Glu166. The grid box was constructed to accommodate the entire highlighted area, with the center dimensions being determined: x=−11, y=1, z=45, and size x=32, y=35, z=33. The Gasteiger charges were added to the protein with AutoDock tools and the docking simulation was performed with the AutoDock Vina program with exhaustiveness equal to 8. The ligands were prepared using OpenBabel. Substance **2** has obtained the best result making hydrogen bonding with His41 equals to 2.60Å whereas Cys145 was 2.68Å and the interaction energy of -6.3 Kcal/mol with the $3CL_{pro}$. **1** presented -6.5 Kcal/mol and 2.77Å/Cys145; **3** 3.01Å/Cys145, 2.19Å/Glu166 and -7.2 Kcal/mol. Moreover **4** demonstrated, 2.92Å/His41 and -5.1 Kcal/mol; **5**: 2.41Å /His41, 2.68Å/Cys145, 2.42Å/Glu166 and -8.1 Kcal/mol; **6**: 2.11Å/Glu166 and -6.4 Kcal/mol. Through the analyzes carried out, we intend to suggest these natural substances as possible inhibitors of $3CL_{pro}$ due previous interaction results, aiming the treatment for covid-19.
Keywords: 3CLpro, flavonoids, *in silico.* **Supported by:** CAPES

KA.03 - Study of molecular mechanisms involved in neurological disorders caused by NS1 protein of Zika virus

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The nonstructural protein 1 (NS1) has applications such as diagnostic biomarker, viral antigen and antibody recognition. Therefore, NS1 is a potential candidate for compose a vaccine against Zika virus. This protein is a highly conserved protein among flaviviruses with function is related to virial replication. The main goal is to understand the function of the NS1 protein, classify the different sequences in circulation in the world and suggest new protein sequences with epitopes optimized for diagnosis and composition of an effective vaccine against the Zika virus. In this study, the Normal Modes Analysis (NMA) was applied to investigate the flexibility of NS1 protein and mutants. NMA is an efficient methodology to predict flexibility and global movements. For the calculations, we used Charmm program. The results showed that the NS1 wild types structures availably in Protein Data Bank (PDBs ID 5GS6, 5K6K and 5IY3) had minor changes in the quaternary structure. However, mutations such as the one at position 146 that changes a charged amino acid negatively (glutamic acid – E) by a positively charged one (lysine – K) can alter regions important in the protein. With NMA, we calculated the movements of NS1 wild type and mutants of proteins from Brazil and other countries. In this study, it is possible to observe structural differences in the three-dimensional structures available in the PDB. These changes may be indicative of the occurrence of microcephaly for the structure detected in Brazil. Regarding mutations, it is possible to notice that the 5K6K structure is more susceptible to mutations than the 5GS6 structure, with exception of the Y122H mutation that changes the flexibility of both structures. **Keywords:** molecular simulations, NS1, ZIKA

KA.04 - Drug design and repurposing with DockThor-VS web server: virtual screening focusing on SARS-CoV-2 therapeutic targets and their non-synonym variants

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The COVID-19 caused by the SARS-CoV-2 virus was declared as a pandemic disease in March 2020 by the World Health Organization (WHO). Docking methodologies have been widely used for both new drug development and drug repurposing to find effective treatments against this disease. In this work, we present the developments implemented in the DockThor-VS web server to provide a virtual screening (VS) platform with curated structures of potential therapeutic targets from SARS-CoV-2 incorporating genetic information regarding relevant non-synonymous variations. The web server facilitates repurposing VS experiments providing curated libraries of currently available drugs on the market. The DockThor docking program was specially developed to deal with highly flexible ligands, being recently validated to dock highly flexible peptides. The program uses a phenotypic crowding-based multiple solution steady-state genetic algorithm as the search method and a scoring function based on the MMFF94S force field to score the docked poses. The affinity prediction and ranking of distinct ligands are performed with the empirical linear model (trained using 2959 protein-ligand complexes), DockTScore scoring function, recently developed by our research group. Currently, DockThor-VS provides ready-for-docking 3D structures for wild type and selected mutations for Nsp3, Nsp5, Nsp12, Nsp15, N protein and Spike. We performed VS experiments of FDA-approved drugs considering the therapeutic targets available at the web server to assess the impact of considering different structures and mutations in the identification of possible new treatments of SARS-CoV-2 infection. The DockThor-VS is freely available at www.dockthor.lncc.br. Guest users are allowed to submit VS experiments with up to 200 compounds, whereas registered users with approved projects can submit up to 5,000 compounds per job. The web server utilises the computational facilities of the Brazilian high-performance platform (SINAPAD, https://www.lncc.br/sinapad/) and the supercomputer SDumont (https://sdumont.lncc.br/).
Keywords: virtual screening, molecular docking, COVID-19
Supported by: CNPq, CAPES, Faperj

KA.05 - Artificial intelligence applied to the structural study of a protein complex related to the nuclear import of the porcine circovirus type 2

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In recent years, Brazil has achieved a prominent position in pork production. Although intensive breeding methods provide high productivity, there are many challenges in porcine farming, including infectious diseases, such as those caused by the viral pathogen Porcine Circovirus Type 2 (PCV2). The nuclear import of this virus occurs by the classic pathway, with the formation of a complex between the protein Importin-α (Impα) from the host and the Nuclear Localization Sequence (NLS) of the viral capsid protein. This study aims to characterize this complex (Impα/NLS) using computational methods based on artificial intelligence and deep learning in order to identify structural patterns related to the interaction between the PCV2 NLS and Impα. To carry out the deep learning calculation, a training dataset was constituted using homologous complexes. These structures were submitted to molecular dynamics (MD) simulations and the calculated structures were used to generate RGB two-dimensional images (200 x 200 pixels) able to codify information as atom pair distances and electric and Lennard-Jones potential. Based on these data, we expect to classify the most likely Impα/NLS complexes and identifying their essential interface contacts. Therefore, our results could contribute to a better comprehension of the PCV2 biology and help the future development of antiviral drugs and/or other products of biotechnological interest. Additionally, the artificial intelligence method proposed in this work is potentially disruptive and could be used to clarify the general molecular mechanisms related to the classical pathway of nuclear transport in eukaryotes.
Keywords: Importin-alpha, Nuclear Import, Deep Learning. **Supported by:** FAPESP

KA.06 - The Effect of Long-Range Electrostatic Treatment on the Sampling of Molecular Simulations of pDMAEMA Polymer Brushes

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Polymer brushes are a class of polymers bonded to a surface at one end of the polymer chain. The pDMAEMA, poly(dimethylaminoethyl methacrylate) brush can be used for the rational design of gene delivery vectors. Classic computational models of pDMAEMA and other polymer brushes were created and validated in our research group (Langmuir. 2019, 35: 5037-5049). Recently, we have found that the use of distinct long-range electrostatic (LRE) treatments can interfere with the conformational behavior of the brush. We have used the GROMOS-derived atomic parameters to perform molecular dynamics simulations of 90-mers long pDMAEMA brushes with 4, 9 and 16 chains in different protonation states. To investigate the pDMAEMA behavior without the high density of the brush, we simulated systems containing a single fully protonated polymer chain with 30 or 192 monomers and a system containing 40 protonated free monomers. The systems were solvated with SPC water and chloride ions were added to neutralize the charged moieties of the polymer. Two LRE treatments were tested: the Particle Mesh Ewald (PME) and the Reaction Field (RF). We found that brushes simulated with PME have a greater thickness and greater radius of gyration (Rg) of the polymer chains than those simulated with RF. This effect is accentuated in fully protonated brushes (~16 nm for PME and ~7 nm for RF). Free chains simulated with the PME have also shown a larger Rg compared to simulations with RF. The system containing only monomers in solution showed differences in the peak intensities of its RDFs for different electrostatic treatments. These differences are accentuated in the brushes. The present results show that the choice of LRE treatment influences the conformational sampling and non-bonded interactions in simulations of pDMAEMA polymer brushes, polymer chains and monomers. This should be carefully considered in atomistic simulations of long brushes.
Keywords: Molecular dynamics simulations, Long-range artefacts, Atomic parameters
Supported by: CNPq, CAPES, FACEPE and FNDE

KA.07 - Fractal dimension applied in the analysis of 17-β-Estradiol chronic effects on the female rat's brain activity

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The 17-β-estradiol acts as a modulator in brain function through its receptors located in various regions of the brain. This steroid hormone is related to behavior and cognition functions and is associated with disorders in the central nervous system. The present work aims to evaluate whether chronic exposure to 17-β-estradiol promotes changes in brain electrical activity. Eighteen female Wistar rats (60-day-old) were distributed into three experimental groups: I - estradiol benzoate (20 µg/kg; 0.1 ml sc; sid); II - peanut oil (0.1 ml sc; sid); III - 0.9% sodium chloride (0.1 ml sc; sid). The respective substances were administered subcutaneously for 90 consecutive days (License CEUA-UFRPE nº 075/2019). The record of electrocorticograms (ECoG) was obtained two weeks later, during free moving. Data were processed and analyzed using the box-counting fractal dimension (FD). In all experimental groups, the FD for ECoG was lower (I= 0.968 ± 0.049; II= 0.986 ± 0.124; III= 0.941 ± 0.053) than those observed for each brain wave. Additionally, the fractality observed in the different brain waves present in ECoG is characterized by higher FD values associated with faster waves, as beta (I= 2.943 ± 0.006; II= 2.942 ± 0.005; III= 2.942 ± 0.008) and alpha (I= 2.695 ± 0.025; II= 2.697 ± 0.024; III= 2.699 ± 0.014), followed by slower theta (I= 2.280 ± 0.030; II= 2.283 ± 0.032; III= 2.280 ± 0.018) and delta (I= 1.472 ± 0.024; II= 1.452 ± 0.049; III= 1.463 ± 0.031) rhythms. There was no significant difference between the experimental groups ($p > 0.05$). The administration of 17-β-estradiol during 90 days in young rats did not alter the animals' electrocorticographic patterns. We can consider the absence of chronic effects on the animals' brain activity is because of its rapid metabolism and negative feedback on the biological system.
Keywords: ECoG, Electrophysiology, Mathematical Methods

KA.08 - *In silico* analysis of natural products from Brazilian biodiversity in COVID-19 treatment: NuBBEDB against SARSCoV2 PLpro

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SARS-CoV-2 emerged as a new human pathogen with rapid spread that may cause COVID-19 (WHO,2021). SARS-CoV-2 PL^{pro} is a potential target for coronavirus inhibitors, being essential on virus replication. Natural Products Database ($NUBBE_{DB}$) was created as the first natural product library from Brazilian biodiversity being a valuable resource for studies. Residues Asn267, Gln269 and Tyr268 are related to protein flexibility, being defined as the main inhibition residues in the search for PL^{pro} inhibitors (Russo et al.2020). This work aims to find a PL^{pro} inhibitor on $NUBBE_{DB}$, using *in silico* strategies. PL^{pro} crystal structure (PDBid:7JRN) was obtained from Protein Data Bank and processed by PDB2PQR server to assess the pKa prediction at pH7.4. GRL0617 redocking and molecular docking were performed with AutoDock Vina (Vina) and GOLD software's. Parameters were defined based on GRL0617 inhibitor center, found on crystal structure. Grid centers x=13, y=−9, z=30, sizes x=30, y=30, z=30; were used in all cases; exhaustiveness=100, number-of-modes=20 were applied in Vina. Vina redocking presented bind energy of −9.6 kcal/mol and RMSD 0.45356Å between the best pose and the crystal original bind. GOLD redocking are presented through the score for the GOLD/CHEMPLP and fitness for the GOLD/Gold score. Redocking with GOLD/CHEMPLP scored 90.41, RMSD 0.537Å, and GOLD/Goldscore, fitness 59.35, RMSD 0.651Å, both compared to GRL0617. Molecular docking was performed against $NUBBE_{DB}$ and a penalty score, based on molecular docking molecule's classification, was developed aiming to compare the molecular docking outputs for each score function. The better the molecule ranks in the docking result; the less penalty will be suffered. Molecules with ≤0.3 penalty score will be selected to proceed in the study. The results will be reclassified based on the distances in Å from Tyr268, described as the main inhibitor residue. Further analyses, such as interaction profile, ADMET and molecular dynamics will be performed.
Keywords: PLpro, SARS-CoV-2, Natural Products; **Supported by:** CAPES

KA.09 - Modeling the Reactivity of Iron-Sulfur Proteins

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Iron-sulfur (FeS) clusters are essential metal cofactors, comprising the largest class of metalloenzymes. They are involved in a wide variety of catalytic functions such as natural photosynthesis and cellular respiration. From structural and electronic points of view, FeS clusters sit between transition metal atoms and solids. Their electronic structures show many low-lying and near-degenerate states that may cross, leading to multiple-state reactivity. In polynuclear FeS clusters, strong electron correlation and long-range spin coupling effects complicate enormously the theoretical description. Here we describe the reactivity of FeS clusters including environmental effects with quantum mechanical/molecular mechanical (QM/MM) potentials [1] to model Fe-S bare dissociation and substitution reactions in aqueous solution and in protein environments. We show that sextet and quartet spin states cross during bare dissociation in a protein desolvated microenvironment with an homolytic Fe-S bond cleavage mechanism[3]. For water substitution at neutral and acid media, however, no spin-crossings are observed and bond cleavage is heterolytic due to stabilization of the sextet ground state by solvation effects and leaving-group protonation[2,4]. These results help to understand the catalytic mechanisms, stability and biogenesis of iron-sulfur proteins. [1] Ferric-thiolate bond dissociation studied with electronic structure calculations. Arantes GM and Field MJ. J. Phys. Chem. A, 119, 10084-10090, 2015; [2] Modelling the hydrolysis of iron-sulfur clusters. Teixeira MH, Curtolo F, Camilo SG, Field MJ, Zheng P, Li H and Arantes GM. J. Chem. Inf. Model., 60, 653-660, 2020; [3] Homolytic cleavage of Fe-S bonds in rubredoxin under mechanical stress. Arantes GM, Bhattacharjee A, Field MJ. Angew. Chem. Int. Ed., 52, 8144-8146, 2013; [4] Force-induced chemical reactions on the metal centre in a single metalloprotein molecule. Zheng P, Arantes GM, Field MJ e Li H. Nat. Commun., 6:7569, 2015;
Keywords: iron-sulfur clusters, computer simulation, bioinorganic chemistry
Supported by: FAPESP and CNPq

KA.10 - Stability of the Delta variant of SARS-CoV-2 and prediction of mutations that maximize antibody-antigen interaction

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The COVID-19 pandemic is of unprecedented impact since the 1918 Spanish flu. The most recent strain of concern of SARS-CoV-2 is Delta (B.1.617.2), responsible for an increase in infections in India and reported with a large increase in viral transmissibility. We studied the stability of the Delta variant mutations. Finally, was performed a computational screening of mutations that maximize the antibody-antigen interaction. We initially performed the stability prediction of the L452R and T478K mutations using the "Residue Scanning" module in the Schrödinger Maestro 2021-2 software. Then, we use the "Affinity Maturation" functionality, whereby the Monte Carlo optimization method we find the mutations that maximize the stability of ACE2-RBD (PDB ID: 6M0J) and antibody-antigen (PDB ID: 7BWJ) binding. Throughout the interpretation of the results, it was considered that the negative sign denotes an increase in stabilization and affinity. We found that the Delta variant L452R mutation achieved stability at -8.161 kcal/mol and affinity of -0.182 kcal/mol for ACE2-RBD. Although the T478K mutation showed a destabilization at +19.490 kcal/mol, it induced an increase in affinity at -5.046 kcal/mol. In this way, we can see an evolution in search of greater structural stability. These values are relatively far from what the virus could actually achieve in terms of stability, where we obtained the maximized value at -96.252 kcal/mol. This, therefore, indicates that SARS-CoV-2 has been seeking marginal stability as a form of adaptation. Regarding the maximization of the antibody-antigen interaction, we found that in terms of stability T333H, A363M, V510I with -33.543 kcal/mol. In terms of affinity, we obtained T333H, V445Q, H519R with -10.501 kcal/mol. Finally, we are currently running molecular dynamics simulations to confirm these conclusions. Therefore, we hope with these results to maximize the effectiveness of the next generation of vaccines so that they are protected against emerging variants.
Keywords: Delta variant, Monte Carlo, SARS-CoV-2. **Supported by:** CNPq

KA.11 - *In silico* identification of a novel open reading frame in the pseudogene MAP_RS22950 from Mycobacterium avium paratuberculosis

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Mycobacterium avium paratuberculosis (MAP) is the causative agent of Johne's disease in ruminants, a chronic granulomatous inflammatory bowel disease, and strongly associated with Crohn's disease in humans. Similar to the closely related *Mycobacterium tuberculosis (Mtb)*, MAP encodes a functional protein tyrosine phosphatase A, PtpA, but no protein tyrosine phosphatase B, PtpB sequence is annotated as a pseudogene. PtpA and PtpB from *Mtb* are two secreted virulence factors essential for its pathogenicity, and hence they become interesting targets for anti-tuberculosis drug development. Based on novel open reading frames (nORFs) discovery, we aimed to further investigate the sequence of the pseudogene MAP_RS22950 for the presence of possible ORFs. Using the ORF finder (NCBI), we identified 11 potential ORFs for the pseudogene. To search for encoding hypothetical proteins each translated ORF was analyzed by BLASTp, we found 2 ORFs encoding proteins with similarities (< 80%) to tyrosine phosphatases. Multiple sequence alignments using Clustal Omega showed that ORF4 (193 amino acids) has the catalytic site of PTPs, the P-loop, but does not have the FPD-loop. Neighbor-joining phylogenetic tree generated with MEGA X showed that the closest related proteins of ORF4 were PTPs from *Mycobacterium avium*, *Mycobacterium lepraemurium* and *Mycobacterium timonense*. The sequence alignment (Clustal Omega) of ORF4 against a PTP from *Mycobacterium intracellulare* revealed that the FPD-loop would be replaced by the amino acid residues Ala8, Pro9 and Thr10. The theoretical 3D model of ORF4 predicted by SWISS-MODEL was related to the Mtb PtpB (PDB: 2oz5). The temple covered 85% of ORF4 sequence (range 30 to 193) and showed GMQE and QMEAN score of 0.77 and −0.03, respectively. Further experimental validation of the existence and functional roles of these ORFs need to be performed to validate the *in silico* data, and thus contribute to a better understanding of MAP pseudogene.
Keywords: Mycobacterium, Paratuberculosis, ptpB. **Supported by:** UFPI

KA.12 - Experimental and computational studies of essential oils insecticidal action on Calliphoridae flies and identification of their possible molecular targets

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Flies in livestock are a huge problem and identification of targets and new compounds to control are crucial in the development of insecticides. This work aims to identify, through bioinformatic and bioassays, new plant compounds that could be used as fly killers. Essential oils were obtained with Clevenger apparatus/gas chromatography and from literature performing 170 database structures/ligands. A laboratory bioassay on blowfly using essential oil of *Baccharis dracunculifolia* shows, until now, that its essential oil has an LC50 of 3,33 µL/cm2. It is observed a pupae size reduction and adult abnormalities. We have performed *in silico* experiments to identify the compound and target which affects fly metabolism. Seven fly metabolism targets were modeled. Molecular docking and two score functions have been used to rank compounds with high affinity to receptors. A chemical-physical computational database of high-affinity compounds was made and the euclidean distance of stereochemical restrictions for target and distance of plant essential oils studied in our bio essays was calculated. Comparing receptors, Octopamine and Agonist Ultraspiracle are most typical presenting the unique stereochemical character as a potential target for selective insecticide. The lowest euclidean distance from other targets was obtained with the Ecdysone receptor suggesting that compounds acting on it could act on the other targets too; it is known that terpenoids act on a myriad of targets. Considering plant species and euclidean distance, *B. dracunculifolia*'s oil is predicted to interact with different targets, in agreement with insecticide and anti-metamorphosis effects documented. Computational tools could explain bioassay data by the high affinity of oil compounds by Acetylcholinesterase and Antagonist Octopamine affecting the metamorphic cycle binding to Juvenile Hormone and Ecdysone receptors. In general, *B. dracunculifolia*'s oil is the most promising source for new insecticides, corroborating with bioassays and the literature signaling anti-metamorphic and neurotoxic effects.
Keywords: Computational biology, essential oils, blowfly. **Supported by:** EPAMIG/UFSJ

KA.13 - Unveiling Mutation Effects on the Structural Dynamics of the Main Protease from SARS-CoV-2 with Hybrid Simulation Methods

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The 2019 coronavirus disease (COVID-19) pandemic, caused by the human severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) strain, is the most health crisis in the past 100 years. Macromolecules adopt several favored conformations in solution depending on their structure and shape, determining their dynamics and function. Integrated methods combining the lowest-frequency movements obtained by Normal Mode Analysis, faster movements from Molecular Dynamics are necessary to establish the correlation between complex structural dynamics of macromolecules and their function. The aim of this study was to characterize the structural dynamics and global motions of Mpro Protease of SARS-CoV-2 wild-type (WT) and of its 48 mutants, including several mutations that appear in five importants variants of concern. PyMOL software was used to generate 48 mutants with single point mutations reported by Amamuddy and colleagues (2020). These mutants were minimized and calculated the first collective modes with an all-atom approach. Twenty seven mutants with a significant difference of RMSF compared to WT were selected and energetically relaxed conformations were generated along the lowest frequency normal modes with the Hybrid method VMOD. After, these structures were analyzed considering several aspects: flexibility, global movements, dyad catalytic and the accessible area for the catalytic site. We used several tools: analysis of variance (ANOVA) test, scripts developed in Python and R-studio, PDBePISA (Proteins, Interfaces, Structures and Assemblies) and TKSA-MC. The results suggested that a single mutation cause a significant change in the flexibility and collective motions of Mpro, reflecting in importants structural characteristics: energy, electrostatic contribution for free energy, energetically accessible conformations, SASA of important regions as the interface of the dimer and the distance of dyad catalytic. Four mutants (K90R, N151D, P108S and P99L) showed significant energy and SASA reduction and could be more stable. Hence, the mutants present low conservation of these motions compared with WT. **Keywords:** Variants of concern, Normal Modes, Molecular Dynamics. **Supported by:** CNPq, CAPES, Fapesp and UFABC

KA.14 - Computational Study of Synthetic Aromatic Aldehydes as Potential Inhibitors of GABA-AT

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Synthetic aromatic aldehydes are consumed worldwide. They are structurally similar to pyridoxal 5'-phosphate (PLP), a B6 vitamer and essential cofactor of neurological disorders-related enzymes. We investigated 17 aromatic aldehydes as potential cofactors of gamma-aminobutyric acid aminotransferase (GABA-AT), a PLP-enzyme, using a computational model. We aimed to obtain and validate a computational model of GABA-AT from *Saccharomyces cerevisiae*. The model was subsequently used in docking procedures to evaluate covalent and non-covalent interactions between the aldehydes and the model. A tridimensional structure for P17649 GABAT_YEAST was obtained by homology modeling and compared to the template P80147 GABAT_PIG (PDB 1ohv). Target and template sequences were aligned by Clustal Omega web server. Modeller 9.24 was used to obtain 100 model structures for P17649 GABAT_YEAST. All models were submitted to the composite scoring function QMEAN and Ramachandran plots were analyzed with RAMPAGE to estimate quality. A web-based computational prediction of protonation states at pH value 8.3 in the best model and in the template was performed by H^{++} server. Covalent docking (CD) by GOLD plataform was performed with aldehydes and PLP, using the best model and the template. M95 was selected as best model according to its QMEAN value (-2,64) and Ramachandran plot statistics revealed 93.9% of residues in the preferred region. CD was simultaneously performed by two investigators, to compare and validate the classifications. The PLP score values for both M95 and the template varied between 110.6 and 113.3. All the aldehydes presented score values between 99.0 and 118.4, significantly equivalent to PLP results. Methoxybenzaldehyde derivatives displayed values in the 115.6-118.4 range, the higher scores in comparison to PLP. The results stimulate subsequent *in vitro* assays aiming yeast lifespan modulation, paving the way for aldehyde-based development of human GABA-AT inhibitors as drug candidates for neurological disorders.
Keywords: covalent docking, neurological disorders, synthetic aromatic aldehydes. **Supported by:** FAPEMIG, CNPq and CAPES

KA.15 - Niferidil drug for the treatment of short QT syndrome: experiment *in silico*

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Computational tools based on mathematical models have collaborated in the discovery and repositioning of antiarrhythmic drugs, helping in decision-making and speeding up the obtaining of results. In this context, we developed the computational tool PharmaLab, which simulates the pharmacological action in the mouse ventricular myocyte. The new proposed model was based in Mullins & Bondarenko (2013) and pharmacological dynamical based on Hill's equation. The short QT syndrome (SQTS) was simulated as an application of the computational tool. According to Campuzano et al. (2018), this disease alters the cardiac Action Potential (AP) and generates a faster repolarization. The conductance of the fast potassium current (G_{Kr} = 0.078mS/µF) was multiplied by a factor of 1000 to simulate this condition. To correct the SQTS, it was simulated the application of the drug Niferidil, which acts on the main potassium currents (I_{Ktof}, I_{Kur} and I_{Kss}). The concentrations applied of Niferidil were 25µM, and 50µM. The experiment *in silico* showed that SQTS mainly changes the time of 90% of repolarization of AP (APD90) reducing the value from 10.04ms (control) to 8.29ms (test). It was found that the drug Niferidil corrects the APD90 for the concentrations applied, 9.92ms and 11.04ms, respectively. However, it was observated an increase in the time of 50% of repolarization of AP (APD50), this increase being 8.28% and 16.56% compared to control for the concentrations of 25µM and 50µM, respectively. Although new tests are needed to confirm the effects of this drug on AP. The results obtained with the simulations are in accordance with the data reported by Abramochkin et al. (2015).

Keywords: *in silico* experiment, drug, cardiovascular disease. **Supported by:** CAPES

KA.16 - Gene family characterization of superoxide dismutase responsible for tolerance to oxidative stress in *Jatropha curcas* L.

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Jatropha curcas L. (physic nut) is an easily propagated, rapid growth oilseed adapted to semi-arid regions that are low in nutrients. The plant has defense mechanisms which provides resistance to abiotic stress caused by temperature, salinity, drought, heavy metals, among others. The tolerance level is correlated with the expression of antioxidant enzymes such as superoxide dismutase (SOD), which catalyzes the decomposition of O_2^- to O_2 and H_2O_2. The aim of this study was to characterize the superoxide dismutase gene family in *J. curcas* (JcSOD) and to verify phylogenetic relationship with other species. Sequences of JcSOD and four other species (*Ricinus communis, Glycine max, Helianthus annuus and Gossypium hirsutum*) were retrieved from the NCBI database, followed by confirmation of the presence of JcSOD domains by Simple Modular Architecture Research Tool (SMART). Alignment was performed with the MUltiple Sequence Comparison by Log-Expectation (MUSCLE) and the verification of the phylogenetic relationships by the Software Molecular Evolutionary Genetics Analysis (MEGAX). The subcellular locations were predicted by the protein subCELlular LOcalization prediction (CELLO) and the conserved motifs in the sequences by the Multiple Em for Motif Elicitation (MEME). The JcSOD genes were characterized based on the phylogenetic analysis, subcellular locations and distribution of conserved protein motifs in relation to the SOD genes of the other analyzed species. The five species contain conserved domains, several SOD isoforms that differ in their metallic ion at the active site, however all species had Cu/ZnSOD and FeSOD in common. It is concluded that SOD is an important enzyme in plant stress tolerance, and the results provide basis for further functional research on the SOD gene family, which is the first line of defense and one of the most efficient antioxidant enzymes against reactive oxygen species in *J. curcas*.

Keywords: Antioxidant enzymes, Phylogeny, Physic nut
Supported by: CNPq, FAPESB, UFBA

KA.17 - Computational Modeling of ZIKV MTase NS5-SAH Complex

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The Zika virus (ZIKV) has emerged as an international public health concern due to its serious symptoms, notably the evidence that has accumulated to conclude that infection during pregnancy is a major cause of microcephaly and other severe fetal brain defects. Parallel to the development of an efficacious vaccine, research on chemotherapy focusing on specific proteins are excellent alternatives for the inhibition of viral replication. The NS5 protein of ZIKV is one of the most important and conserved Flaviviridae enzyme, which contains two domains: a N-terminal methyltransferase (MTase) and a C-terminal RNA polymerase. The function of the MTase domain depends on an S-Adenosyl methionine (SAM) cofactor that acts as a methyl group donor, and is thus converted to S-Adenosyl Homocysteine (SAH). The MTase domain methylates the viral RNA and prevents it from being recognized by the host's immune system. Therefore, NS5 MTase can be considered a promising target for drug design. To investigate the NS5 MTase domain in complex with SAH (PDB ID: 5NJU) through Molecular Dynamics (MD) simulations to design an inhibitor for this enzyme. MD simulations were performed using AMBER18 package in three different conditions: (I) protein without SAH solvated with 70% water and 30% ethanol. This mixed solvation set-up was carried out using the Packmol software, (II) protein without SAH in simple aqueous system and (III) protein-SAH complex in water. The mixed solvent induced a quick structural stabilization of NS5 MTase even without SAH binding. Conversely, the apo protein remains more flexible in water solvent. The map of mixed solvent binding microsites on the protein surface showed important regions to explore with pharmacophoric groups for the design of an inhibitor. We suggested a conformational change around the active site on the unliganded NS5 in water, highlighting that a cavity is open and can be explored as a target for allosteric inhibitors.

Keywords: Molecular Dynamics, NS5, ZikV
Supported by: FAPESP, CNPq and CAPES

KA.18 - Molecular docking experiments from different isoforms of the human importin alpha with nuclear localization signals

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The Nuclear Import Classical Pathway, which import proteins that contain the classical nuclear localization sequences (NLSs) are transported into the nucleus by the importin-α/β heterodimer. Importin-α (Impα) contains the NLS recognition site and, importin-β (Impβ) mediates transport through the nuclear membrane pore. Several proteins related to DNA repair are transported to the cell nucleus by the classical NLS-import pathway and among them, there is a heterodimer formed by the proteins MLH1 and PMS2. These proteins belong to the mismatch repair pathway (MMR) which is responsible for repairing error in DNA replication. Previous studies from the heterodimer interacting with Mus musculus Impα (MmImpα) have shown that this complex binds with high affinity. To better understand the importance of the NLS residues, four mutated MLH1 NLSs were chosen to perform many structural assays, including the molecular docking. The aim is to compare if the human Impα (HsImpα1 and 3) interacts to the MLH1 mutated NLSs in the same way as the Mus musculus Impα. Molecular docking using the MLH1 and mutated MHL1 peptides and crystal structures of the HsImpα1 and HsImpα3 was performed by the Autodock Vina tool, present in the software PyRx. The best complexes obtained were chosen by two criterias, the antiparallel conformation of NLS related to the Impα models and Gibbs free energy expressed in kcal/mol. The HsImpα1 interacts with the MLH1 peptides in a very similar way as the MmImpα, as expected, since they share a very high sequence identity. In contrast, the HsImpα3 only bind to these peptides in the major site, due to natural mutations of some key residues in the minor site. We were able through comparisons to reveal the specificity of the Impα interactions in the Classical Nuclear Import Pathway, and the HsImpα1 and HsImpα3 bind in a different way to the NLSs.

Keywords: Importin, Molecular Docking, Nuclear Localization Signal
Supported by: FAPESP, CNPq, CAPES

KA.19 - Computational Study of Synthetic Aromatic Aldehydes as Potential Inhibitors of GABA-AT

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Synthetic aromatic aldehydes are consumed worldwide. They are structurally similar to pyridoxal 5'-phosphate (PLP), a B6 vitamer and essential cofactor of neurological disorders-related enzymes. We investigated 17 aromatic aldehydes as potential cofactors of gamma-aminobutyric acid aminotransferase (GABA-AT), a PLP-enzyme, using a computational model. We aimed to obtain and validate a computational model of GABA- AT from *Saccharomyces cerevisiae*. The model was subsequently used in docking procedures to evaluate covalent and non-covalent interactions between the aldehydes and the model. A tridimensional structure for P17649 GABAT_YEAST was obtained by homology modeling and compared to the template P80147 GABAT_PIG (PDB 1ohv). Target and template sequences were aligned by Clustal Omega web server. Modeller 9.24 was used to obtain 100 model structures for P17649 GABAT_YEAST. All models were submitted to the composite scoring function QMEAN and Ramachandran plots were analyzed with RAMPAGE to estimate quality. A web-based computational prediction of protonation states at pH value 8.3 in the best model and in the template was performed by H^{++} server. Covalent docking (CD) by GOLD plataform was performed with aldehydes and PLP, using the best model and the template. M95 was selected as best model according to its QMEAN value (-2,64) and Ramachandran plot statistics revealed 93.9% of residues in the preferred region. CD was simultaneously performed by two investigators, to compare and validate the classifications. The PLP score values for both M95 and the template varied between 110.6 and 113.3. All the aldehydes presented score values between 99.0 and 118.4, significantly equivalent to PLP results. Methoxybenzaldehyde derivatives displayed values in the 115.6-118.4 range, the higher scores in comparison to PLP. The results stimulate subsequent *in vitro* assays aiming yeast lifespan modulation, paving the way for aldehyde-based development of human GABA-AT inhibitors as drug candidates for neurological disorders.
Keywords: Covalent docking, Neurological disorders, Synthetic aromatic aldehydes
Supported by: FAPEMIG, CNPq and CAPES

KA.20 - Development of Coarse-grained model for protein complex

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Molecular simulations are useful to investigate dynamics and statics of biomolecules. Coarse-grained (CG) models can reduce computational cost of large-scale and long-time simulation for biomolecules. CG models for single protein have been developed to understand protein folding. CG models for protein complex should be developed to understand protein complex formation, which takes much computational cost than protein folding. In this work, we develop a CG model for protein complex system and apply our CG model to formation of protein complex. In our CG model, intra-molecular interaction was represented by the Go-like model, in which each amino acid residue was described by a single CG particle. Inter-molecular interaction was described by effective interaction between two CG particles, which was calculated as free energy profile as a function of the distance between two amino acid side chain analogs in an explicit water solvent by using all-atom molecular dynamics simulations with the Thermodynamic Integration method. Potential function and parameters were determined from the free energy calculation and applied to CG simulations for protein complex system. We performed the Langevin dynamics simulations for formation of protein complex systems, such as GCN4-pLI tetramer, cyclin D3 and CDK4 complex, and cytochrome f and plastocyanin complex systems. We showed that protein structure complex obtained from our CG simulation was consistent with the structure obtained from experiments. Complex formation pathway was investigated for GCN4-pLI tetramer. Unfolded intermediate of CDK4 was suggested during the functional cycle. Estimated rate constant of electron transfer from cytochrome f and plastocyanin was consistent with an experimental result. We conclude that our CG model is useful to understand protein complex formation.
Keywords: molecular simulation, coarse-grained model, Langevin dynamics

KA.21 - Halogen bonding in membrane-ligand recognition: Insights from computational biophysics

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Halogen bonds (XBs) are noncovalent interactions where halogenated species interact with electronegative acceptors through a region of positive electrostatic potential, named σ–hole [1]. These interactions are known to mediate molecular recognition phenomena in biology, namely protein-ligand complexes or nucleic acids, and hence have found increasing application in drug discovery [2]. In this context, the ability to establish molecular interactions such as hydrogen bonds is a known factor determining the permeability of druglike molecules across biological membranes and may ultimately modulate their pharmacological properties. In contrast, the role of other interactions such as XBs has been largely overlooked, despite halogenated compounds being prevalent in drug discovery and development. Therefore, the eventual role of halogen bonding targeting phospholipid oxygen acceptors merits further investigation. In this work, we used molecular dynamics simulations, explicitly accounting for the σ–hole in halogenated ligands [3], to probe the existence of halogen-phospholipid interactions in a bilayer environment. The results [4] provide direct evidence for the formation of favorable XB interactions, as well as insights into the role of halogen bonding in the partitioning of halogenated molecules across biomembranes. [1] P. J. Costa, Phys. Sci. Rev. 2017, 2, 20170136. [2] P. J. Costa, R. Nunes, D. Vila-Viçosa, Expert Opin. Drug Discov. 2019, 14, 805. [3] R. Nunes, D. Vila-Viçosa, M. Machuqueiro, P. J. Costa, J. Chem. Theory Comput. 2018, 5383. [4] R. S. Nunes, D. Vila-Viçosa, P. J. Costa, J. Am. Chem. Soc. 2021, 143, 4253. Acknowledgements: Fundação para a Ciência e a Tecnologia (FCT), Portugal is acknowledged for funding through doctoral grant SFRH/BD/116614/2016 and projects UIDB/04046/2020, UIDP/04046/2020. This work was also supported by FCT, Programa Operacional Regional de Lisboa (Lisboa 2020), Portugal 2020, FEDER/FN, and the European Union under projects LISBOA-01-0145-FEDER-028455, PTDC/QUI-QFI/28455/2017.
Keywords: halogen bonding, membrane-ligand interactions, molecular dynamics simulations

KA.22 - Parkinson's Disease – *In silico* Analysis and Molecular Dynamics of Mutations of a-Syn Protein

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Parkinson's disease (PD) is among the most prevalent neurodegenerative diseases that still have no cure. PD is characterized by typical motor symptoms that include resting tremor, postural instability, bradykinesia. 5-10% of the cases of PD have a familial origin, with mutations in the SNCA gene, which encodes the a-Syn. In this work, we aim to evaluate the effects on the a-Syn protein caused by mutations associated with PD development. Effects of mutations on protein function were predicted using the algorithms SIFT, PolyPhen-2, PhD-SNP, PANTHER, PMUT, PROVEAN, and MutPred. The structural evolutionary conservation analysis of a-Syn was performed with the ConSurf algorithm. The *in silico* mutagenesis was performed using VMD 1.9.3 mutator plugin. The molecular dynamics (MD) simulations of the WT a-Syn and its variants A30P, A53T, and G51D were performed in triplicates using the GROMACS 2018.6 package with an AMBER99SB-ILDN force field and a TIP3P water triclinic box. The molecular systems were neutralized by adding Na+ and Cl− ions and minimized for 5000 steps. After system minimization, NVT and NPT ensembles were performed at 1 atm and 300K for 100 ps. The A30P mutation was predicted to be deleterious by all algorithms. Consurf results revealed that mutations A30P, E46K, H50Q, G51D, A53E, A53T occur in a highly-conserved sites. MD analysis pointed to flexibility decrease at the N-terminal region of the analyzed variants and flexibility increase in their C-terminal regions compared to the WT. The secondary structure analysis suggested alterations in the G51D variant, particularly by increasing the number of β-sheets formed. Mutation G51D is known to increase a-Syn's propensity to form b-pleated sheets, which increases its tendency to form toxic protein aggregates. This work suggests that mutation of a-Syn affects the structure and function of the protein, which could be related to the development of PD.
Keywords: Alpha-synuclein, *In silico*, Parkinson's Disease.
Supported by: CAPES, DAAD, FINEP, NVIDIA, CNPq, FAPERJ and UNIRIO

KA.23 - Computational studies on inhibition of vivapains of *Plasmodium vivax*

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Malaria is one of the most worldwide spreaded diseases, killing 400 thousand people among the 200 million infected per year. Two parasite species cause together 95% of all its cases, which are Plasmodium falciparum and P. vivax. Increasing resistance to treatment against P. vivax infection difficults its combat around the world. New biological targets and pharmacological molecules must be explored to overcome that. In P. vivax, the proteins responsible for breaking hemoglobin, providing amino acids for the replication of the parasite inside lysosomes are the vivapains (VPs). Here, we study the anti-malarial targets enzymes VPs, specially the vivapain-4, aiming to explore its catalytic and allosteric sites for guiding virtual screening assays to identify potential new inhibitors. Since their structures have not yet been solved experimentally, comparative modelling of their sequences has been made with Modeller v9.21 based on the structure of their homologous enzyme, falcipain-3 from P. falciparum, found through the BLASTp server. To map protein pockets and important residues, different systems were run in replicates for 1 μs (water or mixture of water/ethanol solvents), which were analysed in Amber18. Such mapping was also accessed by the FTMAP server (for docking of fragments on the surface of the enzyme). LASSBio drug repository was the source for the virtual screening process, using AutoDock Vina, and further evaluation with RFScore and MM/GBSA. Mapping of pockets through conformational clusters, as well as from the MD with mixed solvents allowed us to identify important residues for ligand binding interactions in both catalytic and allosteric sites. The Tyr159 and Trp208 are examples, that combined with VS evaluation from MM/GBSA, led us to identify the most interesting ligands, further analysed by MD simulations. The results among the different strategies allowed the identification of potential lead molecules for binding sites of VP4 and perspectives for optimisation.

Keywords: malaria, drug design, molecular dynamics simulations
Supported by: CAPES

KA.24 - Unravelling how phosphorylation patterns affect the collective motions and the transition states of hDat.

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The human dopamine transporter (hDAT) is a member of the subfamily of monoamine transporters which show association with different biological functions such as memory, state of well-being and motor activity that result from the regulation of Dopamine in the sympathetic cleft,this function is highly regulated by the phosphorylations that occur in it. This study aims to evaluate the effects and molecular mechanism involved with hDAT and dopamine transport (absorption and efflux) under various phosphorylation conditions described in the experimentally reported by several papers. We show how the phosphorylation regulates the m collective motions and the transitions of hDat states as: open , closed and intermediate (outward-facing open, inward-facing open, holo - occluded). In the first moment, we built and validated a hDat model using homology and threading modeling for different parts of the protein. In the first moment, we investigate four different phosphorylation conditions using Normal Modes and Hybrid methods (a CHARMM facility VMOD and MDeNM-Molecular Dynamics with Normal Modes Excited) to verify the collective motions and conformational space for them and define which motions lead to the states. Finally, we used the Markov Chain model to calculate the transition between the states and the free energy barrier. We observed that some motions promote preferentially one of the three states. When we do not phosphorylate the SER12, the Open states (outward-facing open and IF inward-facing open) are promoted. On the other hand, with SER12 phosphorylated and SER333 not, the intermediate (holo - occluded) states previled. A single phosphorylation alters the collective motions and, consequently, the states populated by the proteins and the free energy barrier between them. In this way, we correlate how different phosphorylations affect the speed of Dopamine uptake and efflux in the nerve cell from the standpoint of collective motions.

Keywords: Nomral Modes, Free Energy, Human Dopamine Transport

KA.25 - Allosteric inhibition study of p38 MAPK enzyme using computational biology techniques

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Recently, more and more research has demonstrated the important roles of p38 MAPK in the development of chronic inflammation and cancer. Allosteric inhibitors, such as BIRB796, have been developed, leading to conformational changes that avoid the ATP binding, preventing the catalytic activity of p38 MAPK. The present work aims to study the allosteric inhibition of p38α MAPK through molecular dynamics (MD), analysis of correlated movements, and solvent mapping techniques. Structures, dynamics and energies of protein-ligand interaction were characterized by simulations of MD of the p38α MAPK in complex with 5 compounds candidates as allosteric inhibitors, using the compound BIRB796 as reference. Discontinuities were investigated at the interface between the protein and the most promising ligand in mixed solvent (water 70% / ethanol 30%) and molecular probing with small ligands. The characterization of new allosteric cavities in the protein was also carried out through MD simulations in mixed solvent (water 70% / ethanol 30%), analysis of correlated movements and molecular probing with small ligands. The compound LASSBio-1494 showed the best results of free energy of binding between the compounds of LASSBio. Through simulations in ethanol/water and analysis by molecular probes was possible to map solvent molecules stabilized in microsites around the LASSBio-1494 ligand, which will be useful for the proposal of chemical groups to be aggregated in the ligand improving the free energy of interaction with the protein. Through analysis of cavities on the protein surface, potential allosteric sites were identified, with the most promising being characterized, which demonstrated residues with a high prevalence of hydrogen bonds with water and/or ethanol in the simulations of MD and with fragments in the analysis by molecular probes. LASSBio-1494 obtained the most promising results and It was possible to identify potential allosteric sites. The data from this study will be used to propose new p38α inhibitors.
Keywords: drug design, Molecular Dynamics, p38 MAPK inhibition. **Supported by:** CAPES

KA.26 - Exploring the conformational landscape of the 33-mer peptide: a computational approach

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The 33-mer peptide is a digestion product of the gluten α2-gliadin protein. This proline- and glutamine-rich peptide is a putative trigger of the immune response in coeliac disease patients after gluten ingestion. In addition, in the small intestine, 33-mer is deamidated in three glutamines, which allows its recognition by the immune system, initiating the inflammation process. There is experimental evidence that both peptides (the WT and the deamidated peptide) form aggregates in physiologically relevant conditions, and it has been hypothesized that their oligomerization might be involved in the development of the disease. However, an accurate and complete structural description of the peptide and the initial oligomers has remained elusive. This work aims to explore computationally the conformational space of both the 33-mer peptide and its deamidated form as a first step to understand their aggregation properties and their influence on gluten-related diseases. The methods and force fields to assess Intrinsically Disordered Peptides' (IDPs) structures have evolved fast-paced in the last years. We performed classical molecular dynamics (MD) simulations with GROMACS2020 using the atomistic AMBER03WS force field (1.5 μs each peptide) and the coarse-grained SIRAH force field (30 μs per peptide), which have been successfully employed to study other IDPs. The starting structures for both simulations were elongated peptides obtained from an experimentally based modelling scheme. From atomistic unbiased MD simulations, we obtained mainly a wide range of elongated structures. However, through the coarse-grained approach, we were able to assess possible folded and unfolded states. In both representations, the WT peptide visited more compact states than the deamidated one. The representative structures of both types of simulations showed good agreement with the experimental data available. Both the 33-mer peptide and its deamidated form presented several stable structural arrangements, which should be considered to assess the complete aggregation process.
Keywords: Coeliac Disease, IDPs, Molecular Dynamics
Supported by: CONICET, CIC, UNS, de.NBI for computational resources.

KA.27 - The Hitchhiker's Guide to the Periplasm: Unexpected Molecular Interactions of Antibiotics in *E. coli*
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Gram-negative bacterial cell envelopes are host to a variety of biomolecules, being delimited by two lipid membranes, namely the inner membrane (IM) and the outer membrane (OM), along with the cell wall, a peptidoglycan layer that divides the periplasmic space. In recent years, complex models have emerged with the development of atomistic models for the OM and the cell wall components, which is allowing us to access novel conformational dynamics that were previously unreachable. In this regard, studies involving an accurate depiction of the bacterial envelope, as well as describing antibiotic mechanisms of action at the molecular level, can provide invaluable insights for experimental efforts to tackle the current antibiotic resistance crisis. Here, we present our work on large scale atomistic-level molecular dynamics simulations of the *E. coli* cell envelope, with a particular focus on the development of complex cell envelope models and the path taken by polymyxin B1 (PMB1) through various models of the periplasm, aiming to understand how it negotiates the periplasmic space to arrive at the inner membrane. Systems sizes oscillate between 600,000 to 700,000 atoms, due to the presence of OM and cell wall, plus 6 to 9 proteins, water, ions, and antibiotic molecules. Our simulations reveal that PMB1 forms transient interactions with proteins that are free in solution as well as lipoproteins anchored to the outer membrane. Furthermore, the lipid moiety of PMB1 enter the hydrophobic cavities of some lipoprotein carriers. Data obtained also shows that PMB1 binds strongly to the cell wall, diffusing on its surface and altering local morphology, while also allowing us to evaluate possible cell wall crossing mechanisms. Together the results show that PMB1 has a winding, obstacle-ridden path to the inner membrane. It is likely that this observation is true for other antibiotics that rely on diffusion rather than transporters.
Keywords: molecular dynamics, cell envelope, antibiotics

KA.28 - *In silico* Analysis of the A4V and D90A Variants of Human SOD1 related to Amyotrophic Lateral Sclerosis
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Amyotrophic lateral sclerosis (ALS) is the most frequent motor neurodegenerative disorder in adults. Missense mutations in superoxide dismutase 1 (SOD1), a major cytoplasmic antioxidant enzyme, are associated with the development of ALS. The A4V and D90A variants account for approximately half of all ALS-SOD1 cases in the United States and Europe. This work aims to characterize *in silico* the structural and functional effects of A4V and D90A variants on human SOD1 protein. Three-dimensional structures of A4V and D90A protein variants were computationally modeled in the VMD-1.9.1 package using the experimentally determined structure of wild-type SOD1 (PDB ID: 2C9V) as the template. Molecular dynamics (MD) simulations of the wild-type SOD1 protein and its variants A4V and D90A were performed in triplicates using the GROMACS-2018.8 package and AMBER99SB-ILDN force-field. TIP3P water molecules were added to a dodecahedral box system, which was neutralized by the addition of Na+ Cl- ions and then minimized. The system also had its temperature and pressure equilibrated at 1atm and 300K before the start of the simulations, which lasted 300ns. The MD trajectories were concatenated, and the following parameters were analyzed using GROMACS distribution programs: root-mean-square deviation, root-mean-square fluctuation, B-factor, radius of gyration, solvent accessible surface area, secondary structure, and essential dynamics. The MD analyses pointed to changes in flexibility and essential dynamics in the regions corresponding to the electrostatic and metal-binding loops of the variants, which could impact substrate guidance towards the active site and their enzymatic activity. Our findings pointed to structural alterations in A4V and D90A variants that could have functional implications for SOD1 and explain their association with the development of ALS.
Keywords: Amyotrophic Lateral Sclerosis, Superoxide Dismutase 1, *In silico*
Supported by: FAPERJ, CAPES, DAAD, FINEP, NVIDIA, CNPq and UNIRIO

KA.29 - Evaluation of chemical elements distribution and their inter-elemental correlations in tumor progression

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Cancer is considered one of the most complex and fatal diseases worldwide. New approaches to study tumor progression and growth are relevant subjects of research. In this context, the particular role of chemical elements in cancer progression is a subject still not fully explored that presents opportunities for investigation. The main goal of our study is to assess the distribution of chemical elements in cancer progression as well as to discover correlations between elements, observing both the primary tumor and the distant tissues that the tumor cells may affect. For simulating tumor progression in vivo, murine Lewis lung carcinoma cells were injected in C57BL/6 mice and data indicating the presence, concentration, and location of different elements in distinct tissues, in both control and experimental groups, were obtained in a time frame of 5 weeks of tumor progression. The data were collected via Synchrotron Radiation X-Ray Fluorescence in the Brazilian Synchrotron Light Laboratory (LNLS). In order to extract relevant information inherent to the voluminous available data, we adopt statistical analysis. With this work, it was possible to observe the elements' relevance for biological processes of normal, as well as tumor cells, during its tumor progression. Thus, it was possible to notice indications of tumor influence on distant tissues as well as highlight the importance of elements and their correlations for the tissues, including for processes of tumor progression, such as growth and cellular migration, angiogenesis, among others. This work also confirmed information found in the literature and featured results apparently not yet observed. Moreover, elements and correlations of relevance for more investigation, regarding their role in the processes described, were highlighted to bring to light explanations for such observations not yet noted.

Keywords: elemental distribution, tumor progression, X-Ray fluorescence

KA.30 - POLYana: a new software for rheological study of polymeric colloidal materials

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POLYAna is a new executable software developed by SISLIBIO group for rheological analysis of hydrogel and organogel systems and other colloidal materials (nanoparticles and micelles). The software development aims to facilitate the analysis of rheology data associated to both temperature- and frequency-dependent analysis, viscosity and curve flow profiles. The software development aims to facilitate the analysis of rheology data associated to both temperature- and frequency-dependent analysis, viscosity and curve flow profiles. From raw data, several models are applied like power-law model for frequency response and curve flow, Boltzmann law to calculate gelation temperature and viscosity response under temperature, Maxwell model to study interchain relationships in addition to other models such as Bingham model, Cross model, and Herschel-Bulkley are also available. POLYana outputs calculates rheological parameters like consistency, adhesion, hysteresis, flow index, G'/G'' ratio. To validate results obtained from POLYana, same data were analyzed by applying other programs and same mathematical models. In this sense, rheological analysis of Poloxamer 407 in water solution (15 %) were performed: from temperature-dependent G' and G'' analysis were obtained gelation temperature of 45.46 ± 0.02 °C, η_0 = 0.08 ± 0.03 mPa*s, η_max = (32.44 ± 0.17) mPa*s and dη/dT = (1.27 ± 0.02) mPa*s/°C by fitting Boltzmann law (R2 = 0.998), which are similar to results obtained by others softwares and found in literature. From temperature-dependent G' and G'' analysis, it gets adhesion value of (1647.15 ± 18.01) mPa*sn calculated from power-law model (R2 = 0.869), also similar to PRISM results. Also, other Poloxamer concentrations and hydrogels types have been evaluated, showing close numbers to that previously reported. In order to stablish structural relationships, one of POLYana tools is also to analyze small-angle neutron scattering (SANS) and develop Monte Carlo simulation for SANS and rheological analysis, simultaneously.

Keywords: Colloidal materials , Rheology, Software

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KA.31 - Strategies of Ranking methods for Virtual Screening in SARS-CoV-2 PLpro

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In 2019 strange cases of unidentified pneumonia led to the discovery of a new species of coronavirus, the SARS-CoV-2 responsible for COVID-19 pandemic. One of its most important proteins is the PLpro for its ubiquitin and deISGylating capabilities. Considering the costs of developing new drugs, repositioning already approved drugs is an excellent strategy in fighting COVID-19. We used the drugs approved by the Brazilian Health Regulatory Agency ANVISA using *in silico* techniques to try to predict interaction between these compounds and the PLpro. The importance of this work is based on the huge amount of money and time needed to develop a novel drug, while repositioning is cheaper and faster. Perform Virtual Screening and use different strategies for ranking the results to determine possible candidates to inhibit SARS-CoV-2 PLpro. Virtual Screening was performed by AutoDock Vina using parameters defined in redock with the exception of generation of 20 poses. After VS the candidates whose energy was lower than -8.0 kcal/mol were selected. For ADMETox prediction, SwissADME, admeSAR and pkCSM were used with PAINS and AMES being considered as limiting factors. The distance between the molecules and Y268 from PLpro was measured using PyMOL and molecular weight was used like a filter. The compounds were ranked based on these criteria. From the original 273 compounds, 44 had the correct energy range. From those, 36 passed the AMES and PAINS test who were ranked and organized in accordance with their usage. Vitamins, unpromising or problematic drugs were excluded. Using ADMETox prediction and information about distance, pocket volume and literature we organize possible drugs to be tested *in vitro* against PLpro of SARS-CoV-2 and HEK-293 lineage cells.

Keywords: SARS-CoV-2, PLpro, Virtual Screening

Supported by: CNPq and CAPES

KA.32 - Immunogenic characterization of the antigen group (gag) gene of strains of the human immunodeficiency virus type 1 (HIV-1) circulating in Brazil,

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A large genetic variability of HIV represents a major obstacle to the control of infection by the host immune system and to the development of effective drugs and vaccines. CTLs recognizing epitopes within the HIV antigen (gag) group gene have been associated with the initial control of infection and these epitopes, uses in current approaches to developing an HIV vaccine. This study aims to investigate a variability of the non-gag HLA class I ligand binding epitopes of circulating HIV-1 strains in Brazil, available in a publicly accessible database. For this purpose, an allelic variability and predominance of HLA class I in the Brazilian population was determined in order to identify the epitopes of the gag gene of circulating HIV-1 strains in Brazil. One was evaluated by target cell (CTL / CD8) and one screened by an assessed target cell (CTL /CD8), the already defined gene (gag) and the most prevalent HLA alleles in the Brazilian population. The Allele Frequency Net Database (AFND) was used, as gag regions were removed from through the electronic address https://www.hiv.lanl.gov, with the defined genes (gag) and the alleles most prevalent in the Brazilian population. The Immune Epitope Database (IEDB) was used to determine which are the best Brazilian HLA-linked epitopes. From this research a table with a description of the non-Brazilian circulating epitopes was generated for each HLA class I allele to the HIV-1 subtype and position of the epitope in relation to the gag gene. Thus, it is concluded that the variability of HLA class I ligand epitopes in the gag gene found, generated a sum of importance data to characterize and describe the non-circulating epitopes in Brazil and to show the studies involved with these epitopes not the world, for future future vaccine referrals in Brazilian territory.

Keywords: HIV, Epitopes, Brazil

NB - Biociências Nucleares

NB.01 - Synthesis of a iodine-131-labeled derivative of laminin-111, [131I]I-YIKVAV, and its assessment as a potential radiopharmaceutical for breast cancer diagnosis

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Peptide sequences derived from laminin-111 regulate gene expression in breast tumor cells, including the bioactive peptide IKVAV. Here, we evaluated the potential of the YIKVAV modified fragment radiolabeled with iodine-131 by *in vitro* interaction with human breast cancer cells and *ex vivo* biodistribution in mice. The YIKVAV modified fragment (25 µg) was radiolabeled with [^{131} I]NaI (11.1–14.8 MBq) using the chloramine T method (reaction time=90 s). The radiochemical yield was evaluated by ascending chromatography using TLC-SG strips and acetonitrile/water (95:5), as eluent. The radiopeptide, [^{131}I]I-YIGSR, was incubated with 2x10 ^{6} MDA-MB-231 and MCF-7 cells at 37°C under agitation (500 rpm). *In vitro* binding and internalization were assessed at 1, 4, and 24 h post-incubation. To evaluate the biodistribution profile, the [^{131}I]I-YIKVAV was intravenously injected into normal nude female Balb/c mice. *Ex vivo* biodistribution was performed at 0.5 and 2 h after injection. The data revealed radiochemical yield >90% (n=10). The *In vitro* data showed high affinity of the radiopeptide to both human breast cancer cells. The binding percentages were 5.65±0.68, 7.15±0.64, and 7.34±1.17, and the internalization percentages were 68.22±3.70, 73.37±3.73, and 52.00±6.10, at 1, 4, and 24 h, respectively, for MDA-MB-231 cells (n=5). The assay with MCF-7 cells showed binding percentages of 15.21±1.54, 18.10±1.63, and 13.09±2.23, and internalization percentages of 78.42±2.95, 79.23±3.62, and 59.71±5.57, at 1, 4, and 24 h, respectively (n=5). The biodistribution data showed rapid blood clearance and low accumulation of the radiopeptide in the evaluated organs (%ID^{131}I]I-YIKVAV (n=3). Further biodistribution in breast tumor-bearing mice will be performed in order to elucidate in vivo tumor uptake. Therefore, [^{131} I]I-YIKVAV showed high affinity to breast cancer cells and the biodistribution profile revealed that the radiopeptide do not accumulate in any organ compatible with breast cancer primary tumor or its metastasis.

Keywords: [131I]I-YIKVAV, Breast cancer, Derivative of laminin-111
Supported by: FAPESP, CAPES, and FAP-FCMSCSP

NB.02 - Effective methodology for maintaining Toxoplasma gondii *in vitro* using paramagnetic iron nanoparticles to support three-dimensional cell culture

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Toxoplasma gondii is a protozoan parasite that infects approximately one billion people worldwide. Upon infection, the host may die due to latent infection or presence with chronic cysts in brain, retina or muscle tissue. Humans can become infected consuming water or foods contaminated with oocysts or eating undercooked meat. Its virulent form is difficult to replicate *in vitro*, requiring additional steps using experimental animals. The use of nanotechnology can contribute to this *in vitro* production, through the three-dimensional cultivation of mouse fibroblast cells (NIH/3T3 ATCC® CRL-1658™) and nanoparticles synthesized with radiation. The objective of this work was to demonstrate the three-dimensional culture of fibroblast cells aggregated to nanoparticles for inoculation the *T. gondii*. This methodology was created to facilitate parasite management and replication. For the production of nanoparticles, the work used concentrations of iron sulfate II heptahydrate ($Fe_2SO^4.7H_2O$, CAS 7782-63-0) and glycine (NH_2CH_2COOH, CAS 56-40-6) diluted in ultrapure water free of O_2 at pH 12. This solution was irradiated by electron beam of the IPEN / CNEN-SP Radiation Technology Center in doses of at least 15 and at most 30kGy. Paramagnetic iron oxide nanoparticles (PION's) were then adsorbed on cell membranes, and cells were kept together by a magnetic field. Structured spheroids (4 day of culture) were infected with 106 parasites (RH strain) and the infection was evaluated by transmission electron microscopy. Tachyzoites were found inside 3T3 cells, assuring that the spheroid can be a suitable culture substrate to *T. gondii in vitro* propagation. A three-dimensional methodology for *in vitro* cultivation of the parasite is perhaps the key for applications in the study of toxoplasmosis, as it has a fast, cheap, efficient production (yield and reduction of contamination).
Keywords: Toxoplasma gondii, Three-dimensional cell culture, Nanoparticles
Supported by: FAPESP (2017/50332-0) & IPEN/CNEN-SP

NB.03 - Beneficial effects of fructo-oligosaccharides (FOS) and arginine on the intestinal mucositis, induced by 5- fluorouracil (5-FU)

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Mucositis is one of the most common complications in patients undergoing chemotherapy or radiotherapy. The use of compounds with action on the immune system and intestinal microbiota may be a beneficial alternative for the prevention and/or treatment of mucositis. So, the aim of this study was to evaluate the effects of FOS and arginine on intestinal damage in experimental mucositis. Balb/c mice were randomized into 4 groups: CTL (without mucositis + saline), MUC (mucositis + saline), FOS (mucositis + supplementation with FOS – 1st until 10th day) and ARG (mucositis + supplementation with arginine – 1st until 10th day). On the 7th day, mucositis was induced with an intraperitoneal injection of 300 mg/kg 5-FU. After 72 h, weight variation, intestine length, intestinal permeability (IP), morphometry and histopathology analysis were evaluated by ANOVA two way test. Significance level was set at $p < 0.05$. The MUC group showed lost weight, reduced intestine length and increased IP ($p < 0.05$). Results showed presence of tissue damage, inflammatory cells and ulcerations in the ileum of animals of MUC group. FOS and arginine supplementation reduced lost weight, intestinal permeability and maintained the intestine length at physiologic levels ($p < 0.05$). However, arginine was more effective in reducing tissue damage and maintaining villus height in the ileum compared with FOS group. In conclusion, the present results show that FOS and arginine restored intestinal barrier, decreased lost weight and the inflammation induced by mucositis. These immunomodulators could be important adjuvants in the prevention and treatment of mucositis.
Keywords: arginine, fructo-oligosaccharides, mucositis
Supported by: CNPq, FAPEMIG and CAPES

NB.04 - Tucumã extract: phytochemical characterization, acute and subacute oral toxicity studies in Wistar rats

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Tucumã (Arecaceae) is a native palm tree from Brazil, Its fruits are widely consumed by the local population as food and traditionally used to treat the respiratory system, infections, infestations and digestive system disorders. Even though they are natural, its misuse can lead to nocive effects on people. In this scenario, toxicity studies are essential to ensure the safety use of these substances. Therefore, the aim of this study was to perform the phytochemical characterization of tucumã fruits extract (TFE) and to evaluate its toxicity through acute and repeated-doses toxicity studies in female and male rats. TFE was analyzed by HPLC and GC/MS. Acute toxicity consisted in the administration of TFE at a single dose of 2000 mg/kg in female rats. The repeated-doses study was performed in male and female rats, which received TFE for 28 days at doses of 200, 400 and 600 mg/kg. After euthanasia, blood was collected by cardiac puncture for the hematological and biochemical analyses. Liver and kidney were removed to analyze the histopathology and oxidative damage markers/enzymatic activity. The phytochemical analyses evidenced the presence of carotenoids, flavonoids, unsaturated and saturated fatty acids, and triterpenes. The single dose administration of TFE did not induce mortality nor any sign of toxicity. Thus, TFE was classified as safe in acute toxicity. Regarding the repeated-doses study, the 28 days treatment with TFE at the highest dose showed renal toxicity evidenced by histopathological analysis in male rats, all the other parameters were not altered. In female rats, no signs of toxicity were observed. Therefore, the results suggest that TFE did not induce toxicity after exposure to a single or repeated doses in female rats. However, in males it may be considered safe when given repeatedly in low doses. **Keywords:** Carotenoids, Arecaceae, Safety. **Supported by:** CAPES [#001]

NB.06 - Acute myeloid leukemia as late response of hematopoietic tissue to ionizing radiation

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Leukemia is a malignant disease originating in the bone marrow. An important concern in this regard is with the doses of ionizing radiation that we receive throughout life, since exposure can occur through diagnosis or treatment of diseases, or even in an occupational way. IR is capable of producing ionization through the ejection of electrons from the orbit of atoms, caused by the radiolysis of water, reaching the DNA molecules, either directly or by releasing free radicals. It is also known that radiation induces carcinogenic processes by damage to DNA, and that, due to its high proliferation activity, Bone marrow is one of the most sensitive tissues to radiation, being Acute Myeloid Leukemia, known to be a radiogenic subtype. Our aim was to systematically review the literature on the subject in order to update concepts. In the present work, a review of articles was developed in order to reread one of the most important phenomena in radiobiology, which are the biological effects of ionizing radiation on hematopoietic tissue and its molecular aspects. It is also known that there is a positive correlation between the radiation dose and the development of leukemia. However, this correlation is multifactorial, depending on many variables, such as the type of diet, lifestyle, body homeostasis, metabolism, ethnicity (genetic characteristic), age of the organism and also characteristics of the radiation itself. Finally, it is also possible to observe the mechanisms of radioinduced damage directly in blood cells. Radiation also reaches cells indirectly, through radiolysis of water molecules present in metabolic reactions, which enables the formation of free radicals. All this phenomenon can cause the breakage or mutations in the DNA molecule, with the potential to initiate a cancerous process.

Keywords: ionizing radiation; bone marrow ; leukemia.

NB.07 - Molecular docking study of copaiba oil interacting with the spike protein of SARS-CoV-2
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COVID-19 triggered by SARS-CoV-2 has caused hundreds of thousands of deaths worldwide. Organic and inorganic compounds have been tested as potential inhibitors of this lethal virus. For these tests, several techniques are used to design molecules of biological interest for drug composition, in which molecular coupling plays an important role. In the present work, the compounds kaurenoic, copalic and beta-caryophyllene acids that form the copaiba oil were studied as anti-inflammatory and as SARS-CoV-2 inhibitor. The universal force field (UFF) was selected to perform the calculations. After obtaining the best conformation of the geometries, the structures were submitted to a new optimization at the DFT level using the DMOL3 Code, where the generalized gradient approximation (GGA) considers all the electrons of the molecules. Molecular docking showed alkyl, pi-alkyl, conventional H-bond, unfavorable bump, and Van der Waals interactions. The calculated electrostatic potential maps showed the nucleophilic and electrophilic regions. The negative binding energies obtained for the three acids suggest the stability of the complexes. The minimum energy states for -caryophyllene are lower than the other compounds analyzed, and it can be predicted that this is the most stable. From the results obtained, it can be inferred that the acids that form the copaiba oil can be used as an inhibitor of COVID-19.
Keywords: Copaiba oil, COVID-19, DFT, Molecular docking
Supported by: FAPESPA, CNPq and CAPES

NB.08 - Influence of image reconstruction protocol on PET image with 11C
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The small animals positron emission tomography (PET) scanner from Molecular Imaging Laboratory (LIM/CDTN) is dedicated to pre-clinical studies on new ^{18}F and ^{11}C-based radiopharmaceuticals and novel applications for well-known radiopharmaceuticals. According NEMA NU 4-2008 publication, several tests must be performed to ensure the performance of small animal PET scanners. The aim of this work was to evaluate the influence of image reconstruction protocols on the image quality, accuracy of attenuation and scatter corrections parameters for ^{11}C PET images. A PET image of the Image Quality Phantom filled with ^{11}C-PK-11195 was acquired and reconstructed using forty-nine different protocols. The reconstruction variables evaluated were the algorithms (FBP, MLEM-3D, OSEM-2D), the resolution mode (high/standard) and the number of iterations (10 to 150). Uniformity, spill-over ratio (SOR) and recovery coefficients (CR) tests were adapted from NEMA NU 4-2008 and performed for each reconstructed image. PMOD® software was used for image analysis. FBP based protocol generated noisier images compared to iterative algorithms (MLEM-3D or OSEM-2D) based protocols. The increase in the number of iterations resulted in higher standard deviation of the analyzed parameters for all reconstructed images. MLEM-3D and OSEM-2D based protocols generated similar results when number of iterations and resolution mode were identical. SOR and RC mean values remained practically stable when the number of iterations ranged from 40 to 150. This study allowed the evaluation of different image reconstruction protocols on important parameters of ^{11}C PET image quality. Additionally, a standard image reconstruction protocol (MLEM-3D algorithm, 40 iterations, standard resolution mode) for ^{11}C images reconstruction was defined to be adopted in LIM/CDTN laboratorial routine.
Keywords: PET, Image quality control, Carbon-11

NB.09 - Application of 99mTc-DTPA for monitoring the intestinal barrier in the pharmacological repositioning of atorvastatin in the treatment of enteric mucositis

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Pharmacological repositioning seeks to find new uses for existing drugs. Nuclear medicine is a dynamic and versatile specialty, allowing a functional and molecular assessment of organic systems. Mucositis is a side effect of chemotherapy characterized by intense inflammation in the gastrointestinal tract, making treatment a challenge. Statins that are inhibitors of cholesterol biosynthesis and that have anti-inflammatory effects that can be used to control enteric mucositis. The assessment of intestinal permeability using 99mTc-DTPA is a precise and simple method that allows the identification of changes in the gastrointestinal barrier caused by mucositis. The objective of this work is to evaluate the potential of atorvastatin in protecting the gastrointestinal barrier in a model of enteric mucositis. Male BALB/c mice weighing 20-25g (n=6) received intraperitoneal injections of 5-FU (30 mg/kg/day) for 5 days, and were treated with atorvastatin (10 mg/kg) given via oral gavage for 7 days and the mucositis group received 0.9% NaCl. Control group mice received intraperitoneal injection and oral gavage of 0.9% NaCl. On the 7th day of the protocol, the animals were anesthetized and euthanized, the ileum was collected for the analysis of MPO, EPO and histology, and the blood collected for the quantification of 99mTc-DTPA. Animals with mucositis showed a significant increase in intestinal permeability, MPO and EPO ($p<0.05$) and the treatment with atorvastatin promoted a reduction in intestinal permeability, also associated with an improvement in small intestine mucosal architecture, suppression of enzyme levels EPO and MPO. Our results indicate that atorvastatin was able to prevent adverse mucositis-induced damage to the intestinal mucosa, proving to be a therapeutic strategy to aid in the treatment of intestinal mucositis.

Keywords: Gastrointestinal mucositis, Atorvastatin, Pharmacological repositioning. **Supported by:** CAPES

NB.10 - Radiomodifying effect of black grape juice on hematologic alterations induced by whole brain irradiation in rats

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Whole brain irradiation (WBI) causes a variety of secondary side-effects including anorexia, immunosuppression and osteoradionecrosis. Some natural compounds produced by plants are able to reduce the secondary side effect of irradiation because they react with free radicals (FRs) and reactive oxygen species (ROSs). In the present study we evaluated the radiomodifying effect of black grape juice (BGJ) in animal model of fractionated whole brain irradiation. Forty male rats (200–250 g) were exposed to eight sessions of cranial X-ray irradiation. The total dose absorbed was 32 Gy delivered over 2 weeks. Four groups were defined: (a) NG: non-irradiated, glucose and fructose solution-supplemented (GFS); (b) NJ: non-irradiated, BGJ-supplemented; (c) RG: irradiated, GFS-supplemented; and (d) RJ: irradiated, BGJ-supplemented. Rats received daily BGJ or GFS dosing by gavage starting 4 days before, continuing during, and ending 4 days after WBI. Two months after the last dose of WBI, the rats were killed by exsanguination under deep anesthesia induced by i.p. injecting 200 mg/kg body weight pentobarbital. The carotid was cannulated and blood samples were collected in tubes containing EDTA. The hematological parameters analyzed were: white blood cell count (WBC), red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), platelet count (PLT), plateletcrit (PCT), mean platelet volume (MPV), and platelet distribution width (PDW). Data were analyzed using ANOVA. BGJ increased WBC by 118% when compared to that in the irradiated RG. The red blood cell count (RBC) also decreased after irradiation. BGJ increased RBC by 8.3% when compared to that in the RG. HCT increased in BGJ group. The BGJ was able to reduce the hematological syndrome in rats expose to WBI. The BGJ is rich in phenolic compounds which' reduce oxidative stress induce irradiation. **Keywords:** black grape juice, whole brain irradiation, hematological parameters
Supported by: CAPES, Uni. León, UFSM

NB.11 - Comparative analysis of image quality parameters in three PET systems in Brazil

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Positron emission tomography (PET) is widely used in preclinical studies, generating images applied to biochemical, metabolic and functional study of organs and tissues of small animals. A comprehensive evaluation of the PET scanner intrinsic parameters is important to optimize the acquired images, providing more reliable qualitative and quantitative analyses. To evaluate Image Quality (IQ) parameters of Triumph® LabPET-4 systems installed in three different Brazilian preclinical molecular imaging centers. Experiments were carried out at the centers: C1 - Molecular Imaging Laboratory (LIM) of the CDTN/CNEN; C2-Laboratory of Nuclear Medicine (LIM-43) of HCFMUSP; C3-Preclinical Research Center of the Brain Institute (InsCer) at PUC-RS. IQ phantom PET images were acquired as recommended in NEMA NU 4-2008 standard (^{18}F-FDG, 3.7 MBq, 20 minutes). Image reconstructions were performed in the system where they were acquired using the same reconstruction protocol (MLEM-3D algorithm, 20 iterations and no high-resolution mode). Data was processed using PMOD® software. The IQ parameters: (i) uniformity, (ii) spill-over ratio (SOR) and (iii) recovery coefficients (RC) were evaluated and compared. For Uniformity test, the percentage standard deviations of mean activity concentration were 7.8%; 7.3% and 6.4% for Centers 1, 2 and 3 respectively. Cold chambers RSO values in the systems 1, 2 and 3 were respectively 0.16, 0.19 and 0.21 for water; 0.26; 0.28 and 0.30 for air. The RC's for rod diameters from 1 to 5 mm ranged from 0.08 to 0.91 for the three centers. Results revealed that the three PET systems have appropriate quality parameters for pre-clinical studies, presenting values compatible with international standards for this type of image. This study was able to reveals the performance of preclinical PET system of different Brazilian imaging centers and may support the standardization of a National Quality Control Program. **Keywords:** image quality, PET, NEMA

NB.12 - Photobiomodulation modulates gene expression of pro-inflammatory markers in an experimental model of acute arthritis.

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Rheumatoid arthritis is an autoimmune, inflammatory disease that causes pain and joint destruction, and its treatment is performed with anti-inflammatory drugs and resources such as photobiomodulation (PBM) with low-level laser. The aim of this study was to evaluate the effects of photobiomodulation on iNOS and C3 gene expression in an experimental model of acute arthritis. This study was aprproved by CEUA-FHO 077/2017. Eighteen female Wistar rats (200±10g), were kept in light-dark cycles of 12 hours with food and water ad libitum. Animals (n=18) were assigned into three groups (n=6), Control (no induction), Sham (induced arthritis) and PBM (arthritis and low-level laser). Arthritis induction was performed with 200μg of Zymosan injected in the right knee of the animals and twenty-four hours after induction, photobiomodulation was performed with low-level laser by single dose therapy with the following parameters, λ=808nm, 25mW nominal power, fluency of 20J/cm2, beam area of 0.02mm2, time of 33s and total energy of 0.825J. Animals were euthanized after 7 days of arthritis induction by anesthetic overdose and cardiac exsanguination. Synovia samples were submitted to RNA extraction, cDNA synthesis and gene expression was evaluated by real-time PCR (2-ΔΔCt) for β-actin (constitutive), iNOS and C3 genes. The gene expression, median; min;max 2-ΔΔCt values, of iNOS was higher with PBM therapy, Control 0.8749 (0.8154; 0.9362), Sham 0.8836 (0.8311; 0.9363), PBM 1.095 (0.9476; 1.184) with significant differences between PBMxSham p=0.0022. No statistical differences were observed with PBM in C3 gene expression, Control 0.8770 (0.8220; 0.8960), Sham 0.8815 (0.8720; 0.9630), PBM 0.9329 (0.9184; 1.060). Photobiomodulation up regulated iNOS gene expression, which refers to secondary mitochondrial stimulation that can promote an increase of antioxidant enzyme balance, while C3 levels remained constant and it is suggested that there is no activation of the complement system in the period studied.
Keywords: Arthritis rheumatoid, Low-level laser therapy, Gene expression
Supported by: Propesq-FHO

NB.13 - A simple and quick method to generate *in vitro* tridimensional tumor bodies from a human breast adenocarcinoma (MCF7) using magnetic aggregation technique

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Tumor physiology studies have to rely on efficient and representative models, as animal-based or *in vitro* tridimensional cell constructs. The work used magnetite (Fe3O4) nanoparticles produced by electron-beam induced chemical reduction to give cells the ability to form aggregates when submitted to a magnetic field, and thus to produce micro tumors *in vitro*. The work aimed to produce human breast adenocarcinoma mini tumors (BAMT's) *in vitro*. Paramagnetic iron oxide nanoparticles (PION's) were synthesized through electron-beam induced Fe3+ reduction and subsequent coprecipitation. Due to its poly-L-lysine coating, PION's were adsorbed on cell membranes of MCF7 (human breast adenocarcinoma). Cells were seeded in 24-well cell culture plates pre-treated overnight with Pluronic® F-127 to prevent cell adhesion and kept in culture conditions under magnetic fields for at least 6 days. BAMT's were differentially stained with Hoescht 33342 and ethidium bromide and imaged by wide-field fluorescence microscopy. BAMT's appeared as integer and well-defined cellular aggregates, with sparse dead cells stained by ethidium bromide. These structures can be further used for *in vitro* tumor studies, as BAMT's are supposed to be more reliable models than monolayer cultures. Treatment of wells with poloxamer caused a mild to moderated cell-repellent effect, similar to those found in commercially available products, only by a fraction of the cost. The experiments succesfully produced mini tumors prone to be used in *in vitro* studies.

Keywords: breast cancer, 3d culture, magnetic
Supported by: FAPESP (2017/50332-0) & IPEN/CNEN-SP

NB.14 - Development of a Female Mouse Computational Model Based on CT Images for Dosimetric Assays

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Small animals, such as mice, are used in biodistribution studies and innumerous preclinical investigations involving ionizing radiation. Longitudinal preclinical studies with five or more image procedures (MicroCT and/or PET/SPECT) are not uncommon. However, the cumulated absorbed doses in mice organs and their influence in experimental results is often neglected. Accurate calculation of absorbed doses in mice organs are needed to evaluate potential radiobiological effects that may interfere with in vivo experiments. Based on a previous study of a male mouse computational model known as DM_BRA, this paper is focused on the development of FM_BRA, a female mouse computational model. Develop and implement for the MCNP code a female computational mouse model for mice radiopharmaceutical dosimetry. A set of Micro-CT images of a female mouse kindly available at (https://www.youtube.com/watch?v=-Xg921NVFSs) was selected for the segmentation process. Forty-seven coronal slices were manually segmented using AdobePhotoshop®. In these images each color corresponds to a numerical code that identifies each organ. After the segmentation process, the images were converted into a ".raw" 3D file format. An in house C++ program was used to convert the 3D image into the computational model in the MCNP format. The new FM_BRA was segmented with 20 tissues/organs. The model matrix has (156 x 366 x 105) voxels and the voxels dimensions are (0.25 x 0.25 x 0.25) mm3. Elemental composition and density of human organs were used in MCNP setup of the model. The total mass of the model is 26.3 g. The masses of segmented organs were compatible with the values found in the literature. A new female mice model was successfully developed and implemented for MCNP. A set of S-values for dosimetry of positron emitting radioisotopes will be available soon.

Keywords: female mouse model, mice dosimetry, Monte Carlo
Supported by: CNPq

NB.15 - Radiochemical and biological properties of peptides designed to interact with EGF receptor: Relevance for glioblastoma

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Radiolabeled peptides with high specificity for receptors expressed on tumor cells hold great promise as diagnostic and therapeutic biomarkers. The main objective of this study was to evaluate the radiochemical and biological properties of two [131I]I-peptides, as well as their interaction with the epidermal growth factor receptor (EGFR), which is overexpressed in a wide variety of tumors, including glioblastoma. The peptide EEEEYFELV and its analogue DEDEYFELV, both designed to interact with EGFR, were chemically synthesized, purified and radiolabeled with iodine-131 ([131I]-NaI). Radioiodination was evaluated and optimized using the chloramine-T methodology. Stability, serum protein binding and partition coefficient were evaluated for both radioconjugates. In addition, the binding and uptake fraction of radiopeptides synthesized with rat glioblastoma cells (C6) and with rat brain homogenates from a glioblastoma-induced model were evaluated and ex vivo biodistribution studies were performed. Under optimized radiolabeling conditions, the peptides showed an average radiochemical yield of 90-95%. Stability studies showed that both peptides remained stable for up to 24 h in reaction medium, saline solution, and human serum. [131I]I-peptides have hydrophilic characteristics and have a binding percentage to serum proteins around 50%, which is highly compatible with clinical applications; showed binding and internalization capacity both in tumor cells (C6) and in rat brain tissues after tumor induction. Biodistribution studies corroborated cell culture studies and confirmed the different binding characteristics derived from a simple two-amino acid change (Glu→Asp1,3) in their sequences. The results obtained are consistent enough to motivate further studies. The peptides were efficiently synthesized and the tested radiolabeling strategies showed successful results. Moreover, all the peptides demonstrated affinity for the tumor cells evaluated. These results obtained in this study are consistent to adapt in the clinical application.

Keywords: Glioblastoma, Peptides, Radioiodination
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NB.16 - Metabolic and Structural Signatures in Corticobasal Syndrome: A Multimodal PET/MRI Study

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Corticobasal syndrome (CBS) is a progressive neurological disorder related to multiple pathologies, including four-repeat tauopathies, such as corticobasal degeneration and progressive supranuclear palsy, and Alzheimer's disease (AD). Speech and language are commonly impaired, encompassing a broad spectrum of deficits. To investigate CBS speech and language impairment patterns considering a multimodal imaging approach. They underwent positron emission tomography with [^{18}F-]fluorodeoxyglucose (FDG-PET) and [^{11}C]Pittsburgh Compound-B (PIB-PET) on a hybrid PET-MRI machine to assess their amyloid status. PIB-PET images were classified based on visual and semi-quantitative analyses. Quantitative group analyses were performed on atrophy patterns on MRI were investigated using voxel-based morphometry (VBM) and FDG-PET data. Thirty healthy participants were recruited as imaging controls. PIB-PET was classified as negative (CBS-Amyloid – group) in n=18/31 and positive (CBS-Amyloid+ group) in n=13/31 patients. The frequency of dysarthria was significantly higher in the CBS-A– group than in the CBS-A+ group (55.6 vs. 7.7%, p = 0.008). They showed brain atrophy mainly at the putamen and opercular frontal gyrus. Relative to the phonemic verbal fluency, we found a positive correlation at the left frontal opercular gyrus (p = 0.0003, R^2 = 0.3685), the inferior (p = 0.0004, R^2 = 0.3537), and the middle temporal gyri (p = 0.0001, R^2 = 0.3993). There was a positive correlation between [^{18}F]FDG uptake and semantic verbal fluency at the left inferior (p = 0.006, R^2 = 0.2326), middle (0.0054, R^2 = 0.2376), and superior temporal gyri (p = 0.0066, R^2 = 0.2276). Metabolic and structural signatures depicted from this feature provide further insights into the motor speech production network and are also helpful to differentiate CBS variants. In the spectrum of language impairment profile, dysarthria might be helpful to distinguish CBS patients not related to AD.

Keywords: Corticobasal syndrome, amyloid-PET, fluorodeoxyglucose
Supported by: FAPESP

SE - Science Education

SE.01 - Didactic games in Biochemistry: alternative approaches to content absorption

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Biochemistry is considered by many students as a subject with a high degree of difficulty in view of the massive amount of content, which stimulates the development of new teaching methodologies, generating new approaches to the learning process. Among these methodologies are educational games, which allows the student to interact with content in an alternative way, in addition to stimulating skills such as creativity, reasoning and group work. Thus, the objective of this work was the development of three educational games as an alternative approach to teaching the main contents of the discipline. The games “Quem sou eu, bioquímico?”, “Aprendendo bioquímica por bem ou por mau-mau” and “Lipoprotein Game” were applied to students of Biological Sciences, Pharmacy and Chemistry graduation courses at the Federal University of São Paulo (UNIFESP) - Campus Diadema. During the proposed activities, students were subjected to different assessment methods such as forms of satisfaction and progress within the course in relation to each point explored, accompanied by teachers and monitors. Subsequent assessment of students’ perceptions with questionnaires revealed enhanced interest in the subject and a better perfomance in the tests was noticed, suggesting that the use of diversified approaches can be effective in the learning of Biochemistry.
Keywords: Didactic games, Biochemistry, teaching methodologies

SE.02 - Metagame: a proposal for teaching biochemistry

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Biochemistry is an essential subject in most of the undergraduate courses related to health and biologics fields. However, many students find it difficult to understand because of the fragmented way in which this subject is taught. In this context, many educational innovations have been developed, especially games. Games are always in evidence for their ludic and dynamic way of teaching contents and also for stimulating student’s critical thinking. The objective of this work was to create and develop a card game that would allow the integration of metabolism content in a way that would allow undergraduate students from biomedical and biological areas to understand the connection between the topics covered in the biochemistry subject and their applications on a daily basis. The game consists of a total of 35 cards, among which five correspond to the “tissue” cards. The other 30 cards correspond to the “problem situation” cards. The created game was called Metagame, which proposes to work biochemistry from mammalian metabolism contents in a dynamic, integrated and contextualized way, which can be used as an evaluative or fixation activity. Some suggestions for applying and a real example of using the game in a nutrition class are presented to show the practical use of Metagame as a paradidactic instrument to construct biochemistry knowledge.Metagame facilitates the understanding of biochemical concepts considered complex and that are important throughout the curriculum structure of undergraduate courses in the areas of health and biological sciences.
Keywords: didactic model , ludic, metabolism

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Borges, G.S.M.: *CD-44*
Borges, H.O.S.: *CB-19*
Borges, I.B.: *EC-08*
Borges, J.C.: *CD-02, DA-04, DA-07, DA-30, DA-59, DA-63, HA-18*
Bortolotto, V.C.: *CD-19, FB-03, FB-08, GD-09, GD-17, HA-13*
Bosissevitch, I.: *EB-17*
Bottini, M.: *AA-09, AA-13, AA-17*
Bouwstra, J.: *SP-01.03*
Bovo, A.C.: *JB-05*
Braga, C.A: *HA-09, HA-38*
Braga, E.S.: *FB-06*
Braga, I.C.C.: *JB-02*

Brandalize, A.P.C.: *CB-27*
Brandani, B.G.: *DA-41*
Brandão, W.F.: *EA-16*
Brasil, M.S.: *CB-29*
Breitkreitz, M.C.: *CD-29, CD-36, CD-47*
Bressan, C.B.C.: *EA-02*
Brito, I.L.: *BA-01, GA-08, JB-09*
Brito, J.S.: *GD-10*
Brogini, S.: *JB-11*
Brum, H.: *EC-15*
Brum, P.: *HA-40*
Brunetto, S.: *CD-30*
Brunger, A.: *SP-04.01*
Bruno, L.: *EA-19*
Bruno, M.L.: *GD-20*
Bryant, C.E.: *JB-06*
Buccini, D.F.: *SP-06.04*
Buchet, R.: *AA-13, AA-17*
Buchpiguel, C.: *NB-11*
Buckeridge, M.S.: *EA-10*
Buemo, P.S.A.: *CD-33*
Bugg, T.D.H.: *SP-06.05*
Burgardt, N.I.: *BA-10*
Burtoloso, A.C.B.: *CD-02*
Busch, L.: *SP-10.03*
Buschiazzo, A.: *SP-04.03*
Bustamante, C.: *PL-02.*
Butt, A.: *GD-18*
Butt, A.M.: *CA-04, HA-44*

Cabral Filho, P.E.: *EB-10, EB-15*
Cabral, B.C.A.: *HA-10*
Cabral, F.V.: *EB-03*
Cabrera, M.P.: *EB-10, EB-15*
Cabrera, V.I.M.: *GC-02*
Caires, A.R.L.: *EB-13*
Calaça, G.N.: *CA-08*
Calderon, L.A.: *DB-09*
Calienni, M.N.: *CB-05*
Calzada, V.: *SP-14.03*
Camargo, I.L.B.C.: *DA-35*
Camargo, M.L.A.: *KA-15*
Camargo, N.S.: *EC-09*
Cambri, G.: *GA-03*
Cambui, C.C.N: *CD-38*
Campanella, J.E.M.: *DA-07*
Campo Grande, G.C.: *GB-13*
Camporeale, G.: *SP-16.02*
Campos, B.M.R.: *GB-04*
Campos, M.S.: *FB-07*
Campos, R.M.: *DA-19, HA-17*
Campos, V.F.: *CD-15*
Campos-Da-Paz, M: *CB-04*
Cancelliero, G.S.: *HA-46*
Cândido, E.S.: *SP-06.04*
Candido, S.L.: *CB-05*
Cané, L.: *BB-01*
Canessa Fortuna, A.: *BA-06*
Cannavan, F.S.: *GC-03*
Cantu-Jungles, T.M.: *GB-02*
Capille, N.V.M.: *GB-08, GB-12*
Capitanio, M.: *SP-12.03*
Caponi, S.: *JB-11*
Caracelli, I.: *DA-37*
Caramori, S.S.: *CC-07*
Cardinali, M.A.: *JB-11*
Cardone, C.: *DA-16*
Cardoso, G.D.: *DA-56*
Cardoso, I.A.: *DA-17, DB-07*
Cardoso, L.S.: *GD-15*
Cardoso, M.H.S.: *SP-06.04*
Cardoso, S.C.: *GB-09, KA-29*
Cardoso, V.N.: *NB-03*
Cardozo, R.: *BA-15*
Carias, R.B.V.: *CB-07*
Carli, A.P.: *CB-06, JB-03*
Carli, G.P.: *JB-03*
Carlini, C.R.: *GC-10*
Carlos, R.M.: *CA-01, CD-09*
Carmo, F.A.: *DA-65*
Carmo, M.E.G.: *EB-16*
Carmona, E.M.: *BA-03*
Carneiro, C.G.: *NB-16*
Carneiro, F.A.: *EA-01*
Carneiro, L.A.B.C.: *DA-28*
Carneiro, L.T.: *GC-11*
Carnero, L.A.R.: *HA-15*
Carpanez, A.G.: *GB-06*
Carraro, M.F.: *DA-19, HA-17*
Carreira, R.B.: *CD-22*
Carretero, G.P.: *BA-09*
Caruso, B.: *AA-29*
Caruso, I.P.: *CD-02*
Caruso, Í.P.: *DB-02*
Caruso-Neves, C.: *GC-01, HA-19*
Carvalho, A.E.: *JB-07*
Carvalho, A.O.: *BB-03, GD-13*
Carvalho, C.R.O.: *HA-12*
Carvalho, F.A.: *FB-11*
Carvalho, F.C.: *KA-12*
Carvalho, F.V.: *CD-03, CD-06, CD-28, CD-30, CD-36, GD-08, GD-19*
Carvalho, G.Q.: *HA-48*
Carvalho, H.: *EC-11*
Carvalho, H.F.: *SPBN-09.01*
Carvalho, K.M.: *GD-02*
Carvalho, L.A.C.: *AA-07*
Carvalho, L.E.D.: *JA-02*
Carvalho, M: *SPBN-12.04*
Carvalho, P.E.: *CB-06, JB-03*
Carvalho, P.P.D.: *HA-31*
Carvalho-Assef, A.P.D.: *DA-69*
Casadei, B.R.: *CB-03*
Casali, B.C.: *HA-41*
Casali, E.: *EA-09*
Casavilca, L.A.: *BA-09*
Casciaro, B.: *JB-13*
Caseau, C.M.: *DA-16*
Caselli, F.: *JB-13*
Casini, A: *BA-13*
Casoti, R.: *NB-04*
Cassali, G.D.: *NB-03*
Cassanego, G.B.: *NB-04*
Castellini, H.: *GB-07*
Castilho, C.G.R.: *HA-42*
Castilho, M.S.: *CD-11, DB-14*
Castro R.N.: *HA-36*
Castro, R.D.: *GD-01, GE-09, KA-16*
Castro, R.D.C.: *GD-16, GD-20*
Castro, R.J.C.: *SPBN-06.02*
Castro, S.B.R.: *CB-06, JB-03*
Castro, S.R.: *CD-03, CD-47*
Cater, R.: *SP-13.03*
Cavalari, N.T.: *DB-12*
Cavalcante, G.A.: *HA-07, HA-08*
Cavalcanti, D.M.L.P.: *FB-05*
Cavalcanti, J.R.L.P.: *HA-34, HA-43, JA-08*
Cavalcanti, R.M.: *AA-23*
Cavalcanti, R.R.M.: *AA-01*
Cavalheiro, M.C.M.: *GC-16*

Cavalheiro, R.P.: *HA-20*
Cavazzutti, G.F.: *DA-46*
Caviglia, A.: *BA-02, BA-04*
Cepkenovic, B.: *EB-07*
Cesaro, G.: *DA-27*
Chagas, A.N.: *GE-04*
Chaimovich, H.: *AA-02, AA-07*
Chain, M.O.: *CD-40, GC-11, HA-05*
Chakrabort, R.: *DA-64*
Chambergo, F.S: *CC-08, CC-11*
Chan, H. S.: *SP-14.02*
Chan, L.Y.: *SP-06.04*
Chattopadhyay, A.: *SP-17.01*
Chattopadhyay, K.: *DA-64*
Chaves, D.S.A: *EC-03*
Chaves, J.S.: *CD-17*
Chaves, V.E.: *EA-04, EA-07, JA-02*
Chaves-Filho, A.B.: *EC-01*
Chedraoui-Silva, S.: *HA-46*
Chen, I.: *SP-13.03*
Cheohen, C.F.A.R.: *KA-08, KA-31*
Cherene, M.B.: *BB-03, GD-13*
Chevriau, J.: *BA-06*
Chiaratti, M.R.: *SP-09.02*
Chiarelli-Neto, O.: *EB-04*
Chipot, C.: *SP-13.04*
Chirdo, F.G.: *SP-24.04*
Christine Carbone: *SP-14.01*
Ciancaglini, P.: *AA-08, AA-13, AA-09, AA-12, AA-17, AA-19, AA-24, AA-32*
Cilli, E.M.: *DA-35*
Ciociola, T.:
Cipriani, T.R.: *EA-09, GB-02, GB-10, GB-11, GB-13*
Cirauqui, N.: *DA-40*
Civera, M.: *SP-05.01*
Claudio, M.C.: *CC-14*
Clemens, D.: *CD-29*
Cline, E.N.: *HA-17*
Codognato, D.C.K.: *EB-17*
Coelho, A.M.: *EC-05*
Coelho, C.De M.: *DA-03*
Coelho, L.C.B.B.: *CB-13, EB-15, GD-03*
Cogo, S.C.: *GA-03*
Colaço, M.V.: *SPBN-12.03*
Colman, A.B.: *SE-02*
Comério, N.Z.: *EB-04*
Conceição, N.H.: *GE-01, GE-09, KA-16*
Constantino, C.: *AA-27*
Constantino, C.J.L.: *AA-11, AA-25*
Contatori, C.G.S.: *EB-01*
Contessoto, V.G.: *DA-22*
Contreras-Martel, C.: *SP-02.04*
Coppa, C.: *SP-05.01*
Coqueiro, R.S: *EC-13*
Cordeiro, L.M.C.: *GB-02*
Cordeiro, Y.: *DA-70*
Cornélio, M.L.: *CD-01*
Corradi, G.R.: *BA-05*
Corradi, V.: *SP-19.01*
Correa, E.J.A.: *KA-12*
Corrêa-Castro, G: *JB-04*
Correia, C.R.S.T.B.: *CA-07, DA-25, JA-06*
Correia, M.T.S.: *EB-10, EB-15*
Correia, T.M.C.: *EC-16*
Correia, T.M.L.: *EC-10, EC-13*
Cortes, V.F.: *BB-02, JA-02*
Coskuner-Weber, O.: *SP-16.03*
Costa, A.G.: *HA-22*
Costa, A.R.: *CB-37*
Costa, C.: *BA-15*
Costa, C.A.R.: *DA-71*
Costa, D.C.: *EC-05*
Costa, G.C.A.: *DA-58, GA-07*
Costa, G.S.: *EA-04*
Costa, J.: *NB-11*
Costa, L.S.C.: *KA-04*
Costa, M.C.R.: *GE-08*
Costa, M.F.D: *GD-18, HA-44, HA-45*
Costa, M.I.C: *DA-62*
Costa, M.J.: *SP-21.03*
Costa, P.A.C.: *CD-27*
Costa, P.A.C.C.: *CD-43*
Costa, P.J.: *KA-21*
Costa, S.L.: *CA-04, CD-22, GD-18, HA-44. HA-45*
Costa, S.L.: *CD-07*
Costa, T.R.: *DA-10*
Costabel, M.D.: *KA-26*
Costa-Filho, A.J.: *AA-12, AA-31, CD-32, CD-34, DA-08, DA-17, DA-49*
Costa-Junior, D.A.: *HA-48*
Cota, G.: *JB-04*
Coutinho, A.: *AA-16, DA-73*
Couto, V.M.: *CD-47*
Covali-Pontes, H.R.: *BA-01, GA-04, GA-08, JB-09*
Craik, D.J.: *SP-06.04*
Crea, F.: *AA-14*
Cruz, G.C.F.: *DA-67*
Cruz, M.A.: *AA-17*
Cruz, M.A.E.: *AA-19*
Cruz, T.A.: *EC-06*
Cuccovia, I.M.: *AA-02 AA-07, BA-09*
Cunha Filho, E.: *NB-06*
Cunha Lima, S.T.: *CB-32*
Cunha, A.F.: *SP-05.02*
Cunha, E.M.F.: *DA-05*
Cunha, E.S.: *EC-07, EC-15*
Cunha, M.M.L: *DB-12, CC-12, EA-16*
Cunha, T.M.: *HA-27*
Cunha-Neto, E.: *HA-15*
Curado, M.P.: *SPBN-04.03*
Curto, L.M.: *DA-31*
Curtolo, F.: *KA-09*
Custódio, F.L.: *KA-04*

Da Costa, F.P.: *DA-70*
Da Cruz, L.A: *GC-04*
Da Fonseca, C.A.R.: *CD-15*
Da Hora, N.R.S.: *GE-03*
Da Luz, B.B.: *GB-13*
Da Rosa, G.: *SP-14.03*
Da Silva, A.N.R.: *KA-22*
Da Silva, B.B.: *DB-01*
Da Silva, E.R.: *DB-01*
Da Silva, G.D.: *NB-02*
Da Silva, G.F.: *CB-17, CB-21, CC-13, CC-15*
Da Silva, I.V.: *FB-11*
Da Silva, J.O: *CB-04*
Da Silva, M.L.: *DA-13, DA-38*
Da Silva, P.M.: *FB-11*
Da Silva, V.D.A: *HA-44*
Da Silva, V.G.: *DA-38*
Da-Cruz, A.M.: *JB-04*

Dagostin, L.S.: *EA-02*
Dahleh, M.M.M.: *CD-19, FB-03, FB-08, GD-09, GD-17*
Dallagnelo, E.G.: *NB-06*
Dallari, D.: *JB-11*
Dall'igna, D.M.: *EA-13, JA-03*
Dametto, M.: *KA-25*
Damiani, A.P.: *EA-02*
Danesi, C.C: *NB-04*
Dans, P.D.: *SP-14.03*
Dantas, D.C.C.P.: *CA-06, HA-08*
Dantas, P.H.: *GD-02*
Dapueto, R.: *SPBN-10.02*
Dardenne, L.E.: *KA-04*
Dartora, N.: *GD-12, GE-05*
Das, S.: *DA-26*
Dáu, J.B.T.: *CB-07*
David, J.M.: *GD-18, HA-44*
David, J.P.: *HA-44*
David, V.: *DA-15*
Daza Millone, M.A.: *BB-01*
De Almeida, R.M.M.: *HA-28*
De Andrade, A.V.: *CC-13*
De Andrade, M.F.: *JB-07*
De Araujo, D.R.: *CB-25, KA-30*
De Araújo, F.M.: *HA-45*
De Assis, L.V.M.: *SP-03.04*
De Ávila, M.C.: *DA-26*
De Barboza, M.F.: *NB-01*
De Castro, R.D.: *GD-15, GE-01*
De Freitas, J.S.: *CC-14*
De Jesus, L.B.: *HA-44, HA-45*
De La Fuente, C.: *SP-06.04*
De La Mora, E.: *SP-22.02*
De Lanna, C.A.: *GC-13, GC-17*
De Luca, G.: *EB-05*
De Lucca, F.L.: *HA-42*
De Marco, R.: *KA-01*
De Melo, B.A.G.: *SP-06.01*
De Melo, L.D.B.: *CD-40*
De Mesquita, J.F.: *KA-22, KA-28*
De Mori, R.M.: *DA-17*
De Moura, J.F.: *DA-29*
De Ninno, A.: *JB-13*
De Oliveira, A.C.B.: *CD-10*
De Oliveira, A.C.R.: *JA-05*
De Oliveira, A.H.C.: *DA-05*
De Oliveira, C.F.R.: *CB-14*
De Oliveira, G.A.P.: *DA-70*
De Oliveira, J.V.R.: *HA-45*
De Oliveira, L.A.S.: *HA-38*
De Palma, G.Z.: *BA-06*
De Paula, E.: *CD-03, CD-06, CD-28, CD-29, CD-30, CD-36, CD-47, GD-08*
De Paula, H.: *CD-21*
De Paula, N.D.: *CD-35*
De Prat Gay, G.: *SP-16.02*
De Rossi, M.C.: *EA-19*
De Sairre, M.I.: *CD-21*
De Sautu, M.: *DA-42*
De Sousa, G.S.: *DA-70*
De Souza, A.D.: *CD-29*
De Souza, A.R.: *DA-37*
De Souza, E.A.: *EA-18*
De Souza, V.B.: *CC-10*
Dehez, F.: *SP-13.04*
Del Mastro, N.L.: *KLBN-05*
Delfino, J.M.: *DA-11, DA-31*
Delgado André, N.: *HA-42*
Delorme, V.: *DA-69*
De-Melo, L.D.B.: *GC-11*
Demicheli, C.: *CD-10*
Demitroff, N.: *SP-16.02*
Demo, G.: *SP-14.01*
Demócedes, C.: *CB-36*
De-Moraes, G.N.: *GC-11*
Denadai, A.M.L.: *CB-06, CC-01*
Deserno, M.: *SP-22.03*
Dessen, A.: *SP-02.04*
Dey, S.: *DA-64*
Di Cicco, A.: *SP-22.02*
Di Lella, S.: *DA-74*
Di Santo, C.C.: *DA-03*
Dias E Vasconcellos, P.: *DA-21*
Dias Neto, E.: *SP-11.03*
Dias, M.: *HA-03*
Dias, W.B.: *HA-19*
Dias-Baptista, I.M.F.: *HA-26*
Dimova, R.: *AA-14, AA-30*
Dimova, R.: *SP-07.02*
Diogo, Í.L.: *JA-02*
Dixon, N.: *SP-06.05*
Do Nascimento, P.F.: *NB-13*
Dodero, V.I.: *KA-26*
Dodero, V.I.: *SP-24.04*
Dohra, H.: *BB-05, BB-06*
Dolan. E.L.: *SP-21.01*
Domingos, R.M.: *DA-23*
Domingues, R.S.: *KA-14, KA-19*
Domingues, W.B.: *CD-15*
Domont, G.: *GA-02*
Donato, M.: *EB-12*
Dores-Silva, P.R.: *DA-30*
Dornelles, R.C.: *NB-04*
Dos Anjos, J.V.: *CD-16*
Dos Reis, R.: *KA-01*
Dos Santos, K.B.: *KA-04*
Dos Santos, L.A.: *GD-13*
Dos Santos, M,: *CB-04*
Dos Santos, R.A.: *CD-21*
Dreyer, T.R.: *DA-39*
Du, G.: *SP-01.03*
Duarte, P.: *SPBN-10.02*
Ducatti, D.R.B.: *GB-11*
Durante, A.C.R.: *NB-01*
Dutra, A.C.L.: *HA-28*
Dutra, B.A.: *GE-08*
Dutra, C.L.: *AA-07*

Eaton, P.: *BB-12*
Echeverri, L.M.S.: *CB-09, CB-11, CB-17 CB-18, CB-21*
Efremov, R.G.: *SP-13.02*
Elias, M.A.: *CB-20*
Elifio-Esposito, S.: *GA-03*
Ellena, J.A.: *CD-34*
Elvis Franca: *SPBN-06.03*
Emanuelli, T.: *NB-04*
Emery, F.: *CD-11*
Engelmann, A.M.: *NB-04*
Engstrand, L.: *SP-11.01*
Enguita, F.J.: *BB-11*
Epifânio, N.M.M.: *EC-03*
Escriou, V.: *JB-06*
Esquisatto, M.A.M.: *NB-12*
Ester, V.G.: *CC-07*
Esteves, M.E.A.: *KA-02*
Estrada, J.E.: *DA-15*
Estrin, D.: *BA-06*
Estrozi, L.F.: *SP-02.04*
Evangelista, N.N.: *AA-31*
Ewins, E.J.: *GE-10*

Fabri, L.M.: *DA-53, DA-57*
Fabri, M.L.: *DA-62*
Fabrino, H.J.F.: *CD-10*
Faça, V.M.: *DA-19, HA-21, HA-29*
Facchin, G.: *CD-32*
Facchin, G.: *CD-34*
Facelli, J.C.: *BA-07*
Fagundes, T.M.: *GC-11, HA-05*
Fairley, J.K.: *HA-26*
Faleiros R.O.: *DA-02*
Falomir Lockhart, L.J.: *DA-46*
Fantin, C.: *HA-22*
Farah, C.: *GC-06*
Faria, B.Q.: *EA-12*
Faria, D.: *NB-11*
Faria, F.A.M.: *GB-02, GB-10, GB-11*
Faria, G.H.L.: *HA-25*
Faria, M.T.: *CB-02*
Faria, R.L.: *EC-01*
Farias, J.A.M.: *GD-14*
Farias, J.P.C.: *KA-11*
Farias, J.P: *CB-35*
Farias, L.P.: *HA-23*
Farina, M.: *DA-44*
Favarin, B.: *CD-32*
Favarin, B.Z.: *AA-12, AA-17, AA-24*
Fedorov, A.: *AA-16*
Feijoó, C.G.: *CD-06, CD-28*
Feitos, B.R.: *CC-10*
Felix, A.L.: *DA-70*
Fenerich, B.A.: *HA-29*
Fernades, J.: *JA-01*
Fernandes, A.Z.N.: *SP-05.02*
Fernandes, B.V.: *GE-02, GE-04*
Fernandes, C.J.C.: *SP-06.03*
Fernandes, E.J.: *CD-19, FB-01, FB-09*
Fernandes, J.: *CB-34, EC-02*
Fernandes, K.M.: *GD-03*
Fernandes, L.A.: *CB-09, CB-11, CB-17, CB-21, CC-15*
Fernandes, M.M.L.: *BA-01, GA-04, GA-08, JB-09*
Fernandes, M.T.P: *HA-12*
Fernandes, R.F.: *DB-11*
Fernandes, S.O.A.: *NB-03*
Fernandes, T.: *HA-08*
Fernandes, T.A.A.M: *CA-06, DA-10, HA-07*
Fernandes-Siqueira, L.O.: *EA-17*
Fernández Casafuz, A.B.: *EA-19*
Fernandez, L.G.: *CA-04, GD-01, GD-15, GD-16, GD-20, GE-01, GE-09, KA-16*
Fernández, M.: *BA-02*
Ferrari, E.: *EA-09*
Ferraro, M.B.: *BA-07*
Ferraz, P.J.C.: *GB-05*
Ferreia, N.C.: *DB-10*
Ferreira De Mattos, G.: *BA-15*
Ferreira, A.C.: *HA-24*
Ferreira, A.M.T: *GC-04, GC-09, GC-14, JB-05*
Ferreira, A.T.R.N: *GC-15*
Ferreira, A.V.: *NB-08, NB-14*
Ferreira, A.V.F.: *CB-02, CB-04*
Ferreira, C.R.: *AA-19*
Ferreira, F.B.: *CD-02*
Ferreira, G.F.: *CC-01, CB-16, CD-37*
Ferreira, H.A.S.: *CD-27*
Ferreira, H.A.S.: *CD-43*
Ferreira, J.C.B.: *SP-18.02*
Ferreira, L.A.M.: *CD-44*
Ferreira, M.R.: *SP-06.03*
Ferreira, M.R.A.: *CB-13*
Ferreira, M.S.: *KA-23*
Ferreira, N.A.: *DA-51*
Ferreira, P.F.: *GD-11*
Ferreira, P.R.: *SPBN-06.01*
Ferreira, R.S.: *CA-04*
Ferreira, S.A.O.: *CB-13*
Ferreira, S.F.: *GD-13*
Ferreira. B.C.L.: *CA-04*
Ferreira-Gomes, M.: *FA-03*
Ferreira-Gomes, M.S.: *DA-42*
Ferreiro, D.U.: *DA-33, DA-76*
Ferrera, L.: *BA-11*
Ferretti, G.D.S.: *DA-52, DA-70*
Ferrolho, J.F.: *CD-07*
Festuccia, W.T.L.: *EC-10*
Fidalgo, G.: *SPBN-12.03*
Figueira, A.C.M.: *HA-03*
Figueiredo, R.C.B.Q.: *EB-03*
Finardi, A.J.: *HA-26*
Fini, M.: *JB-11*
Fioretto, D.: *JB-11*
Fiorini-Rosado, A.: *BB-07, GC-08, GD-06, GE-07*
Fodera, V.: *DA-54*
Fogaça, M.M.C.: *CB-28*
Folly, M.L.C.: *HA-04*
Fonin, A.B.: *SP-16.04*
Fonseca, M.L.: *GC-12, GC-19*
Fonseca, N.P: *HA-29*
Fonseca, R.C.: *GE-08*
Fonseca. L.F: *DA-13*
Font, J.: *SP-13.03*
Fontana, N.A.: *DA-49*
Fontes, A.: *EB-03, EB-10, EB-15*
Fontes, C.F.L.: *DA-02*
Fontes, M.R.M.: *DA-07, DA-39, KA-18*
Forini, M.M.L.: *EB-13*
Fortuna, A.F.: *JA-07*
Fraga, L.A.O.: *HA-26, JB-01*
Fraifeld, F.: *SPBN-06.01*
Frallicciardi, J.: *GE-10*
França Junior, N.: *GA-03*
Franceschi, N.: *SP-22.02*
Franco M.S.F: *EB-02*
Franco, M.K.K.D.: *CD-29, CA-02, KA-30*
Franco, O.L.: *CD-04*
Franco, O.L.: *SP-06.04*
Freddi, P.: *CD-34*
Freira, A.: *BA-15*
Freire, M.C.L.C.: *DA-20*
Freitas De Lima, F.: *GD-08*
Freitas, B.L.: *HA-12*
Freitas, J.S.: *GE-08*
Freitas, K.H.: *CB-13*
Freitas, M.L.: *EB-04*
Freitas, R.: *KA-10*
Freitas, R.B.: *NB-10*
Frezard, F.J.G.: *CD-10, CD-27, CD-43, CD-44*
Froes, T.: *CD-11*
Froes, T.Q.: *DB-14*
Fronza, M.G.: *HA-13*
Fuhler, G.: *SP-06.03*
Fujita, F.: *AA-15*
Fujiwara, R.T.: *CD-44*

Fukuda, C.A.: *KA-18*
Funguetto-Ribeiro, A.C.: *CD-19*
Furlanetto, A.L.D.M.: *EC-15*
Fuscaldi, L.L.: *NB-01, NB-15*
Fuscaldi, L.L.: *SPBN-05.03*

Gadelha, R.: *NB-11*
Galdino, A.S.: *CB-02, CB-04, CC-16*
Galeano, R.M.S.: *CB-29, CC-04*
Galheigo, M.M.: *KA-04*
Galisteo Jr., A.J.: *NB-02*
Galizia, L.: *BA-04*
Galízio, N.C.: *CB-24*
Gallo, M.: *EA-09*
Galman, J.L.: *SP-06.05*
Galparsoro, D.F.: *DA-54*
Galpern E.A.: *DA-76*
Galvao, T.C.: *DA-69*
Gambini, J.P.: *SPBN-10.02*
Gangloff, M.: *JB-06*
Gangloff, M.: *SP-24.01*
Gárate, J.A.: *BA-02*
Garbelotti, C.V.: *DA-28, EA-03*
Garcez, F.R.: *GC-04*
Garcia, B.B. M.: *DA-71*
Garcia, C.S.: *GC-14*
García, E.: *CD-34*
Garcia, M.L.C.: *CA-01*
García-López, J.P.: *CD-06, CD-28*
Garçon D.P.: *DA-02 DA-53, DA-57, DA-62*
Gardini, L.: *SP-12.03*
Garofalo, F.: *CD-26*
Garófalo, M.A.R.: *EA-07*
Gasparini, P.: *KA-13*
Gasparotto, J.: *HA-28*
Gava, L.M.: *DA-04, DA-59*
Gavazzo, P.: *BA-11*
Gavernet, L.: *CD-26*
Gawriljuk, V.O.: *DB-11*
Gay, N.J.: *JB-06*
Gay, N.J.: *SP-24.01*
Gelain, D.P.: *FB-07, HA-28, HA-40*
Generoso, S.V.: *NB-03*
Generoso, W.: *DA-68*
Generoso, W.C: *DA-26, DA-55*
Genu, V.F.: *EA-05*
Georgiev, V.: *AA-14*
Gerardino, A.: *JB-13*
Gerheim-Silva, J.V.: *GB-06*
Geronimo, G.: *CD-03, CD-06, CD-28, CD-36, GD-08*
Gervasoni, J.L: *KLBN-05.*
Ghezzi, B.: *EA-09*
Ghosh, B.: *DA-09*
Giannesi, G.C.: *CB-29, CC-04, CC-06, CC-09*
Gibson, M.O.: *BA-04*
Gil, M.L.S.: *DA-49*
Gil, P.O.: *BB-02*
Giménez, E.: *DA-46*
Giordano, R.J.: *CD-38, CD-42, FB-02, HA-37*
Giordano, R.J.: *HA-15*
Giraldi-Guimarães, A.: *DA-38*
Girardi, C.S.: *FB-07, HA-28*
Giron, C.C.: *HA-06*
Gismene, C.: *DB-06, DB-13*
Giudice, M.D.: *DA-04, DA-59*
Glienke, N.N.: *CC-04*
Glynne-Jones, P.: *GA-05*
Godoi, R.R.: *CC-16*
Godoy, A.S.: *DB-11*
Godoy, J.M.: *SPBN-06.01*
Goes, C.G.: *EA-05*
Goldbeck, R.: *SP-06.05*
Gomes, A.A.: *CB-09, CB-11, CB-17, CB-21*
Gomes, A.A.S.: *DA-07*
Gomes, A.C.: *NB-02*
Gomes, B.M: *CC-08, CC-11*
Gomes, B.S.: *NB-12*
Gomes, D.E.B.: *KA-23*
Gomes, F.: *EA-05*
Gomes, F.: *SP-23.03*
Gomes, H.M.: *JB-10*
Gomes, P.: *BB-12*
Gomes, P.S.F.C: *DA-21*
Gomes, R.H.M.: *KA-05*
Gomes, V.M.: *BB-03, GD-13*
Gomes-Silva, A.: *JB-04*
Gómez, G.E.: *DA-11*
Gómez-Fernández, J.C.: *AA-22*
Gonçalves, A.B.: *NB-12*
Gonçalves, D.B.: *CC-16*
Gonçalves, L.M.: *AA-07*
Gonçalves, O.H.: *FB-08*
Gonçalves, S.: *CD-04*
Gonçalves, S.: *SP-06.04*
Gontijo, R.M.G.: *NB-08*
González Flecha, F.L.: *BA-10, BA-12*
González Lebrero R.M.: *BA-12*
Gonzalez, A.A.: *GE-04*
Gonzalez, A.J.: *KLBN-01.*
González, C.: *BA-02*
Gonzalez, C.: *BA-03*
Goroso, D.G.: *KA-15*
Govender, P.: *GD-24*
Govoni, M.: *JB-11*
Gozzer, L.T.: *HA-41*
Grafmueller, A.: *AA-14*
Grahl, M.V.C.: *GC-10*
Granjon, T.: *AA-08, AA-13*
Grasselli, M.: *SP-01.02*
Grassi, M.L.: *HA-21*
Gratton, E.: *SP-12.02*
Greaves, T.: *CA-10*
Greggio, S.: *NB-11*
Grego, K.F.: *CB-24, CB-31, CB-38*
Grille, L.: *SP-14.03*
Grillo, D.C.N.: *EC-03*
Grillo, R.: *EB-13*
Gronenborn, A.M.: *PL-08.*
Gualberto, S.A.: *GE-08*
Guarnieri, L.O.: *EB-08*
Guastaferri, F.: *BA-04*
Guatimosim, S.: *CD-27, CD-43*
Guedes, I.A.: *KA-04*
Gueiros-Filho, F.J.: *SP-05.02*
Guerra, G.P.: *CD-19, FB-03, FB-08, FB-09*
Guerra, J.F.C.: *EA-12*
Guerra, M.O.: *GB-06*
Guerreiro, A.T.G.: *CD-18*
Guex, C.G.: *NB-04*
Guimaraes, B.G.: *DA-27*
Guimaraes, L.C: *CD-27, CD-43*
Guimarães, L.S.: *SPBN-01.01*
Guimarães, N.C.A.: *CB-29, CC-04*

Guimaraes, P.P.G.: *CD-27, CD-43, CD-44*
Gurtovenko, A.A.: *SP-19.02*
Gusmão, A.C.M.M.: *EC-10*
Gutfilen, B.: *SPBN-05.04*
Gutierrez, R.F.: *DA-43*
Guzmán, F.: *BB-01*
Guzzo, C.R.: *GC-06*

Haas, S.E.: *CD-19*
Hackenberger, C.: *DA-74*
Hadizadeh, N.: *DA-36*
Hamasaki, M.: *SP-18.01*
Han, S.W.: *DA-71*
Haouz, A.: *DA-27*
Hatakeyama, D.: *CB-31*
Hatzinikolaou, D.G.: *CC-05*
Hayashi, K.: *GA-12*
Heberle, J.: *AA-14*
Henderson, R.: *PL-01.*
Hendges, F.B.: *BB-07, GC-08, GE-07*
Henrique, C.W.B: *CB-26*
Herlax, V.: *AA-04, BB-01*
Herrera, G.: *SP-24.04*
Hespanhol, J.T.: *GC-06*
Hicks, K.G.: *DB-07*
Hilbig, A.: *HA-28*
Hochhegger, B.: *SPBN-09.02*
Hoffmann, L.: *HA-10, HA-32*
Hohmann, S.: *DA-48*
Honorio, K.M.: *CD-20, CD-21*
Hoppe, J.B.: *CA-04, GD-23*
Hossain, F.: *BB-05*
Hosseinzadeh, R.: *EB-19*
Hoylaerts, M.: *AA-09*
Hözer, R.M.: *GD-23*
Huber, R.G.: *BB-11*
Huff, G.M.: *GC-08, GD-06*

Iacomini, M.: *GB-02, GB-13*
Iagher, F.: *JA-05*
Ide, Y.: *FA-04*
Igartúa, D.E.: *CD-47*
Imbert, L.: *SP-02.04*
Imelio, J.A.: *SP-04.03*
Inaba, J.: *CA-08*
Inague, A.: *HA-37*
Isaurralde, F.: *SPBN-10.02*
Ishii, S.: *JA-04*
Itri, R.: *AA-16, CB-25, DA-42, EB-09, EB-12*
Izumi, K.S.: *DA-60*
Izzi, B.: *AA-09*
Jacob, C.M.B.: *JB-05*
Jadranin, M.: *FB-06*
Jain, S.: *DA-09*
Jakobus, B.: *DA-70*
Jalilian, A.R.: *SPBN-02.03*
Jandova, J.: *SP-10.02*
Janet-Maitre, M.: *SP-02.04*
Janmey, P.A.: *SP-07.03*
Janner, D.E.: *FB-01, FB-09*
Jaques, J.A.S.: *BA-01, GA-04, GA-08, JB-09*
Jara, G.: *BA-07*
Jasmin: *. CB-07*
Jerala, R.: *SP-24.03*
Jeronymo, L.R.: *HA-24*
Jesus, B.A.P.: *CB-09, CB-11, CB-17 CB-21*
Jesus, H.N.R.: *GC-18*
Jiacomini, I.G.: *DA-29*
Jiskoot, W.: *SP-01.03*
Job, V.: *SP-02.04*
Jolly, M.: *SP-16.01*
Jovin, T.M.: *DA-46*
Juliano, M.A.: *AA-02*
Junior, N.N.: *DA-10*
Júnior, P.N.O.: *EB-09*
Junqueira, C.H.: *EB-11*
Junqueira, H.C.: *CA-03*
Junqueira, M.: *GA-02*
Justo, G.Z.: *GB-05, SE-01*
Justo, V.E.M.S.: *GD-06, GC-08*

Kadowaki, M.K.: *CC-03*
Kalume, D.E.: *GD-21*
Kappo, A.P.: *DB-03*
Karguth, N.: *BB-11*
Karl, A.L.M.: *KA-04*
Kartunnen, M.: *SP-19.02*
Kashchuk A.V.: *SP-12.03*
Kashiwazaki, K.: *JA-04*
Kava, E.: *DA-08*
Kawaguchi, K.: *KA-20*
Kellerman, G.: *CA-02*
Kenmotsu, T.: *AA-15*
Kennedy-Feitosa, E.: *CB-36*
Kent, B.: *CD-29*
Kesminiene, A.: *SPBN-04.02*
Kettelhut, I.C.: *EA-07*
Ketzer, L.A: *CB-33, EA-17*
Khalid, S.: *KA-27*
Khalid, S.: *SP-19.03*
Khorsandi, K.: *EB-19*
Khouri, R.: *HA-23*
Kinupp, V.F.: *GD-02*
Kiraly, V.T.R.: *DA-07*
Klassen, G.: *CA-08*
Klein, C.P.: *GD-23*
Klein, E.: *JB-10*
Klein, F.: *BA-16*
Klein, M.K.: *GE-02, GE-04*
Klein, N.K.: *GE-02, GE-04*
Klein, W.L.: *HA-17*
Kleiz-Ferreira, J.M.: *DA-40*
Knittel, T.L.: *EA-18*
Kobayashi, T.J.: *FA-05*
Kobayashi, T.J.: *FB-04*
Kock, F.V.C.: *CD-09*
Koide, T.: *DA-25, FB-10*
Koiwai, K.: *AA-10*
Korostelev, A.: *SP-14.01*
Kotnala, S.: *SP-16.01*
Kozlov, M.: *SP-07.01*
Kreirmerman, I.: *SPBN-10.02*
Krempser, E.: *KA-04*
Krmpot, A.: *JB-12*
Kruger, W.M.A.: *GC-19*
Krylov, N.A.: *SP-13.02*
Kulkarni, P.: *SP-16.01*
Kumari, S.: *BB-10*
Kumayama, S.: *JA-04*
Kupko, N.: *SP-13.02*
Kuschnir, R.: *JB-04*
Kuznetsova, I.M.: *SP-16.04*

Laaksonen, A.: *HA-06*
Lacerda, C.D.: *AA-02*
Lacerda, P.: *DB-14*
Lachagès, A.M.: *JB-06*
Lafarge, E.: *BA-08*
Lago, J.H.G: *GC-02*
Lam, P.Y.: *SP-05.03*
Lanfredi, G.P.: *HA-21*
Lang, K.L.: *CB-16, CD-37*
Langel, U.: *DA-71*
Lanza, J.S.: *CD-10*
Lapierre, L.: *SP-18.03*
Lassounskaia, E.: *BB-03*

Latini, A.: *HA-49*
Latorre, R.: *PL-07.*
Lauster, D.: *DA-74*
Lautherbach, N.E.S.: *EA-07*
Lavor, T.S.: *EB-06*
Leake, M.: *DA-48*
Leal Da Silva, M.: *KA-08*
Leal, C.M.: *KA-02*
Leal, D.M.F.: *AA-04*
Leal, E.C.: *JA-02*
Leal, T.C.: *DA-35*
Leech, A.: *DA-05*
Legrand, P.: *DA-27*
Leitão, A.: *HA-18*
Leitão, G.G.: *KA-02*
Leitão, S.G.: *KA-02*
Leite, C.: *CD-34*
Leite, C.S: *NB-14*
Leite, R.C.: *CB-01*
Leite, R.S.A.: *BB-12*
Leite, V. B. P.: *SP-16.01*
Leite, V.B.P.: *DA-33*
Lemes, J.B.P.: *CD-06*
Lemos, B.B.: *CD-15*
Lemos, L.N.: *GC-15*
Lemos-Pinto, M.: *SPBN-08.04*
Leomil, F.S.C.: *AA-30*
Leone, F.A.: *DA-02, DA-53, DA-57, DA-62*
Leoni, S.: *BA-13*
Lessa, T.L.A.S.: *EC-10*
Levy, Y.: *SP-14.04*
Libardi, S.H.: *CD-02, DA-04, DA-59*
Liberalino, J.P.S.: *CA-06, HA-07, HA-08*
Lima Fernandes, P.C.: *GD-08*
Lima, A.N.: *CD-21, KA-03*
Lima, C.C: *CB-36*
Lima, C.K.: *CB-20*
Lima, D.: *CA-08, CD-16*
Lima, J.V.A.: *EB-15*
Lima, L.C.: *GA-06*
Lima, L.H.F.: *KA-12*
Lima, L.M.T.R.: *DA-01*
Lima, M.F.: *CD-01*
Lima, M.M.P.: *NB-13*
Lima, R.D.P.: *EA-11*
Lima, R.S.: *HA-02*
Lima, S.: *SP-04.03*
Lima, S.L.: *CD-22*
Lima, W.G.: *EC-05*
Lima, W.J.N.: *CC-16*
Linhares, S.S.G.: *JA-08*
Lippi, G.L.: *GA-05*
Lira, R.B.: *AA-01, AA-23*
Liria, C.W.: *AA-02*
Lissek, E.N.: *DA-36*
Llanos, M.: *CD-26*
Lohan, S.B.: *SP-10.03*
Londero, L.: *FB-03*
Lonez, C.: *JB-06*
Lopes Filho, G.J.: *NB-12*
Lopes Neto, A.V.: *EA-14*
Lopes, D.O.: *BB-02*
Lopes, R.T.: *SPBN-06.02*
Lopes, U.G.: *GC-11*
Lotierzo, M.: *CB-03*
Lotierzo, M.C.G.: *CD-41*
Loureiro, M.B: *GD-16*
Lourenço, T.: *DB-01*
Lourenço, T.C.: *DA-71*
Loveland, A.: *SP-14.01*
Lu, T.K.: *SP-06.04*
Lucena, L.: *SPBN-08.04*
Lucena, M.N.: *BA-01, DA-02, GA-04, GA-06, GA-08, JB-09, SE-02*
Luchese, C.: *CD-15, CD-17, HA-14*
Lüchmann, K.H.: *CB-20, CB-23*

Macambira, S.G.: *HA-01, JA-07*
Macario, T.M.: *EA-15*
Macedo, A.A.: *CB-22*
Macedo, M.L.R.: *CB-14, GC-07, GC-16, GD-05, GD-04*
Machado, C.R.: *GC-05*
Machado, E.C.: *SPBN-06.02*
Machado, F.K.: *GD-12*
Machado, F.R.: *CD-19, FB-08, GD-09, GD-17*
Machado, I.P: *CD-25*
Machado, J.M.: *CB-02, CB-04*
Machado, L.R.: *DA-15*
Machado, M.: *EC-13*
Machado, N.T.: *HA-01*
Machado, R.F.: *DA-69*
Machado, S.S.: *DA-51*
Machado, T.R: *KA-25*
Machado, W.: *SPBN-06.02*
Machado, V.D.: *BB-07*
Machini, M.T.: *AA-02*
Maciel, I.O.: *GC-11*
Magalhães, M.L.B.: *CB-09, CB-11, CB-17, CB-21, CC-13, CC-15*
Magne, D.: *AA-13*
Magro, A.J.: *KA-05, KA-13*
Maity, S.: *SP-22.02*
Makris, T.: *DA-68*
Makris, T.M.: *DA-26*
Malavolta, L.: *NB-01, NB-15*
Malheiros, B.: *CB-03*
Malizia-Motta, C.L.F.: *DA-01*
Maller, A.: *CC-03*
Malospirito, C.C.: *SP-02.04*
Mamede, M.: *NB-08, NB-11, NB-14*
Mancini, G.: *HA-49*
Mangenot, S.: *SP-22.02*
Mangialavori, I.: *FA-03*
Mangialavori, I.C.: *DA-42*
Mangiarotti A.: *AA-06*
Mangoni, M.L.: *JB-13*
Manica, G.C.M.: *CA-08*
Maniglia, T.C.: *GC-08*
Manzato, V.M.: *CB-38, DA-03, DA-58*
Maranduba, C.M.C.: *GB-06*
Marchini, F.K.: *EC-07*
Marciano, C.L.: *CC-06, CC-09*
Marcolongo-Pereira, C.: *EB-04*
Marcondes, M.F.M.: *AA-18*
Maria-Ferreira, D.: *GB-13*
Mariani, P.: *CB-25*
Mariano, B.J.: *EC-06*
Marinho, A.O.: *GD-10*
Marinho, L.N.: *GD-02*
Mariutti, R.B.: *DB-06, DB-13*
Marques, A.De L: *DB-02*
Marques, C.: *BA-08*
Marques, I.L.S.: *EB-08*
Marques, J.: *... DA-44*
Marques, J.: *CB-18*
Marques, M.A.: *DA-70*
Martin, C.S.: *AA-25*

Martín, P.: *CD-26*
Martinez, C.S.: *CD-47*
Martinez, G.R.: *EC-07, EC-15*
Martinez-Banaclocha, H.: *JB-06*
Martini, M.F.: *BB-01*
Martins, A.: *SP-02.04*
Martins, A.S.: *BB-11*
Martins, C.C.: *CD-17*
Martins, C.H.G.: *DA-10*
Martins, G.F.: *GD-03*
Martins, I.C.: *BB-11*
Martins, L.G.: *FB-06*
Martins, M.: *CD-20*
Martins, M.C.M.: *EA-10*
Martins, M.H.D.: *CC-07*
Martins, V.S.: *AA-07*
Martins-De-Souza. D.: *SP-08.02*
Maruta, S.: *DA-75*
Masamba, P.: *DB-03*
Mascarenhas, Y.M.: *SPBN-07.03*
Massirer, K.B.: *EA-18*
Masson, A.P.: *DA-19, HA-21, HA-29*
Massoud, R.: *JB-13*
Masui, D.C.: *CC-04, CC-06, CC-09*
Masui, D.G.: *CB-29*
Maté, S.: *BB-01*
Matias, T.A.: *EB-16*
Matos, A.C.B.: *CB-20*
Matos, C.O.: *DB-08*
Matos, P.A.: *EB-16*
Matsuguma, B.E.: *SP-05.02*
Matsukawa, Y.: *FA-04*
Matsuyama, Y.B.: *GC-06*
Mattarelli, M.: *JB-11*
Matté, C.: *GD-23*
Mattos, J.C.: *KA-13*
Mattos, M. J. G.: *HA-18*
Matushita, C.S.: *SPBN-09.02*
Mayama, S.: *FA-04*
Maybeck, V.: *EB-07*
Mayrinck, J.V.R.: *CC-12*
Mazepa, E.: *EC-07, EC-15*
Mazzitelli, L.R.: *BA-05*
Mcdargh, Z.A.: *SP-22.03*
Mcnamara, J.C.: *DA-02, DA-62*
Mebarek, S.: *AA-13, AA-17*
Medeiros, D.: *DA-14*
Medeiros, V.: *KA-04*
Medeiros, V.P.: *GB-06*
Medrado, G.T.: *GD-01, GD-15*
Meichtry, L.B.: *FB-01, GD-09, GD-17, HA-13*
Meichtry, L.M.: *FB-03*
Meinke, M.C.: *SP-10.03*
Meira, M.R.: *GE-08*
Meireles, A.P.A.: *HA-07*
Meireles, D.A.: *EC-16*
Meirelles, E.F.: *GC-10*
Mejia, J.: *GA-05, NB-01, NB-15*
Meleti, M.: *EA-09*
Melian, N.A.: *BA-10*
Mello, E.O.: *BB-03*
Mello, G.S.: *GB-02, GB-10, GB-11*
Mello, L.R.: *CB-15, DA-71, DB-01*
Mello, L.V.: *SP-21.02*
Mello, S.R.: *CB-18, DA-44*
Melo, A.: *AA-16*
Melo, A.M.: *DA-73*
Melo, A.M.M.A.: *CB-13*
Melo, C.M: *GB-04*
Melo, D.R.: *SPBN-07.02*
Melo, F.A.: *CD-02*
Melo, K.R.B.: *HA-20*
Melo, L.D.B.: *HA-05*
Melo, M.C.: *GB-05*
Melo, M.M.: *BB-04*
Melo, M.T.: *HA-10*
Melo, N.: *EB-10*
Melo, R.R.: *DA-50*
Melo, V.S.: *CC-08, CC-11*
Melo-Minardi, R.C.: *KA-12*
Mendes, B.M.: *NB-08, NB-14*
Mendes, F.: *CB-22*
Mendes, J.S.: *EC-06*
Mendes, L.F.: *CD-32, CD-34*
Mendes, L.F.S.: *AA-31, DA-17*
Mendes, N.D: *HA-27*
Mendes, T.F.: *EA-02*
Mendonça, F.F.: *NB-01, NB-15*
Mendonça, L.: *KA-17*
Mendonça, T.C.: *CD-03, CD-06, CD-28, CD-36, GD-08*
Mendoza, D.: *AA-26*
Menegatti, A.C.O.: *DA-24, KA-11*
Menezes, R.P.: *DA-10*
Menna-Barreto, R.F.S.: *CD-40*
Meotti, F.C.: *AA-07, EC-10, EC-16*
Mesnage, S.: *GC-06*
Mesquita, N.C.M.R.: *DA-20*
Mesquita, R.D.: *GC-11*
Micheau, O.: *GA-03*
Micheletto, M.C.: *AA-31*
Michnick, S.: *SP-20.04*
Migliorini, B.M.S.: *GC-09*
Miles, A.J.: *DA-06*
Miletti, L.C.: *CB-18, DA-44*
Millan, J.L.: *AA-09, AA-13, AA-17, AA-32*
Minneci, M.: *SP-05.01*
Minozzo, J.C.: *DA-29*
Miotto, R.: *EB-11*
Miranda, A.C.A.M.: *HA-23*
Miranda, A.C.C.: *NB-01, NB-15*
Miranda, F.R.: *GD-03*
Mitsutake, H.: *CD-29, CD-30*
Miyachiro, M.M.: *SP-02.04*
Miyamoto, A.: *GA-09*
Miyamoto, R.Y.: *DA-50, DA-68*
Miyamoto, S.: *EC-01, HA-37*
Mohanty, A.: *SP-16.01*
Moncrieffe. M.: *SP-24.01*
Mönkäre, J.: *SP-01.03*
Monteiro, J.S.: *FB-02, HA-15, HA-37*
Monteiro-Cunha, J.P.: *FA-01, KA-32*
Montor, W.R.: *NB-01*
Moraes, C.M.: *DA-53, DA-57*
Moraes, I.R.: *KA-18*
Moraes, M.F.D.: *EB-08*
Morais, D.M.: *DA-62*
Morais, N.M.: *HA-08*
Morais-Zani, K.: *CB-24, CB-31, CB-38*
Morales-Navarrete, H.: *FA-02*
Moran, O.: *BA-11*

Morato, L.F.C.: *AA-11*
Moreira, E.L.T.: *HA-44*
Moreira, H.T.: *BB-07, GC-08, GE-07*
Moreira, J.C.: *HA-40*
Moreira, J.C.F.: *CA-04, FB-07, HA-28, JB-10*
Morgante, P.G.: *DA-47*
Mori, M.A.: *EA-18*
Mori, R.L.: *CB-15*
Moritz, M.N.O.: *HA-18*
Moro, R.P.: *EA-18*
Moroto, B.S.: *GD-04*
Morresi, A.: *JB-11*
Morrow, M.: *BB-10*
Mortara, L.: *AA-07*
Mota, D.C.A.M.: *DA-17*
Mota, H.P.: *CD-17*
Mota, L.G.: *SPBN-13.01*
Mota, M.F.: *DA-70*
Motadi, I.R.: *GD-07*
Motta, G.: *HA-20*
Motta, K.P.: *CD-15*
Mottola, M.: *AA-05*
Mottram, J.C.: *DA-05*
Moura, L.D.: *CD-03, CD-06, CD-28, CD-30, CD-36, GD-08*
Moura, M.A: *CB-33*
Moura, T.C.: *CD-02*
Mourão, P.A.S.: *GB-08, GB-12*
Muller, P.: *BA-08*
Munhoz, V.H.O.: *BB-09*
Munsamy, G.: *DB-03*
Murakami, M.T: *DA-26*
Murayama, Y.: *GA-09, JA-04*
Musachio, E.A.S.: *FB-01, FB-03, FB-09, GD-09, GD-17*
Muxel, S.M.: *SP-06.02*
Muzitano, M.F.: *BB-03*

Nader, H.B.: *HA-20*
Nadezhdin, K.D.: *SP-13.02*
Nagae, F.: *DA-41*
Nagao, H.: *KA-20*
Nagem, R.A.P.: *CC-16*
Nakagawa, H.: *JA-04*
Nakamura, A.N.: *DB-11*
Nakamura, K.: *FB-04*
Nakao, L.S.: *EC-15*
Nakashima, S.: *FA-05*
Nanzer, A.L.: *AA-32*
Napoleão, T.H.: *GD-03, GD-10*
Napoleone, A.: *CD-34*
Nascimento, A.C.G.: *NB-13*
Nascimento, A.G.L.C.: *CC-03*
Nascimento, A.S.: *DA-43*
Nascimento, C.M.: *EC-11*
Nascimento, F.D.: *HA-20*
Nascimento, G.N.: *GE-08*
Nascimento, M.S.: *GE-08*
Nascimento, R.P.: *CD-22 HA-44*
Nascimento, R.T.: *HA-16*
Navarro, B.V.: *EA-10*
Navarro, D.M.A.F.: *GD-03*
Navarro-Quiroz, R.: *KA-24*
Navas, J.: *GC-18*
Navegantes, L.C.C.: *EA-07*
Neder, L.: *HA-27*
Neely, A.: *BA-03*
Neiverth-Chagas, A.: *GE-02*
Neta, L.C.: *GD-16*
Neta, L.C.S.: *GD-01*
Neto, A.N.: *CD-40, GC-11*
Netto, A.M.: *SPBN-08.04*
Netto, L.E.S.: *DA-23,EA-05, EC-16, GC-02, SP-23.03*
Neuberger, A.: *SP-13.02*
Neves, G.B.: *CB-18, DA-44*
Neves, N.: *DA-52*
Neves, V.B.S.: *HA-01 ,JA-07*
Nicastro, G.G.: *GC-06*
Nicolás, E.: *CD-26*
Nicolás, M.F.: *KA-04*
Nilsson, P.: *SP-08.03*
Nishizaka, T.: *CA-09*
Nogueira, A.S.: *GC-11*
Nogueira, C.W.: *EA-06*
Nogueira, F.C.S.: *GA-02*
Nogueira, G.O.: *HA-27*
Nogueira, L.M.: *CB-02 ,CB-04*
Nogueira, L.P.: *SPBN-12.03*
Nolwenn, M.: *SP-22.02*
Nonato, M.C.: *CD-11, CD-45, DA-17, DB-07, DB-14*
Noske, G.D.: *DB-11*
Nunes, A.C.C.: *HA-35*
Nunes, F.O.: *GC-04*
Nunes, L.O.: *BB-09*
Nunes, R.S.: *KA-21*
Nunes, S.A.: *HA-01*
Nunes, V.A.: *HA-12*
Nunez, C.V.: *GD-02*
Nury, H.: *SP-13.04*

Odunfa, S.A.: *CC-05*
Offenhäusser, A.: *EB-07*
Okamoto, M.R.Y.: *SPBN-11.02*
Oliva, G.: *DA-20, DB-11*
Oliva, M.L.V.: *EB-10*
Oliveira Jr, A.B.: *DA-22, DA-33*
Oliveira, A.E.A.: *GD-13*
Oliveira, A.M.: *GD-10*
Oliveira, C.F.R.: *GC-07, GC-16, GD-05*
Oliveira, C.G.: *DA-49*
Oliveira, C.L.P.: *DA-23*
Oliveira, C.S.: *DA-59, DA-63*
Oliveira, D.M.: *EB-09*
Oliveira, D.M.P.: *GA-02*
Oliveira, D.R.: *JB-01*
Oliveira, E.: *HA-39*
Oliveira, E.A.M.: *CD-37*
Oliveira, H.C.: *DA-39, KA-18*
Oliveira, H.C.F.: *EA-04*
Oliveira, I.R.A.: *KA-05*
Oliveira, K.M.T.: *KA-10*
Oliveira, L.G.: *SP-06.02*
Oliveira, L.G.F.: *HA-23*
Oliveira, L.M.G.: *CD-22*
Oliveira, L.P.: *EA-10*
Oliveira, M.: *AA-03, AA-27*
Oliveira, M.A.: *GC-02*
Oliveira, M.C: *EB-11*
Oliveira, M.D.L.: *KA-10*
Oliveira, M.P.: *EA-02*
Oliveira, M.R.: *GB-08, GB-12*
Oliveira, R.B.: *EA-13*
Oliveira, R.B.: *JA-03*
Oliveira, R.C.: *FA-01*
Oliveira, R.F.: *HA-34*
Oliveira, R.J.: *CD-02*
Oliveira, R.P.S.: *CA-10*
Oliveira, S.A.: *EC-11*
Oliveira, S.E.: *EA-17*
Oliveira, V.: *AA-18*

Oliveira, V.S.: *EC-06*
Oliveira, W.F.: *EB-10, EB-15*
Oliveira-Filho, A.G.: *DA-23*
Oliveira-Junior, M.S.: *HA-44*
Oliveira-Melo, P.: *CB-36*
Oliviera, E.L.: *GE-08*
Olorunfemi, F.G.: *CD-48*
Onuchic, J.N.: *DA-22*
Opoku, F.: *GD-24*
Orban, J.: *SP-16.01*
Ortiz, K.O.: *DA-24*
Oshiro, K.G.N.: *SP-06.04*
Osorio, R.P.: *EC-12*
Osternack, B.R.: *NB-06*
Outeiro, T.F.: *KA-22*
Ozu, M.: *BA-02, BA-04*

Pacheco, C.A.S.J.: *CD-35*
Pacheco, L.G.C.: *GC-18*
Pacheco, M.E.B.: *HA-09*
Padovani, C.T.J.: *GC-14, JB-05*
Pádua, T.A.: *GC-01*
Paiva, K.: *SPBN-12.03*
Paiva, K.M.: *HA-43*
Paiva, P.M.G.: *GD-03, GD-10*
Paixao, D.A.A.: *DA-50*
Pajić, T.: *JB-12*
Pal, K.: *DA-09*
Pallarola, D.: *AA-04*
Palma, A.S.: *DB-04*
Palmisano, G.: *EA-08*
Palomino, C.M.V.: *HA-20*
Paltian, J.J.: *HA-14*
Pannuzzo. M.: *SP-22.03*
Pant, S.: *SP-13.03*
Pantaleão, S.Q.: *CD-20, CD-21, DB-09*
Pantano, S.: *BA-16, DA-34*
Panzenhagen A.C.: *FB-07*
Paolino, A.: *SPBN-10.02*
Parada, C.A.: *CD-06*
Paredes, B.D.: *HA-44*
Parra, M.F.: *KA-03*
Parra, R.G.: *DA-33*
Parra, V.O.: *SP-09.03*
Parreira, G.M.: *EB-08*
Partipilo, M.: *GE-10*
Pascoal, I.G.: *DA-56*
Pascutti, P.G.: *KA-25. CD-35, DA-21, KA-23*
Passos, P.M.S.: *CA-07, DA-25, JA-06*
Passos, Y.M.: *DA-70*
Patel, A.: *GC-06*
Patriota, L.L.S.: *GD-10*
Patrocínio, A.O.: *EB-16*
Paul, S.S.: *DA-64*
Paula, L.: *JB-04*
Pavão, M.S.G.: *GB-09, KA-29*
Pavone, F.S.: *SP-12.03*
Paz, G.S.: *CB-34*
Paz, M.C.: *CB-02*
Pazenhagem, A.C.: *HA-28*
Pazin, W.: *AA-27*
Pazin, W.M.: *AA-11*
Paz-Marzolo, M.: *HA-19*
Pebay-Peyroula, E.: *SP-13.04*
Pedebos, C.: *KA-27*
Pedebos, C.: *SP-19.03*
Pedersen, P.A.: *BA-13*
Pedrote, M.M.: *DA-70*
Peiter, G.C.: *BB-07, GC-08, GD-06, GE-07*
Peixe, D.G.: *FB-02*
Peixoto De Almeida, M.: *CD-04*
Peixoto, A.S.: *EC-10*
Peixoto, D.: *HA-40*
Pelegrin, C.M.G De: *GD-12*
Pelegrin, P.: *JB-06*
Peppelenbosch, M.P.: *SP-06.03*
Perahia, D.: *KA-13*
Perdomo, R.T.: *CD-08, CD-18*
Pereira, A.K.H.: *CB-36*
Pereira, A.P.R.: *GD-05*
Pereira, A.S.: *FB-08*
Pereira, E.: *CD-04*
Pereira, E.A.: *CD-08*
Pereira, F.C.B.N.: *GC-08, GD-06*
Pereira, F.S.: *CC-13*
Pereira, F.S.O.: *EA-06*
Pereira, G.A.L.: *EB-15*
Pereira, G.R.C.: *KA-22, KA-28*
Pereira, H.J.V.: *DA-51*
Pereira, J.F.B.: *CA-10*
Pereira, J.P.J.: *EA-10*
Pereira, L.M.: *DA-47*
Pereira, M.B.M.: *HA-04, HA-25*
Pereira, M.D.: *EC-09, HA-36*
Pereira, R.: *EC-13, EC-16*
Pereira, T.C.S.: *CA-05*
Pereira, W.O.: *CA-06, HA-08*
Pereira. E.P.L.: *CA-04*
Perez Socas, L.B.: *BB-08*
Perez, C.A.: *SPBN-12.01*
Perez, K.P.: *AA-18*
Pérez-Gil, J.: *SP-17.04*
Perillo, M.A.: *AA-05, AA-29*
Perin, G.: *CD-15*
Perles, L.: *SPBN-08.02*
Peron, J.P.S.: *SP-06.02*
Persinoti, G.F.: *DA-26, DA-50, DA-68*
Pertici I.: *SP-12.03*
Pertinhez, T.A.: *EA-09*
Peruchetti, D.B.: *GC-01,HA-19*
Pessatti, T.B.: *DA-24*
Pessôa, C.A.: *CA-08*
Peters, V.M.: *GB-06*
Peterson, R.T.: *SP-05.03*
Petronilho, E.C.: *DA-70*
Petry, F.S.: *GD-23*
Petukhov, A.V.: *SP-16.04*
Pezzi, M.: *.. EA-09*
Philot, E.A.: *CD-21, KA-03, KA-13, KA-24*
Pianta, D.B.: *SPBN-09.02*
Piccinini, A.: *EA-02*
Piccirillo, E.: *CD-42*
Picco, C.: *BA-11*
Piccoli, J.P.: *DA-35*
Picinato, B.A.: *FB-10*
Pickholz, M.: *BA-07*
Pieropan, F.: *CA-04*
Pietrasanta, L.I.: *GA-11*
Pikula, S.: *AA-08, AA-13*
Pimpão, C.: *BA-13*
Pinhal, M.A.: *HA-24*
Pinhal, M.A.S.: *GB-04, GB-05, HA-20*
Pinheiro, A.A.S.: *GC-01, HA-19*
Pinheiro, A.S.: *DA-01, DA-56*

Pinheiro, F.C.: *CD-19, GD-09, GD-17, HA-13*
Pinheiro, G.: *DB-08*
Pinheiro, G.M.S.: *DA-32*
Pinheiro, M.D.S.: *JB-01, HA-26*
Pinheiro, N.D.: *SE-01*
Pinheiro, N.R.: *HA-46*
Pinto, A.M.F.: *SPBN-01.03*
Pinto, K.H.P.: *FB-01*
Pinto, M.R.: *DA-05*
Pinto, M.S.: *EB-14, EB-01, EB-18*
Pinto, S.S.: *EA-18*
Pires, R.A.: *EC-13*
Pizzuto, M.: *JB-06*
Plácido, A.: *BB-12*
Podolsky-Gondim, G.: *HA-27*
Poetini, M.R.: *FB-09, GD-09, GD-17, HA-13*
Poetini, R.P.: *FB-03*
Pohl, P.: *SP-17.03*
Pohlmann, A.R.: *SP-01.04*
Politi, M.J.: *AA-07*
Polloni, L.: *DA-10*
Polonio, C.M.: *SP-06.02*
Pontelli, M.C.: *HA-27*
Pontes, M.S.: *EB-13*
Poolman, B.: *GE-10*
Porcionatto, M.A.: *SP-06.01*
Porosk, L.: *DA-71*
Portilho, R.: *BA-01*
Portilho, R.S.: *BA-01, JB-09*
Porto, M.S.: *GE-08*
Pougy, K.C.: *DA-01*
Povinelli, A.P.R.: *CD-01*
Prata, B.A.: *CB-01*
Prazeres, P.H.D.M.: *CD-27, CD-43*
Prieto, M.: *AA-16, DA-73*
Prieto, M.J.: *CD-47*
Prigol, M.: *CD-19, FB-01, FB-03, FB-08, FB-09, GD-09, GD-17, HA-13*
Puga, M.A.M.: *JB-05*
Pusch, M.: *BA-11*
Puzer, L.: *CD-24*
Quartieri, E.: *EA-09*
Queiroz, I.F.: *EC-05*
Queiroz, R.C.N.: *KA-13*
Queiroz, R.F.: *EC-10, EC-13, EC-16*
Quines, C.B.: *EA-06*
Rabasović, M.: *JB-12*
Rade, L.: *DA-68*
Rade, L.L.: *DA-26*
Radi, R.: *SP-23.01*
Raíssa-Oliveira, B.: *HA-42*
Raji, H.M.: *CC-05*
Ramalho, S.R.: *GC-07*
Ramos, A.: *SP-23.03*
Ramos, A.F.: *CB-11, CC-15*
Ramos, A.P.: *AA-08, AA-09, AA-12, AA-13, AA-17, AA-19, AA-24, AA-32, EB-17*
Ramos, C.H.I.: *DA-04, DA-32, DA-59, DA-68, DA-30, DB-08*
Ramos, G.S.: *CD-44*
Ramos, J.A.: *HA-10, HA-32*
Ramos, L.: *DA-60*
Ramos, L.F.C.: *GA-02*
Ramos, R.: *JA-01*
Rangarajan, G.: *SP-16.01*
Rebello, L.G.: *EB-04*
Rebouças, J.S.: *EB-03*
Redner, P.: *DA-69*
Reis, A.A.A.S.: *GE-01*
Reis, A.S.: *CD-15, CD-17, HA-14*
Reis, O.A.O.: *CA-03*
Reis, R.M.V.: *HA-42*
Remigante, A.: *BA-11*
Requião, E.: *GD-15*
Resende, J.M.: *BB-09*
Resende, R.R.: *HA-42*
Resende-Lara, P.T.: *KA-03*
Rezende, H.K.: *GE-02, GE-04*
Rezin, G.T.: *EA-02*
Rial Hawila, M.R.: *DA-11*
Ribeiro, E.E.: *NB-04*
Ribeiro, E.H.P.: *EC-04*
Ribeiro, G.F.: *HA-38*
Ribeiro, L.N.M.: *CD-03, CD-29, CD-30, CD-36, CD-47*
Ribeiro, M.: *SP-10.01*
Ribeiro, M.B.: *HA-24*
Ribeiro, M.S.: *EB-01, EB-03, EB-12, EB-14, EB-18*
Ribeiro, P.R.: *CA-04, CA-05, CB-08, CD-07, GD-16, GD-15, GD-19, GD-20, GE-01, GE-03*
Ribeiro, S.B.M.H.A.: *KA-32*
Ricci-Azevedo, R.: *DA-05*
Riccitelli, F.: *JB-13*
Rieder, C.R.M.: *HA-28*
Riger, C.J.: *EC-03, EC-06, EC-09, EC-12, HA-36*
Righetto, G.M.: *DA-35*
Rigueira, J.P.M.: *HA-30*
Rinaldi, D.: *FA-03*
Rios, C.: *GA-01*
Rios, V.M.: *HA-26*
Riquelme, B.: *GB-07*
Riske, K.A.: *AA-01, AA-03, AA-23, AA-30*
Rivera, A.: *CA-04*
Roberto, G.F.: *HA-24*
Robinson, T.: *GE-10*
Rocchi, M.: *JB-11*
Rocha, A.: *CD-32*
Rocha, D.J.P.G.: *GC-18*
Rocha, E.T.: *SPBN-09.04*
Rocha, G.K.: *KA-04*
Rocha, L.: *GC-12*
Rocha, L.S.: *EC-16*
Rocha, L.W.P.S.: *NB-02*
Rocha, M.O.P.: *KA-14*
Rocha, M.P.O.: *KA-19*
Ródio, G.R.: *CB-27*
Rodrigues Da Silva, G.H.: *CD-03, CD-28, CD-36, CD-06, CD-47*
Rodrigues, A.P.: *CD-43*
Rodrigues, J.M.: *DA-51*
Rodrigues, K.C.: *CD-14*
Rodrigues, L.F.C.: *DA-30*
Rodrigues, M.Q.R.B.: *CC-16*
Rodrigues, R.: *GD-13*
Rodrigues, V.M.: *DA-10*
Rodrigues-Costa, F.: *SP-02.04*
Rogério, E.C.: *GC-08*
Rojas, F.I.: *CD-46*
Roman, D.H.H.: *SPBN-09.02*
Roman, E.A: *BA-12*

Romanovich, A.E.: *SP-16.04*
Ronsein, G.E.: *EA-08, EC-14*
Roos, W.H.: *AA-23*
Roos, W.H.: *SP-22.02*
Rosa Fernandes, L.: *EA-08*
Rosa, B.: *CD-34*
Rosado, F.R.: *BB-07, GC-08, GD-06, GE-07*
Rosa-Silva, H.: *HA-40*
Rosset, I.G.: *CB-27, CC-02, GE-07*
Rossi, J.P.: *DA-42, FA-03*
Rossi, R.: *FA-03*
Rossini, O.N.: *CD-31*
Roy, S.: *SP-16.01*
Rueda, S.F.: *DA-74*
Ruiz, G.C.M.: *AA-11*
Ruller, R.: *CC-06, CC-09, DA-50*
Rutter, J.: *DB-07*
Ruysschaert, J.M.: *JB-06, SP-24.02*
Ryan, R.M.: *SP-13.03*
Ryan, T.M.: *CA-10*

Sabino, E.C.: *HA-15*
Sachetto-Martins, G.: *DA-01*
Sacramento, M.: *CD-17*
Sadakane, K.: *AA-15*
Saffioti, N.: *FA-03*
Saffioti, N.A.: *AA-04*
Sagazio, G.: *SPBN-11.01*
Sairre, M.I.: *CD-25*
Saito, C.P.B.: *CB-22, GC-03, GC-15*
Saito, D.: *CB-22, GC-03, GC-15*
Saito, V.I.: *CD-13*
Sakamoto, N.: *JA-04*
Sakuta, H.: *AA-15*
Salbego, C.G.: *CA-04, GD-23*
Saldanha, T.: *EC-06*
Salgia, R.: *SP-16.01*
Salgueiro, M.: *SP-16.02*
Salinas, R.K.: *BA-09*
Salla, D.H.: *EA-02*
Salles, S.: *KA-29*
Salmazo, P.V.: *DA-26*
Salnikov, E.: *BB-09*
Salvador, G.H.M.: *KA-18*
Salvego, C.A.: *EB-14*
Sampaio, S.B.: *CB-01*
Sancatldo, G.: *EB-05*
Sanches, F.S.: *CD-22*
Sant'anna, C.M.R.: *EC-12*
Santa Cruz, G. A.: *KLBN-04.*
Sant'ana, A.N.: *JB-10*
Santana, F.: *CB-01*
Santana-Silva, M.C.: *DB-12*
Sant'anna, C.: *EA-16*
Sant'anna, S.S.: *CB-31*
Santiago, E.F.: *EB-13*
Santiago, H.C.: *HA-42*
Santiago, P.S.: *DA-47, DA-60*
Santisteban, A.N.: *DB-06*
Santisteban, A.R.N.: *DB-13*
Santos, A.A: *EC-10*
Santos, A.G.R.: *EA-06*
Santos, A.K.: *CD-27, CD-43, HA-42*
Santos, A.P.P.: *HA-48*
Santos, A.R.: *CC-06, CC-09*
Santos, B.A.A.: *GD-01, GD-16, GD-20*
Santos, B.G.: *GD-23*
Santos, B.L.: *GD-18*
Santos, B.S.: *EB-03, EB-15*
Santos, C.C.: *GD-18*
Santos, C.C.A.: *CB-36*
Santos, C.M.: *CB-01*
Santos, C.S.: *CA-08, KA-10*
Santos, D.C.: *SE-01*
Santos, D.E.S.: *KA-06*
Santos, D.M.R.C.: *DA-51*
Santos, E.A.: *GC-09*
Santos, E.S.: *DA-47*
Santos, F.L.E.: *DA-04, DA-59*
Santos, I.S.: *HA-46*
Santos, J.F.: *HA-12*
Santos, J.S.: *EB-13*
Santos, K.F.: *EC-03*
Santos, K.R.: *JA-06*
Santos, L.: *FB-07*
Santos, L.: *HA-40*
Santos, L.F.P.: *CB-12*
Santos, L.O.: *GC-13, GC-17*
Santos, L.S.: *CB-13*
Santos, M.: *CC-16*
Santos, M.C.: *GC-02*
Santos, M.Dos: *CB-02*
Santos, M.F.: *GE-02, GE-04*
Santos, N.C.: *BB-11, BB-12, FB-11*
Santos, N.C.: *SP-06.04*
Santos, N.S.: *CD-04*
Santos, P.C.: *GD-01, GD-15, GD-16, GD-20*
Santos, R.P.: *GB-08, GB-12*
Santos, S.X.: *CC-07*
Santos, T.C.: *EC-10*
Santos, T.L.: *BB-09*
Santos, V.F.Dos.: *KA-06*
Santos, V.H.F.: *FB-10*
Santos, W.O.: *NB-07*
Santos-Filho, N.A.: *DA-35*
Santos-Oliveira, J.R.: *JB-04*
Santos-Silva, A.P.: *EA-15*
Saponi, C.F.: *CA-10*
Sardi, J.C.O.: *CB-14, GC-07, GD-04*
Sarkis, G.S.: *CB-22*
Sarmento, M.J.: *FB-11*
Sarmento-Neto, J.F.: *EB-03*
Sarto, C.: *DA-74*
Sattin, S.: *SP-05.01*
Savegnago, L.: *HA-13*
Savio, E.: *SPBN-10.02*
Savio, F.: *BA-15*
Sbano, A.: *JB-08*
Sbaraglini, M.L.: *CD-26*
Scalzo, S.: *CD-43*
Scanavachi, G.: *AA-16, DA-42*
Scandiuzzi, R.T.: *DA-67*
Scherrer, E.C.: *JB-03*
Schipper, P.: *SP-01.03*
Schmidt, M.: *SP-02.02*
Schoehn, G.: *SP-13.04, SP-02.04*
Schroder, A.: *BA-08*
Schuster, M.F.: *GE-05*
Schwarzbaum, P.J.: *AA-04*
Scott, A.L.: *CD-21, DB-09, KA-03, KA-13, KA-24*
Sebastiao, I.: *DA-47, DA-60*
Sebinelli, H.G.: *AA-17*
Sebollela, A.: *DA-19, HA-46, HA-17, HA-27*
Segreto, H.: *SPBN-08.01*
Seixas, F.A.V.: *BB-07, CD-33*
Sejdiu, B.I.: *SP-19.01*

Selistre-De-Araujo, H.S.: *HA-18, HA-35, HA-41*
Sen, R.: *DA-09*
Sen, U.: *DA-09*
Sena, G.: *SPBN-12.03*
Sengupta, J.: *DA-64*
Separovic, F.: *SP-02.01*
Sepulveda, A.F.: *KA-30*
Sergides, M.: *SP-12.03*
Serino-Silva, C.: *CB-38*
Sessa, D.P.: *GC-02*
Setti, G.: *EA-09*
Setubal, J.C.: *FB-02, HA-15, HA-37*
Setubal, J.C.: *SP-11.02*
Sewell, B.T.: *DB-03*
Shashkova, S.: *DA-48*
Shechtman, Y.: *PL-09.*
Shimon, S.M.M.: *. HA-20*
Shin, S.R.: *SP-06.01*
Shpironok, O.G.: *SP-16.04*
Shuma, M.S.: *BB-06*
Sibinelli-Souza, S.: *GC-06*
Sidhu, S.: *SP-20.01*
Sigaut, L.: *GA-11*
Sil, P.: *DA-64*
Silonov, S.A.: *SP-16.04*
Silva E Alves, A.T.: *GD-08*
Silva Jr, W.A.: *SP-11.03*
Silva, A.A.M: *CB-02, CB-04, CC-16*
Silva, A.B.: *GC-09*
Silva, A.B.A.: *HA-29*
Silva, A.R.: *GD-10*
Silva, A.R.M.: *EA-08*
Silva, B.M.: *EB-11*
Silva, B.P.: *EC-10, EC-16*
Silva, C.A.: *DA-45*
Silva, C.F.N.: *EB-11, CA-03*
Silva, C.M.: *HA-48*
Silva, C.P.: *GA-01*
Silva, C.R: *EB-14, EB-18*
Silva, C.S.: *CD-31*
Silva, C.V.: *GE-08*
Silva, D.K.V.: *DA-05*
Silva, E.R.: *CB-15, DA-71*
Silva, F.A.A.: *DA-03, DA-58*
Silva, F.N.: *CC-13*
Silva, F.S.: *JB-03*
Silva, G.F.: *CB-09, CB-11*
Silva, G.H.R.: *CD-30*
Silva, G.M.: *CB-36*
Silva, G.S.: *FB-03*
Silva, Í.C.: *CD-04*
Silva, I.C.: *SP-06.04*
Silva, I.T.: *SP-11.03*
Silva, J.B.: *NB-14*
Silva, J.E.S.: *KA-07*
Silva, J.G.: *JB-03*
Silva, J.H.C.: *CA-04*
Silva, J.J.: *CB-06*
Silva, J.L.: *DB-10, DA-52, DA-61, DA-70*
Silva, J.L.C.: *CC-03*
Silva, J.N.: *KA-10*
Silva, J.O.: *CC-16, CB-02*
Silva, K.C.: *GD-18*
Silva, K.E.: *EA-07*
Silva, L.E.: *EA-02*
Silva, L.L.S.: *GD-03*
Silva, L.S.: *GC-01*
Silva, M.C.: *GE-09*
Silva, M.L.: *HA-02, JB-08, KA-02, KA-31*
Silva, M.M.: *CD-43*
Silva, M.R.: *EA-02*
Silva, M.R.P.: *FB-01*
Silva, N.M.C.: *BB-11*
Silva, N.S.M.: *DA-30, DA-63*
Silva, P.: *AA-18*
Silva, R.: *GC-11*
Silva, R.: *HA-10*
Silva, R.A.: *SP-06.03*
Silva, R.M.V.: *SPBN-08.03*
Silva, R.P.: *EC-10, EC-16*
Silva, R.R.: *KA-15*
Silva, R.R.C.: *KA-07*
Silva, R.S.F.: *CD-40*
Silva, S.C.C.: *GD-03*
Silva, S.M.: *CB-29*
Silva, S.P.: *GD-10*
Silva, T.D.: *KA-18*
Silva, T.P.: *DA-51*
Silva, V.D.A.: *CA-04, CD-07, CD-22, GD-18*
Silva, V.O.: *HA-42*
Silva, W.N.: *CD-27*
Silva, W.W.G.: *KA-07*
Silva, Y.M.: *GA-02*
Silva-Aguiar, R.P.: *GC-01, HA-19*
Silva-Freitas, M.L.: *JB-04*
Silva-Lopez, R.E.: *DA-65, GD-11, GD-21*
Silveira, A.K.: *FB-07, JB-10*
Silveira, G.P.M.: *CB-24, CB-31*
Silveira, I.B.M.: *CD-18*
Silvestre, D.H.: *KA-31*
Silvestrini, V.C.: *HA-29*
Simão, A.N.C.: *SP-11.03*
Simão, R.C.G.: *CC-03*
Simas, A.L.O.: *CC-04*
Simelane, M.B.C.: *GD-24*
Simões, I.T.: *DA-55*
Simões, R.C.: *CB-19*
Simões, R.P.: *KA-05*
Simon, K.A.: *SE-01*
Simons, S.: *GA-03*
Sindelar, C.V.: *SP-04.02*
Sinica, V.: *SP-13.02*
Siqueira, R.D.: *HA-11*
Sirena, D.H.: *JB-10*
Siri, M.: *SP-01.02*
Sito Junior, E.A: *GC-08*
Sizun, C.: *DA-16*
Slochower, D.: *SP-07.03*
Slotboom, D.J.: *GE-10*
Smith, I.: *KA-27*
Soares, L.: *JB-04*
Soares, L.A.L.: *CB-13*
Soares, M.P.: *CD-15*
Soares, N.M.: *HA-28*
Soares, P.S.: *CD-15*
Soares, R.De O.: *KA-25*
Soares, T.A.: *CD-16*
Soares, T.A.: *KA-06*
Sobolevsky, A.I: *SP-13.02*
Sobral, D.V.: *NB-01, NB-15*
Sobti, M.: *SP-13.03*
Somensi, N.: *HA-28*
Sondermann, M.N.: *SPBN-06.02*
Soñora, M.: *DA-34*
Sorrentino, L.: *SP-05.01*
Sosabowski, J.: *SPBN-02.01*
Sousa, A.: *DA-68*
Sousa, A.S.: *DA-26*
Sousa, B.: *SP-16.02*
Sousa, G.M.: *DA-66*
Sousa, G.S.: *DA-52*
Sousa, J.D.: *HA-23*
Sousa, T.: *DA-73*
Souza Alves, C.C.: *CB-06, JB-03*
Souza Jr, D.R.: *EA-08*

Souza S.A.L.: *SPBN-05.04, SPBN-05.02*
Souza, C.O.: *DA-60*
Souza, D. N.: *SPBN-08.03*
Souza, E.M.: *SPBN-06.01*
Souza, E.R.P.: *EC-09, HA-36*
Souza, F.J.: *GC-20*
Souza, G.C.A.: *NB-08*
Souza, I.S.: *EA-02*
Souza, J.P.: *HA-27*
Souza, J.S.: *DA-71*
Souza, K.L: *EC-08*
Souza, K.M.: *CB-09, CB-17, EA-13, GC-05, JA-03*
Souza, K.R.: *BB-09*
Souza, L.C.M.: *KA-16*
Souza, L.E.A.: *CD-23*
Souza, M.C.: *GC-01*
Souza, M.O.: *EC-05*
Souza, R.F.: *GC-06*
Souza, S.G.H.: *GE-02*
Souza, T.H.S.: *EB-03*
Souza, T.P.P.: *CC-16*
Souza-Santos, D.: *SPBN-07.01*
Soveral, G.: *BA-13, FB-11*
Spadoti, L.M.: *GD-08*
Spagnol, V.: *CA-07, DA-25, JA-06*
Spinozzi, F.: *CB-25*
Squina, F.: *SP-06.05*
Stachowiak, J.C.: *SP-22.01*
Stagni, C.: *JB-11*
Stanisic, D.: *FB-06*
Stefani, F.D.: *SP-12.01*
Stella, L.: *JB-13*
Stelling, M.P.: *GB-09, KA-29*
Stelling, M.P.: *SPBN-12.02*
Stephens-Hermes, F.: *DA-69*
Stevanovic, K.: *BA-14*
Stevanović, K.: *JB-12*
Stewart, A.G.: *SP-13.03*
Steyaert, J.: *SP-13.04*
Storsberg, J.: *CA-02*
Strzelecka-Kiliszek, A.: *AA-13*
Subía, M.: *FA-02*
Suita, J.: *SPBN-11.04*
Suzuki, H.: *AA-10*
Suzuki, K.: *SPBN-06.02*
Swaan, P.W.: *SP-01.01*
Tabata, K.: *SP-18.01*
Tadokoro, M.M.: *JB-05*
Tahara, E.B.: *KA-14, KA-19*
Tajkhorshid, E.: *SP-13.03*
Takada, S.: *DA-41*
Talevi, A.: *CD-26*
Talvani, A.: *EC-05*
Tanaka, A.S.: *CB-38, DA-03, DA-58, GA-07*
Tanaka-Azevedo, A.M.: *CB-24, CB-31, CB-38*
Tasca, C.I.: *HA-49*
Tasic, L.: *DA-67, FB-06, HA-33*
Tasso, T.T.: *CA-03*
Taveira, G.B.: *BB-03*
Tavernarakis, N.: *SP-18.04*
Teibo, J.O.: *CD-48, HA-21*
Teixeira, A.: *JB-01*
Teixeira, C.: *BB-12*
Teixeira, C.S.: *CB-30, CB-37*
Teixeira, D.E.: *GC-01*
Teixeira, E.M.: *GD-21*
Teixeira, E.M.G.F.: *GD-11*
Teixeira, F.R.: *CA-07, DA-25, HA-18, JA-06*
Teixeira, I.M.: *KA-04*
Teixeira, M.H.: *KA-09*
Teixeira, O.: *DB-14*
Teixieira-Dutra, M.R.: *JB-04*
Teles, M.F.: *EC-13*
Teles-Souza, J.: *HA-45*
Temprana, C.F.: *SP-01.02*
Terakawa, T.: *DA-41*
Teranishi, H.: *SP-18.01*
Terazima, M.: *SP-03.01*
Terenzi, H.: *DA-24*
Tersariol, I.L.S.: *HA-20*
Teruel, J.A.: *AA-22*
Theodoro, T.R.: *HA-24*
Thomas, G.A.: *KLBN-02.*
Thomaz, D.T.: *HA-49*
Thomé, C.H: *HA-29*
Thompson, M.J.: *SP-13.04*
Tiefensee Ribeiro, C.: *HA-28, HA-40*
Tieleman, P.D.: *SP-19.01*
Tittanegro, T.H.: *HA-03*
Todorović, N.: *BA-14, JB-12*
Tognato, R.C.: *CD-24*
Tognon, R.R.: *HA-04*
Tognon-Ribeiro, R.: *HA-25, HA-47*
Tokatlidis, K.: *SP-23.02*
Tolchard, J.: *DA-06*
Torquato, R.J.S.: *DA-58*
Torquato, R.S.J.: *DA-03*
Torres, H.S.: *CD-39*
Torres, M.T.: *SP-06.04*
Torres-Bonfim, N.E.S.M.: *DB-09, KA-13*
Toscani, A.M.: *DA-46*
Tostes, F.: *EA-01*
Tourlomousis, P.: *JB-06*
Tovar, A.M.F.: *GB-08, GB-12*
Tozetti, I.A.: *GC-04, JB-05*
Traina, F.: *HA-29*
Trajano, E.A.: *DA-40*
Trajtenberg, F.: *SP-04.03*
Tramontina, R.: *SP-06.05*
Tremarin, B.: *CA-02*
Trevisan, A.C.: *SPBN-10.03*
Triboni, E.: *AA-07*
Trinchieri, G.: *PL-03.*
Trindade, D.M.: *SP-02.04*
Trindade, L.M.: *NB-03*
Trindade, V.M.T.: *GD-23*
Trofimov, Y.A.: *SP-13.02*
Troiano, C.: *JB-13*
Trujillo, M.: *DA-23*
Tsai, S.M.: *GC-03, GC-15*
Tschon, M.: *JB-11*
Tsubone, T.: *EB-09*
Tsubone, T.M.: *EB-16*
Tsumoro, K.: *AA-15*
Turner, N.J.: *SP-06.05*
Turoverov, K.K.: *SP-16.04*

Uberti, A.F.: *GC-10*
Uchański, T.: *SP-13.04*
Ueda, H.: *SP-08.01*
Umemura, K.: *FA-04*
Ungri, A.M.: *CB-18*
Urlaub, H.: *DA-46*
Ushiyama, R.: *AA-10*
Uversky, V.N.: *SP-16.04*

Valadares, H.M.S.: *HA-42*
Valadares, Y.M.: *JB-03*
Valente, A.C.M.M.: *HA-25, HA-47*
Valente, T.N.S.: *HA-48*
Valle, G.S.: *CB-07*
Vallejos, V.M.C.: *CD-10*
Vallejos, V.M.R.: *CD-27, CD-44*
Van Der Eerden, B.C.J.: *SP-06.03*
Van Kerckhoven, S.: *AA-09*
Vandenberg, R.: *SP-13.03*
Varal, M.: *FB-07*
Vargas, A.P,: *HA-29*
Vasconcelos, A.A.: *DA-15*
Vasile, F.: *SP-05.01*
Vasques, L.H.: *KA-05*
Vassalli, M.: *GA-05*
Vaz-Busoli, F.A.: *DA-58*
Veiga, M.: *GD-12*
Vela, M.E.: *BB-01*
Velloso-Rodrigues, C.: *HA-30, HA-48*
Venâncio, T.: *CD-09*
Vêncio, R.Z.N.: *FB-10*
Vendruscolo, E.C.G.: *GE-02, GE-04*
Ventura, T.L.B.: *BB-03*
Venturin, G.: *NB-11*
Vergani Junior, C.A.: *EA-18*
Veríssimo, N.V.: *CA-10*
Verly, R.M.: *BB-09*
Veschi, E.A.: *AA-08, AA-13*
Vetri, V.: *DA-54, EB-05*
Viana, C.E.: *FB-08*
Viana, C.T.R.: *CD-43*
Viana, L.S.: *HA-22*
Viani, M.V.: *EA-09*
Vicente, E.F.: *BB-04*
Vidal, A.: *NB-11*
Viegas, R.: *DA-33*
Vieira, D.P.: *NB-02, NB-13*
Vieira, D.P.: *SPBN-05.01*
Vieira, M.M.: *GD-02*
Vieira, M.S.: *HA-42*
Vieira, P.S.: *DA-68*
Vieira, R.P.: *EB-08*
Vieira, R.P.: *KA-14, KA-19*
Vieira, T.C.R.G.: *DA-52, DA-61, DA-70, DB-10*
Vieira-Da-Motta, O.: *BB-03*
Vietri, A.: *KA-26*
Vigato, A.A.: *CD-25*
Vigil, M.A.: *FA-03*
Vila-Viçosa, D.: *KA-21*
Vilela, T.C.: *EA-02*
Vilke, J.M.: *CB-23*
Villela-Nogueira, C.A.: *HA-10*
Visentin, A.: *SP-16.02*
Vital, K.D.: *NB-09*
Vitale, P.A.M.: *BA-09*
Vital-Fujii, G.D.: *EB-11*
Vitali, V.: *BA-06*
Vitor, L.N.A.: *CB-30*
Vivarelli, L.: *JB-11*
Vlachova, V.: *SP-13.02*
Vogel, C.I.G.: *CB-20, EA-13, GC-05, JA-03*
Volpon-Santos, M.: *HA-27*
Von Bilderling, C.: *GA-11*
Von Krüger, W.M.A.: *GC-12, GC-13, GC-17*

Wainwright, M.: *EB-11*
Wallace, B.A.: *DA-06*
Wallace, B.A.: *SP-02.03*
Wang, Y.H.: *SP-07.03*
Ward, R.J.: *DA-28, EA-03*
Wattret, G.: *SP-21.02*
Watts, A.: *PL-10.*
Weber, B.: *DB-03*
Weissenhorn, W.: *SP-22.02*
Werner, M.F.P.: *GB-13*
Whittingham, S.: *SPBN-01.02*
Wichert-Ana, L.: *SPBN-10.03*
Wilhelm, E.A.: *CD-15, CD-17, HA-14*
Wilke, N.: *AA-29*
Wilkinson, A.J.: *SP-17.02*
Wohnrath, K.: *CA-08*
Wolynes, P.G.: *DA-33*
Wondrak, G.T.: *SP-10.02*
Woolley, A.: *SP-03.02*
Wragg, D.: *BA-13*
Wrenger, C.: *DA-43*
Wu, Q.: *SP-13.03*
Wurtele, M.R.A.: *DA-03*
Xavier, D.G.: *JB-01*
Ximenes, V.F.: *DA-37*
Xu, T.: *PL-05.*
Yadav, M.: *DA-09*

Yakimenko, I.: *AA-22*
Yamaga, L.Y.I.: *SPBN-09.03*
Yamazaki, M.: *BB-05, BB-06*
Yáñez, C.: *CD-32, CD-34*
Yatsuda, R.: *EC-10*
Yoguim, M.I.: *DA-37*
Yokaichiya, F.: *CA-02, CD-29, CD-30, KA-30*
Yoneda, J.S.: *CB-25*
Yonekawa, M.K.A.: *CB-29*
Yoshikawa, K.: *AA-15*
Yoshimori, T.: *SP-18.01*
Yoshinaga, M.Y.: *HA-37*

Zaitseva, E.: *BA-08*
Zakharian, E.: *SP-13.02*
Zambuzzi, W.F.: *SP-06.03*
Zamin, L.L.: *GE-05*
Zanchin, N.I.T.: *DA-27*
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Zanoelo, F.F.: *CB-29, CC-04, CC-06, CC-09*
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