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ABSTRACT

# 20th IUPAB International Congress, 45th SBBf Congress and 50th Annual Meeting of SBBq

## Speaker's Abstracts



**IUPAB-SBBf-SBBq Program**
**Keynote Lectures**

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

**Richard Henderson** [1]
[1]MRC Laboratory of Molecular Biology, (Cambridge CB2 0QH, U.K.)

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]
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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

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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-05. - 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-06. - Carbon dioxide redox metabolites in eustress and oxidative distress

**Ohara Augusto** [1]
[1]Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo (SP, Brazil)

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 (CO2) 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

**Ramon Latorre** [1]
[1]Centro Interdisciplinario de Neurociencia de Valparaíso, Facultad de Ciencias, Universidad de Valparaíso (Valparaíso, Chile)

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**

**Angela M. Gronenborn** [1]
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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**

**Yoav Shechtman** [1]
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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]
[1]Biochemistry Department, University of Oxford (Oxford, UK)

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

**Peter W. Swaan** [1]

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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

Macarena Siri[1,2], Carlos Facundo Temprana[1], Mariano Grasselli[1,2], **Silvia del Valle Alonso** [1,2]

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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]
[1]Division of BioTherapeutics, Leiden Academic Centre for Drug Research, Leiden University (Leiden, The Netherlands)

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

**Adriana Raffin Pohlmann** [1]
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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

**Frances Separovic** [1]
[1]School of Chemistry, Bio21 Institute, University of Melbourne (VIC, Australia)

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

**Prof. Bonnie Ann Wallace** [1]
[1]Institute of Structural and Molecular Biology, Birkbeck College, University of London (, UK)

INTRODUCTION
Voltage-gated sodium channels are responsible for the conductance of sodium ions across cell membranes in neurological and cardiovascular tissues.

OBJECTIVES
They are essential targets for drug design, with particular relevance in epilepsy, cardiac conditions and pain diseases.

MATERIALS AND METHODS
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.

DISCUSSION AND RESULTS
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.

CONCLUSION
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

**Masahide Terazima** [1]

[1]Chemistry, Kyoto University (Kyoto, Japan)

INTRODUCTION

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.

OBJECTIVES

In this paper, the principle and studies on blue-light sensor proteins, phototropins (phots), is presented.

MATERIALS AND METHODS

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.

DISCUSSION AND RESULTS

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.

CONCLUSION

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

**Andrew Woolley** [1]

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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

**Matias Zurbriggen** [1]

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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?

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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

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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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INTRODUCTION
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.

OBJECTIVES
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.

MATERIALS AND METHODS
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.

DISCUSSION AND RESULTS
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.

CONCLUSION

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

**Supported by:** Agencia Nacional de Investigacion e Innovacion – Uruguay

### SP-04.04 - Regulation of the photosynthetic AB-GAPDH via self-assembly

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INTRODUCTION

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 heterotetramer formed by A and B-subunits, and the homotetramer 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.

OBJECTIVES

The present study is aimed at disclosing the structural basis of the CTE-dependent regulatory mechanism.

MATERIALS AND METHODS

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.

DISCUSSION AND RESULTS

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.

CONCLUSION

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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INTRODUCTION

*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 extracytoplasmic domains that bind the antibiotic (BlaR1 and MecR1) or that might sense it (VraS and VraT).

OBJECTIVES
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.

MATERIALS AND METHODS
We combined the use of ampicillin-derived photoprobes, reporter strains, recombinant protein expression, phosphorylation assays, western blot, band-shift assays and electron microscopy.

DISCUSSION AND RESULTS
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.

CONCLUSION
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
**Supported by:** ANPCyT, CONICET

## SP-05. 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
**Supported by:** ERC-StG-2017 (grant n. 758108)

### 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-05.03 - Discovery of Nanomolar Myeloperoxidase Inhibitors with Anti-Arthritis Properties: A Computational, in vitro and in vivo study**

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INTRODUCTION

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.

## OBJECTIVES

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.

DISCUSSION AND RESULTS

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.

CONCLUSION

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-05.05 - Interaction of genetically encoded photosensitizers with scintillating nanoparticles for X-PDT**

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INTRODUCTION
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).

OBJECTIVES
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.

MATERIALS AND METHODS
To do so the time-resolved and static fluorescence, TEM, DLS and radioluminescence techniques were used.

DISCUSSION AND RESULTS
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.

CONCLUSION
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-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 superflexible 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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INTRODUCTION

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.

OBJECTIVES

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.

MATERIALS AND METHODS

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.

DISCUSSION AND RESULTS

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.

CONCLUSION
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

## 05104 - 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

## 05022 - Systems biology and biomarkers for human disorders

### 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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INTRODUCTION
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.

OBJECTIVES
This work aimed to investigate possible alterations promoted by HPU in neuroinflammation and tau phosphorylation.

MATERIALS AND METHODS
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.

DISCUSSION AND RESULTS
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.

CONCLUSION
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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INTRODUCTION
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.

OBJECTIVES
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.

MATERIALS AND METHODS
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.

DISCUSSION AND RESULTS
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.

CONCLUSION
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. 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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INTRODUCTION
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.

OBJECTIVES
The aim of this study was to investigate the role of mitofusins in oocytes as well as their impact on fertility and offspring viability.

MATERIALS AND METHODS
Towards that, we used a mouse model with targeted deletion of *Mfn1* and/or *Mfn2* in oocytes.

DISCUSSION AND RESULTS
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*.

CONCLUSION
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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**Introduction:** 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.

**Objectives:** 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).

**Methods and results:** 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.

**Conclusion:** 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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INTRODUCTION
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.

OBJECTIVES
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.

MATERIALS AND METHODS
We applied molecular dynamics simulations and the free energy methods umbrella sampling, metadynamics and linear integration energy.

DISCUSSION AND RESULTS
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.

CONCLUSION
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 ( $^{1}H_2O$), 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
**Supported by:** The work was partly funded by the German Federal Ministry of Education and Research BMBF within the “Advanced UV for Life” projects “BlutRedox” (Grant: 03ZZ0140A) and “VIMRE” (Grants: 03ZZ0146A-D

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

**Tania Mateus Yoshimura** [1], Ismael Pretto Sauter[1], Martha Simões Ribeiro[1]
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INTRODUCTION
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.

OBJECTIVES
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.

DISCUSSION AND RESULTS
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.

CONCLUSION
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-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

**João Carlos Setubal [1]**
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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
**Supported by:** UKVI, MRC

## 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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INTRODUCTION

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.

OBJECTIVES

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.

MATERIALS AND METHODS

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.

DISCUSSION AND RESULTS

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.

CONCLUSION

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-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 $\alpha/\delta$ versus $\alpha/\gamma$ 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 precondensation 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

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### 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

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### SP-17.03 - 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-18.02 - 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 nucleocytoplasmic 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 polyunsaturated 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.

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**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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INTRODUCTION
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).

OBJECTIVES
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.

MATERIALS AND METHODS
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.

DISCUSSION AND RESULTS
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.

CONCLUSION
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 - Variation in GPCR signaling: Implications for drug discovery**
**Madan Babu** [1]
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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-20.02 - 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-20.03 - 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-20.04 - biophysics of peptiplexes based on cell penetrating peptides**

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INTRODUCTION

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].

OBJECTIVES

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.

MATERIALS AND METHODS

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.

DISCUSSION AND RESULTS

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.

CONCLUSION

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-21. IUBMB Symposium: Science Education**

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

**Erin L. Dolan** [1]

[1]Biochemistry & Molecular Biology, University of Georgia (Georgia, United States)

**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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INTRODUCTION
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.

OBJECTIVES
This App shows these steps using images, associated texts and cognitive challenges.

MATERIALS AND METHODS
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.

DISCUSSION AND RESULTS

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.

CONCLUSION

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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INTRODUCTION
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.

OBJECTIVES
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.

MATERIALS AND METHODS
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,

DISCUSSION AND RESULTS
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.

CONCLUSION
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-22.05 - The SARS-CoV-2 nucleocapsid protein N-terminal domain phase separation is triggered by the serine-rich region and modulated by TRS binding**
**Clara Malizia Leal Ferreira da Motta [1]**, Mariana Juliani do Amaral[1], Jéssica Moreira de Azevedo[1], Fábio Ceneviva Lacerda Almeida[1], Marcius da Silva Almeida[1], Anderson de Sá Pinheiro[1]
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INTRODUCTION
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).
OBJECTIVES
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).

MATERIALS AND METHODS
Recombinant N-NTD and N-NTD-SR from SARS-CoV-2 were obtained by expression in E. coli. Images were acquired by DIC microscopy.

DISCUSSION AND RESULTS
N-NTD-SR but not N-NTD formed spherical, micronszied 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.

CONCLUSION
These results provide a mechanism by which SARS-Cov-2 N regulates viral transcription and replication.
**Keywords:** SARS-CoV-2, Nucleocapsid, Phase Separation

## 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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INTRODUCTION
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.

OBJECTIVES
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.

MATERIALS AND METHODS
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.

DISCUSSION AND RESULTS
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.

CONCLUSION
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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INTRODUCTION

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.

OBJECTIVES

In this work, we questioned ourselves if something special in quercetin could explain its high reactivity with Cyt c.

MATERIALS AND METHODS

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.

DISCUSSION AND RESULTS

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.

CONCLUSION

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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INTRODUCTION

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.

OBJECTIVES

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 $PrP^{90-231}$ and $PrP^{120-231}$.

MATERIALS AND METHODS

By microscopy and a range of biophysical techniques, we show that H139 and H176 are potential residues that mediate LLPS.

DISCUSSION AND RESULTS
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.

CONCLUSION
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.

**Nicholas J. Gay** [1], Martin Moncrieffe[1], Monique Gangloff[1]
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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

**Jean Marie RUYSSCHAERT** [1]
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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

**Roman Jerala** [1]
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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.
**SBBn Program**

### 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
**Supported by:** US-NIAID grant #U19-A1067773; US-BARDA HHS01201000008C

**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

## 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-02- Biochemistry and biotechnology innovations for health**

**SPBN-02.03 - 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-03- The protection of the biodiversity using nuclear analytical techniques**

**SPBN-03.01 - 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.

**SPBN-03.03 - Chemical diversity in tree species from Caatinga**

**Elvis Joacir De Franca [1]**
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INTRODUCTION
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.

OBJECTIVES
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.

DISCUSSION AND RESULTS
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.

CONCLUSION
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
**Supported by:** FACEPE, FAPESP, CNPq and CAPES

**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**

**Ausrele Kesminiene [1]**
[1]Environmental and Life style Epidemiology Branch, International Agency for Research on Cancer (Rhône, France)

INTRODUCTION
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.

OBJECTIVES
EPI-CT aims at evaluating cancer risk potentially related to radiation doses from CT in childhood and adolescence.

MATERIALS AND METHODS
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.

DISCUSSION AND RESULTS
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).

CONCLUSION
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**
**Maria Paula Curado** [1]
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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-04.04 - Biodosimetry for assessing human chromosome instability**
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INTRODUCTION
Chromosome instability (CIN), a prevalent pattern of genome instability, is characterized by defects in DNA repair mechanisms, leading to chromosomal breakages and increased cellular radiosensitivity. If present in normal tissues, this increased radiosensitivity can limit cancer radiotherapy in an individual with CIN. Thus, the detection of radiosensitivity levels is crucial for these kinds of patients before starting radiotherapy. For instance, Fanconi Anemia (FA) is a rare autosomal recessive genetic disorder in which individuals present severe CIN, including spontaneous chromosomal aberrations. The standard laboratory tests for FA diagnosis use extremely hazardous substances to induce chromosomal aberrations in peripheral blood lymphocytes, such as diepoxybutane (DEB) or mitomycin C (MMC), which induces much more chromosomal alterations and breaks in FA cells than in normal control. On the other hand, Biodosimetry (biological dosimetry) is a methodology widely applied to correlates radioinduced biological endpoints with the absorbed doses. The scoring of chromosome aberrations from samples of peripheral blood lymphocytes is the most reliable method to evaluate real or suspect personal exposure to ionizing radiation.

OBJECTIVES
This work investigated the application of Biodosimetry as a new methodology for FA diagnosis, based on the scoring of background and radiation-induced chromosome aberrations following the International Atomic Energy Agency guidelines (2011).

MATERIALS AND METHODS
This study analyzed 28 subjects grouped in: normal individuals (14) and DEB-positive patients (14). A 3 mL peripheral blood sample was collected from each subject and irradiated using a 6 MV linear accelerator delivering 2 Gy.

DISCUSSION AND RESULTS
The cytogenetic analyses showed remarkable differences in shape, frequency, and distribution of chromosome aberrations (e.g., dicentrics, fragments, and rearrangements) between the two groups. Hence, it was possible to establish a FA pattern for DEB-positive individuals.

CONCLUSION
This new application of Biodosimetry might provide an alternative approach for assessing chromosome instability, especially for FA diagnosis, that is safer for cytogeneticists and equally reliable as DEB tests.
**Keywords:** Chromosome instability, DEB test, Biodosimetry
**Supported by:** IAEA, CNPq and FACEPE

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

**SPBN-05.01 - 131I-Ixolaris development as a theragnostic agent: metastatic melanoma pre-clinical studies**
**Sergio Augusto Lopes de Souza** [1]

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INTRODUCTION
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).

OBJECTIVES
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.

MATERIALS AND METHODS
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.

DISCUSSION AND RESULTS
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.

CONCLUSION
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-05.02 - 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-05.03 - Derivative from the antimicrobial peptide LyeTx I as potential positron emission tomography (PET) radiopharmaceutical**

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INTRODUCTION
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*.

OBJECTIVES
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.

DISCUSSION AND RESULTS
Three novel shortened derivatives (LyeTx I mn; LyeTx I mnΔK; LyeTx I mnΔKAc) were synthetized and evaluated for their toxicity and biological activity. Among them, LyeTx I mnΔK 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 mnΔK] < $EC_{50}$ [LyeTx I]). Data obtained in a mouse model of septic arthritis (*S. aureus*), showed that LyeTx I mnΔK is able to reduce infection and, then, the inflammatory process and pain. Next, LyeTx I mnΔK 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 mnΔK to identify infection focus (target/non-target = 4.9) and differentiate it from sterile inflammation (target/non-target = 1.3).

CONCLUSION
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 radiological pollution**

**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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INTRODUCTION
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.

OBJECTIVES
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.

MATERIALS AND METHODS
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.

DISCUSSION AND RESULTS
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.

CONCLUSION
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-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 implemented 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

**Yvone M Mascarenhas**
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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

**Luis Augusto Perles** [1]
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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 minibeam, 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-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 applications of positron emission tomography (PET) with 68Ga-PSMA**

**Lilian Yuri Itaya Yamaga**[1]
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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-09.03 - Clinical trials with 18F-PSMA1007**

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INTRODUCTION

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.

OBJECTIVES

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.

MATERIALS AND METHODS

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.

DISCUSSION AND RESULTS

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

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-09.04 - 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

**Alexander Birbrair** [1]
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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

Savio, E; Kreirmerman, I.; Arredondo, F.; Zirbesseger K.; Paolino, A.; Dapueto, R., Isaurralde, F.; Baletta, S.; Duarte, P.; Gambini, J.P.
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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 preliminar 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.**

**Lauro Wichert-Ana [1]**
Ribeirão Preto Medical School, University of São Paulo, Brazil.

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**

Gianna Sagazio[1]
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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-11.02 - Growth and strengthening of the private sector in the Brazilian radiopharmaceutical market: the case of R2IBF**

**DESIREE MORAES ZOUAIN [1]**
[1]R2IBF Radiopharmaceuticals, (Rio de Janeiro, Brazil)

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-11.03 - 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-11.04 - A public facility for alpha emitters production: the intersection between reaserch and the healph public attention**

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INTRODUCTION

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.

OBJECTIVES

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.

MATERIALS AND METHODS
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.

DISCUSSION AND RESULTS
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.

CONCLUSION
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-12.02 - 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

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

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INTRODUCTION
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.

OBJECTIVES
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.

MATERIALS AND METHODS
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.

DISCUSSION AND RESULTS
The first students have already begin to present their dissertation. The employability has increased among students enrolled in the program.

CONCLUSION
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-13.02 - 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-13.03 - 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 postgraduate 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