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**III Latin American
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IX IberoAmerican
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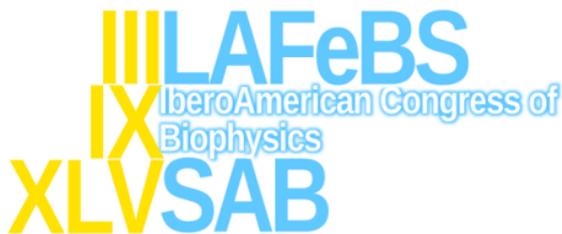
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Main Lectures



Conference Gregorio Weber
Wednesday 23rd November, 13:00hs
Chair: David Jameson

Mechanisms of molecular transport in live cells

Enrico Gratton

Laboratory for Fluorescence Dynamics, department of Biomedical Engineering University of California, Irvine

The coordination of cell functions requires that molecules move in the cell interior to find their partners. In the cell, the mechanisms for molecular motion are poorly understood. While in an isotropic fluid diffusion is the default mechanism of motion, in the cell interior diffusion is hindered by barriers and by transient binding. Also molecules can move by active transport. One universal transport processes which is still debated is the shuttling of molecules between the cell membrane and other locations where the molecules will deliver a signal. Although we have made important progresses in understanding direct motion, less is understood in regard to paths for diffusion and in general the connectivity of the cell interior. In this talk I will discuss the development of tools that could help us in measuring the path that molecules follow in the cell to reach their target.

C02: Conference (SAB, Argentina)

Wednesday 23rd November, 17:30hs

Chair: Gabriela Amodeo

Division of Labor Among the Subunits of a Highly Coordinated Ring ATPase

Carlos Bustamante

Single Molecule Biophysics at UC Berkeley, USA

As part of their infection cycle, many viruses must package their newly replicated genomes inside a protein capsid. Bacteriophage phi29 packages its 6.6 mm long double-stranded DNA using a pentameric ring nano motor that belongs to the ASCE (Additional Strand, Conserved E) superfamily of ATPases. A number of fundamental questions remain as to the coordination of the various subunits in these multimeric rings. The portal motor in bacteriophage phi29 is ideal to investigate these questions and is a remarkable machine that must overcome entropic, electrostatic, and DNA bending energies to package its genome to near-crystalline density inside the capsid. Using optical tweezers, we find that this motor can work against loads of up to 55 piconewtons on average, making it one of the strongest molecular motors ever reported. We establish the force-velocity relationship of the motor. Interestingly, the packaging rate decreases as the prohead fills, indicating that an internal pressure builds up due to DNA compression attaining the value of 6 MegaPascals at the end of the packaging. This pressure,

we show, is used as part of the mechanism of DNA injection in the next infection cycle. We have used high-resolution optical tweezers to characterize the steps and intersubunit coordination of the pentameric ring ATPase responsible for DNA packaging in bacteriophage Phi29. By using non-hydrolyzable ATP analogs and stabilizers of the ADP bound to the motor, we establish where DNA binding, hydrolysis, and phosphate and ADP release occur relative to translocation. We show that while only 4 of the subunits translocate DNA, all 5 bind and hydrolyze ATP, suggesting that the fifth subunit fulfills a regulatory function. Finally, we show that the motor not only can generate force but also torque. We characterize the role played by the special subunit in this process and identify this the symmetry-breaking mechanism. These results represent the most complete studies done to date on these widely distributed class of ring nano motors.

C03: Conference (SBB, Brazil)

Thursday 24th November, 8:30hs

Chair: Silvia del Valle Alonso

Stem Cell Approaches for the Treatment of Renal Diseases

Marcelo Marcos Morales

Universidade Federal do Rio de Janeiro Rio de Janeiro, Brazil

We investigated the regenerative capacity of an intravenous infusion of bone marrow-derived mononuclear cells (BMMCs) and mesenchymal cells in a rodent model of bilateral renal IR injury, diabetic nephropathy and ischemia/reperfusion renal injury and the involvement of inflammatory, anti-inflammatory and other biological markers in this process. Renal function and structure ameliorated after cell infusion. Labelled BMMCs were found in the kidneys at 2 h, 4 h, and 24 h after cell therapy. The expression of inflammatory and biological markers (toll-like receptor 2, toll-like receptor 4, receptor for advanced glycation end products, interleukin 17, high-mobility group box-1, kidney injury molecule-1) were reduced and the expression of anti-inflammatory and antioxidant markers (interleukin 10, Nrf2, and HO-1) were increased in treated animals. The apoptotic index diminished and the proliferation index increased treated animals. The results contribute to the effort to understand the role of different biological players in the morphofunctional renal improvement and cytoprotection in renal injury model subjected to cellular therapy. The results gave us the scientific basis to con-

duct a successfully prospective, non-randomized, single-center longitudinal safety study of Infusion of Bone Marrow Derived Mononuclear Cells in Patients With Focal Segmental Glomerulosclerosis at University Hospital at Federal University of Rio de Janeiro.

C04: Conference (SPB, Portugal)
Thursday 24th November, 14:00hs
Chair: Luis Bagatolli

Analytical solutions for advanced ensemble average FRET in biophysics

Berberan-Santos, Mário¹; Coutinho, Ana^{1,2}; Fernandes, Fábio^{1,3}; Gonzalez-Ros, José⁴; Loura, Luís⁵; Melo, Ana¹; Renart, María Lourdes^{1,4};
Prieto, Manuel¹

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Donor decay ensemble average in FRET studies of biological systems usually only allow qualitative information about topology and structure. However, in systems of high symmetry, exact analytical solutions containing information about specific distances can be obtained, and used to fit high quality time-resolved data (usually within a global analysis methodology).

Several examples illustrate the potential of this approach: i) The trimeric outer membrane protein F (OmpF), is a multi-tryptophan system, which are located both near the center of the channel, and at the

lipid-protein interface. This allows the determination of the site of interaction of the antibiotic ciprofloxacin with OmpF.

ii) Lipid-protein interaction at critical lipid-protein ratio can lead to the formation of mixed fibers. The oligomerization state of the protein in-between the lipid bilayers, can be obtained from FRET homo-transfer modeling, and the inter-bilayer spacing from hetero-FRET from the derivatized protein to lipid acceptor probes.

iii) Protein-lipid selectivity, the preference of a membrane protein for having a specific type of lipid in its near vicinity, can be derived from FRET methodology with intrinsic advantages over the more common ESR approach. A single parameter is fitted, which translates the preference for a specific lipid.

iv) The application of exact solutions for FRET in 2D to microscopy data in order to obtain information about compartmentalization below fluorescence microscope resolution, is hampered by the lack of information on the precise acceptor concentration. This can be overcome in case a system with homogeneous distribution is available, allowing to obtain a direct measure of acceptor confinement. Application to PI(4,5)P₂ in living cells will be described.

v) The tetrameric potassium channel KscA was engineered in order to have only a single tryptophan per monomer. An exact solution for homo-FRET within a square was derived and fitted to anisotropy decays. The recovered distances will be compared with the ones from diffraction data, in both closed and open states, and for different ions. The advantage of this methodology over X-ray diffraction will be dis-

cussed.

Acknowledgments: FCT (Portugal) for projects FAPESP/20107/2014 and RECI/CTM-POL/0342/2012.

C05: Conference (Biophys, Uruguay)

Thursday 24th November, 17:30hs

Chair: Daniel Peluffo

**Reaction of Mn- and Fe-superoxide dismutases with peroxynitrite:
A case study of metal-catalyzed protein tyrosine nitration and
relevance in mitochondrial dysfunction**

Rafael Radi

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Excess biological formation rates of free radicals and oxidants are associated to biomolecular oxidative damage and disease development. Among the key endogenous antioxidant mechanisms in human cells is the mitochondrial, manganese-containing, superoxide dismutase (hsMnSOD). MnSOD is an essential protein in mammals with its primary function being the detoxification of mitochondrial-derived superoxide radical ($O_2^{\bullet-}$). The diffusion-controlled reaction of $O_2^{\bullet-}$ with nitric oxide ($\bullet NO$) leads to the formation of peroxynitrite ($ONOO^-$), an oxidizing and nitrating cytotoxin, known to cause mitochondrial dysfunction. Thus, in mitochondria, MnSOD is a key element controlling the formation of peroxynitrite and therefore, provides protection from its mitochondrial-damaging effects. However, peroxynitrite readily reacts with MnSOD ($k \sim 10^5 \text{ M}^{-1}\text{s}^{-1}$) and promotes its inactivation via a Mn-catalyzed nitration reaction at the active site Tyr34

both *in vitro* and *in vivo*. Then, peroxynitrite-dependent MnSOD nitration and inactivation triggers a pro-oxidant vicious cycle, that ultimately causes severe alterations in mitochondrial, and subsequently cellular, redox homeostasis. Peroxynitrite is also a key cytotoxic effector liberated by immuno-stimulated macrophages to control invading pathogens, including *Trypanosoma cruzi* the causative agent of Chagas Disease. Then, we have also studied the reaction of peroxynitrite with *T. cruzi* mitochondrial (Fe-SODA) and cytosolic (Fe-SODB) Fe-SODs that have similar protein fold to that of mammalian MnSOD. While the primary, secondary and tridimensional structures of the TcFe-SODs are closely related, their sensitivity to peroxynitrite-dependent nitration and inactivation are quite disparate; this is due to the effective repair of the active site tyrosyl radical (Tyr35) in Fe-SODB by intramolecular electron transfer processes, ultimately depending on reducing equivalents of solvent-exposed Cys83. Experimental and computational approaches to study these reactions and their biological consequences will be discussed.

C06: Conference "Confraternización SBE-SAB"

Friday 25th November, 8:30hs

Chair: Gerardo Fidelio

Protein kinases C are regulated by concerted interaction with lipids.

Juan C. Gómez-Fernández and Senena Corbalán-García

Departamento de Bioquímica y Biología Molecular A, Universidad de Murcia, Campus of International Excellence "Campus Mare Nostrum", Murcia, Spain

Classical protein kinases C are known to be important in cell physiology both in terms of health and disease. They are activated by triggering signals that induce their translocation to membranes. The consensus view is that several secondary messengers are involved in this activation, such as cytosolic Ca^{2+} and diacylglycerol. The former bridges the C2 domain to anionic phospholipids as phosphatidylserine in the membrane and diacylglycerol binds to the C1 domain. Both diacylglycerol and the increase in Ca^{2+} concentration are assumed to arise from the extracellular signal that triggers the hydrolysis of phosphatidylinositol-4,5-bisphosphate, however results obtained during the last decade indicate that this phosphoinositide itself is also responsible for modulating classical PKC activity and its localization in the plasma membrane. On the other hand novel protein kinases C are known to be activated by diacylglycerol through the C1 domain and through the interaction with

negatively charged phospholipids through both the C1 and the C2 domains. Recent results show that the interaction with negatively charged phospholipids of the C1 domain is especially important in isoenzymes ϵ and η but less so in δ and θ , due to differences in key aminoacyl residues found between both types of domains.

This work was supported by Ministerio de Economía y Competitividad (Spain), grant (BFU2014-52269) co-financed by the European Regional Development Fund and by grant 19409/PI/14 Fundación Séneca, Gobierno de la Comunidad Autónoma de Murcia.

C07: Conference SAB

Friday 25th November, 17:30hs

Chair: Gabriela Amodeo

Allosterism and Structure in Thermal Transient Receptor Potential Channels

Ramón Latorre

Centro Interdisciplinario de Neurociencia de Valparaíso, Facultad de Ciencias, Universidad de Valparaíso, Chile

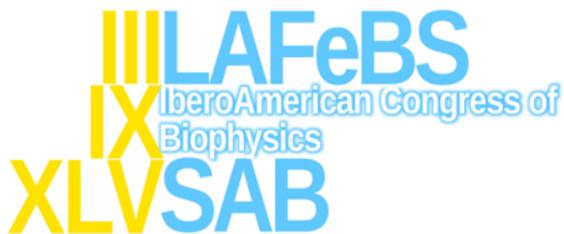
Temperature-activated transient receptor potential (themoTRP) channels behave as polymodal receptors with allosteric gating. We have recently developed an allosteric model that is able to explain the channel the voltage- and temperature-dependent gating in TRPM8, a cold receptor. The most salient results obtained interpreting the electrophysiological data using the allosteric model are: 1. A limiting-slope analysis of the probability of opening (P_o)-voltage curves demonstrate that channel opening is voltage independent; 2. When all voltage sensors are at rest, TRPM8 can be activated by decreasing temperature with a Q_{10} of 25 indicating that the channel is a bona fide temperature receptor and implying the existence of a structure specialized in temperature sensing; 3. TRPM8 channel deactivation proceeds with a double exponential time course suggesting that channel closing is a two steps process Open-Closed-Close. All the channel dependence resides in the deactivation process. Both time constants are temperature dependent

and we show that the total Q_{10} of the overall reaction is obtained by multiplying the Q_{10} of the individual deactivation rate constants. The whole temperature sensitivity of the Po is recovered in the deactivation process.

The structures of the TRPV1 channel (a heat receptor) solved using cryomicroscopy by the group of Julius in the closed and open configuration have greatly helped us to get a better picture about the mechanisms by means of which agonists and temperature gate thermoTRPs. In particular, using these structures as templates, we have been able to identify the PIP2 binding site. We show that PIP2 behave as an agonist of TRPV1 channels and the structure of the PIP2 binding obtained in silico site was confirmed using mutagenesis and electrophysiology. The phosphate groups of PIP2 made contact with positively charged amino acid residues contained in the S4-S5 linker and in the TRP domain of TRPV1. Molecular dynamic simulation and docking of PIP2 to the closed configuration of the channel, suggest that PIP2 open the lower gate by inducing a bending of the α helix formed by S6 and the TRP domain.

Finally, using the structure of TRPV1 and a in silico technique called anisotropic thermal diffusion we offer a possible mechanism by means of which temperature activates TRPV1 channels.

Symposia



Gregorio Weber Special Symposium

Wednesday 23 rd November
Chairs: Jerson Lima da Silva, Francisco Barrantes

Part I: 14:00 - 15:00

Part II: 15:15 - 16:45

Gregorio Weber Special Symposium (Part I)

Chair: Jerson Lima da Silva, Francisco Barrantes

The Fundamental Contributions of Gregorio Weber to Fluorescence and to Protein Chemistry

David M. Jameson

Department of Cell and Molecular Biology, John A. Burns School of Medicine, University of Hawaii, Honolulu, HI 96813

During the last few decades, fluorescence spectroscopy has evolved from a narrow, highly specialized technique into an important discipline widely utilized in the biological, chemical and physical sciences. As in all scientific disciplines, the development of modern fluorescence spectroscopy has benefited from the contributions of many individuals from many countries. However, one individual, Gregorio Weber, can be singled out for his outstanding and far-reaching contributions to this field. Gregorio Weber's research career, spanning more than half a century, was characterized by an unbroken chain of highly original and important contributions to fluorescence spectroscopy and also to protein chemistry. In this talk, I will briefly outline aspects of Gregorio Weber's life and times and discuss some of his more important contributions to these fields. In addition to these seminal contributions, Gregorio Weber also trained and inspired generations of spectroscopists and biophysicists who went on to make important contributions to their fields, including both basic research as well as the commercialization

of fluorescence methodologies and their extension into the clinical and biomedical disciplines.

Gregorio Weber Special Symposium (Part I)

Chair: Jerson Lima da Silva, Francisco Barrantes

How Gregorio Weber's Theory on Conformational Drift and Condensation of Proteins Explains its Prion-like Aggregation Behavior

Jerson L. Silva

Instituto de Bioquímica Médica Leopoldo de Meis, Instituto Nacional de Biologia Estrutural e Bioimagem, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil.

Protein misfolding and prion-like aggregation result in devastating degenerative diseases and cancer. Besides inducing misfolding of native proteins, prion-like proteins bind nucleic acids and other polyanions. Key proteins involved in these diseases, such as $A\beta$, τ , α -synuclein, SOD1, and TDP43 can show a prion-like behavior. Prion-like protein aggregation and amyloidogenesis are condensation reactions. Weber pointed out that reactions involving protein interactions, such as protein association, are different than those reactions that provided the original basis for the application of classical thermodynamics to chemistry. Among other consequences of the dissimilarity between these reactions is the fact that the condensation of proteins – such as folding, oligomerization and aggregation – would be always highly susceptible to pressure and to low temperatures. Gregorio Weber also proposed an elegant hypothesis to explain the hysteresis based on the slow interconversion

between the protein species, termed "conformational drift". Hysteresis would explain why early amyloid fibrils that are pressure sensitive could eventually evolve slowly to species that are more packed and less sensitive to pressure. Studies by our group and others have demonstrated that prion-like behavior of proteins involved in cancer, which is the case of p53. The function of this tumor suppressor protein is lost in more than 50% of human cancers. Our studies have suggested that the formation of prion-like aggregates of mutant p53 is associated with loss-of-function, dominant-negative and gain-of-function (GoF) effects. These aggregates are present in tissue biopsies of breast cancer especially in more aggressive ones. The prion-like properties of p53 aggregates are considered potential targets for drug development. We will discuss how nucleic-acid binding might influence protein misfolding for both disease-related and benign, functional prions and why the line between bad and good amyloids might be more subtle than previously thought. On the pathological side, there are no effective therapies against diseases involving the prion-like aggregation of proteins. An approach that focuses on protein-nucleic acid interactions, which are the key characteristic of these diseases, might reveal new therapeutic targets. Nucleic acids can have opposing effects on protein aggregation, depending on the specific cellular context, either in function or pathology. Thus, this relationship suggests a molecular personification of Dr. Jekyll and Mr. Hyde. (Supported by CNPq, FAPERJ, FINEP and CAPES).

Gregorio Weber Special Symposium (Part II)

Chair: Jerson Lima da Silva, Francisco Barrantes

Gregorio Weber's roots in Argentina and acetylcholine receptor-cholesterol intimate (nanoscale) and long (billion year) relationship

Francisco J. Barrantes

Laboratory of Molecular Neurobiology, Institute of Biomedical Research, UCA-CONICET, A. Moreau de Justo 1600, 1107 Buenos Aires, Argentina

Professor Gregorio Weber's childhood, youth and education up to his first doctoral degree took place in Buenos Aires, and these periods of his life had a deep impact on the shaping of his persona, his cultural habits, and his scientific interests. His great mind and avid quest for knowledge in all spheres of life were undoubtedly nourished by the high standards of the educational system in Argentina at that time, the influence of his family and cultural environment, and the informal training that he received in his home town. I will attempt a brief overview of the crossroad of his personal trajectory and the arena where this took place. The second part of my talk will summarize our current efforts to understand a key molecule in synaptic transmission, the acetylcholine receptor (nAChR). This macromolecule is the paradigm member of the superfamily of rapid, neurotransmitter-gated, pentameric ligand-gated ion channels (pLGIC), an active research subject in Neurobiology, and

focus of potential pharmacological intervention in a number of neurological and neuropsychiatric diseases. pLGIC share a number of phylogenetically conserved structural features. Some of these appear to be related to the modulation exerted by the lipid microenvironment on their physiological activity. I will attempt to illustrate i) the intimate (nanoscale) liaison that the nAChR has maintained with cholesterol over the billion-year time scale, and the effects exerted by this sterol on the cell-surface distribution, endocytic pathway, and ion channel gating of the receptor, and ii) the tools we are currently employing to interrogate their tight and long-lasting relationship.

Gregorio Weber Special Symposium (Part II)

Chair: Jerson Lima da Silva, Francisco Barrantes

Extended Excitation FLIM (eeFLIM)

Thomas M. Jovin, Nathan P. Cook, and Donna Arndt-Jovin

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The usual dogma in the field of fluorescence decay measurements is that "the shorter the excitation pulse the better". We overcome this limitation by recording the integrated emission of the fluorophore excited with a rectangular light pulse substantially longer than the anticipated lifetimes. Sensitive and accurate determinations of the *mean intensity-weighted lifetime* are obtained. Successive determinations (≥ 2) are taken in the region corresponding to constant excitation intensity and at integration times $> 5-6$ the longest lifetime in the sample population. These points fall on a straight line with a slope and position referenced to a companion measurement of a sample with a 0 (e.g. scattered excitation light) or known lifetime, thereby yielding the absolute mean lifetime. That is, the displacement on the integration time (gate width) axis is given by the lifetime. The mixtures can be of arbitrary heterogeneity. For a two-component system (e.g. a binding reaction, FRET), the mean lifetime can be expressed analytically as a function of fractional concentrations. The mean lifetime is very useful, even essential, in numerous other applications, e.g. involving single molecules. We

have implemented eeFLIM in an imaging system with a gated intensified camera and laser diodes (soon LEDs) for excitation. This camera features excellent spatial resolution and linearity (emCCD detector), and powerful software-electronics for multimode acquisition and synchronization. The system is very sensitive, allowing real-time full-field ($1K \times 1K$) FLIM at rates >1 Hz. Some important advantages of eeFLIM are: (1) rectangular excitation pulses (e.g. 10-50 ns) that are easy to generate and have very high pulse energies, yielding intense response signals; (2) utilization of virtually all the emitted light; (3) temporal resolution of tens of ps; long-lived emissions (delayed fluorescence, phosphorescence) can also be measured; (4) very fast lifetime image generation involving only simple, linear, non-iterative calculations; (5) applicability of eeFLIM to single or array detectors and TCSPC detection.

Gregorio Weber Special Symposium (Part II)

Chair: Jerson Lima da Silva, Francisco Barrantes

Super-resolved fluorescence imaging of neurons and *Trypanosoma cruzi*

Fernando D. Stefani

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Super-resolution microscopy, or far-field fluorescence nanoscopy are becoming more and more widely used in biological imaging due to their capability to deliver spatial resolution well beyond the diffraction limit. In this talk, I will first present the key concepts of coordinate-targeted (scanning) and coordinate-stochastic (wide-field) fluorescence nanoscopy. Then, I will illustrate how these methodologies are useful to solve biophysical questions related to neuronal polarity, axonal degeneration, and the interaction of *Trypanosoma cruzi* with the host.

S02: Lipids, Structure and Function (SEB, Spain)

Thursday 24 th November (10:00 - 12:00)

Chairs: Juan Carmelo Gómez Fernández, Gerardo Fidelio

Lipids, Structure and Function (SEB, Spain)

Chair: Juan Carmelo Gómez Fernández, Gerardo Fidelio

Role of lipids in autophagy events

Antón, Zuriñe^{1}, Hervás, Javier H^{1*}, Landajuela, Ane¹, Montes, L. Ruth¹, Hernández-Tiedra, Sonia^{2,3}, Velasco, Guillermo^{2,3}, Goñi, Felix M¹ and Alonso, Alicia¹*

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3. Instituto de Investigaciones Sanitarias San Carlos (IdISSC), Madrid, Spain.

**These two authors contributed equally to this work.*

Autophagy is primarily a cell survival mechanism although depending on the cellular context and the type and duration of the triggering stimuli, this cellular process can also lead to cell death. We have studied the role of two different kinds of lipids on two autophagy events, namely tetrahydrocannabinoid-induced cell autophagy, where the dihydroceramide/ceramide ratio is a crucial factor to regulate cell fate (Hernández- Tiedra et al, 2016) and the role of cardiolipin (CL) on mitophagy (Antón et al, 2016). We have applied quantitative biophysical techniques to the study of CL interaction with various Atg8 human homologues, namely LC3B, GABARAPL2 and GABARAP. We have found that LC3B interacts preferentially with CL over other dianionic lipids, that CL-LC3B binding occurs with positive cooperativ-

ity, and that the CL-LC3B interaction relies only partially on electrostatic forces. CL-induced increased membrane fluidity appears also as an important factor helping LC3B to bind CL. The LC3B C-terminus remains exposed to the hydrophilic environment after protein binding to CL-enriched membranes. In intact U87MG human glioblastoma cells rotenone-induced autophagy leads to LC3B translocation to mitochondria and subsequent delivery of mitochondria to lysosomes.

Acknowledgments

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Lipids, Structure and Function (SEB, Spain)*Chair: Juan Carmelo Gómez Fernández, Gerardo Fidelio***Heterogeneous diffusion, molecular interactions and ergodicity breaking in living cell membranes***Carlo Manzo**ICFO - Institut de Ciències Fotòniques, The Barcelona Institute of Science and Technology, 08860 Castelldefels (Barcelona), Spain*

Molecular diffusion and interactions regulate numerous processes underlying biological functions in living cells. In the last decade, advances in single-molecule fluorescence and super-resolution nanoscopy have allowed the visualization of cellular components at unprecedented spatial and temporal resolution, providing novel insights on a variety of cellular processes. These experiments have revealed that the complexity of the cellular environment often produces large heterogeneity both at the structural and dynamical level, whose implications for the cellular function are not fully understood.

Recently, we have studied the organization and dynamics of DC-SIGN, a transmembrane pathogen- recognition receptor involved in the capture of viruses, bacteria and parasites. By combining stimulated emission depletion (STED) microscopy and single particle tracking, we have found that DC-SIGN displays a multiscale organization in the cell membrane [1]. In addition, its motion deviates from a purely Brownian behavior, exhibiting anomalous diffusion with signatures of weak- ergod-

icity breaking and aging [2, 3]. In contrast to other systems showing analogous behavior, DC- SIGN nonergodicity is not induced by transient immobilization and therefore cannot be modeled as a continuous-time random walk. We model and quantitatively interpret this dynamics within the framework of a new family of stochastic models [3], assuming inhomogeneous Brownian diffusion with random diffusivity on scale-free media.

To explore the molecular causes of DC-SIGN nonergodic subdiffusion, we comparatively studied three mutated forms of the receptor [2], and we are currently extending our investigation from both the experimental and theoretical point of view [4]. These data allow us to correlate receptor motion with molecular structure and interactions, thus establishing a link between nonergodicity and DC- SIGN capability in pathogen capture and internalization. Our findings highlight the fundamental role of disorder in cell membranes and postulate a connection with function regulation.

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Lipids, Structure and Function (SEB, Spain)

Chair: Juan Carmelo Gómez Fernández, Gerardo Fidelio

The Synergistic Effect of Lipids to Enhance the Permeation Induced by the Peptide Polybia-MP1 in Biomembranes.

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Ruggiero Neto, Joao(1)*

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Phosphatidylserine (PS) plays important role in different processes in eukaryotic cells. In healthy cells, this aminophospholipid is found in the inner leaf of plasmatic membrane. In apoptotic cells, however, PS is externalized to the outer leaf signaling for phagocytes and macrophages to promote the clearance of these cells. The outer leaflet of mammalian cells is rich in sphingomyelin (SM) and cholesterol (Chol) that forming liquid-ordered (Lo) domains. In epithelial cancer cells, phosphatidylethanolamine (PE) was also externalized together with PS. The peptide Polybia-MP1 (MP1) was shown to be inhibitor of cancer cell proliferation and selective to leukocytes without harming healthy T lymphocytes. In this presentation the results obtained in the investigation of the effect of PS on the affinity and lytic activity of MP1 in model membranes will be discussed. The effect of PS was investigated in two situations: PS in the presence of PE and PS in the presence of Lo domains. The affinity was assessed by adsorption of MP1 to large

unilamellar vesicles (LUVs). The peptide lytic activity was assessed monitoring either the influx of fluorescent dyes to the lumen of giant unilamellar vesicles (GUVs) or by conventional leakage experiments in LUVs. The findings indicate that the peptide affinity to POPS containing LUVs is larger compared to vesicles lacking this lipid. PE, in larger extension, and Lo domains affected the permeation of the vesicles potentiating the influx and/or efflux of dyes. For PC/PE/PS vesicles the lag time to the onset of the influx is half of that for PC/PE and PC/PS compositions. Otherwise, MP1 induced complete filling to PC/PE/PS vesicles even for the 10kD dye at lower bound peptide concentrations. In vesicles of PC/PS/SM/Chol the permeation was five times larger than in PC/PS vesicles with 70% more negative charge. PS and PE as well as PS and Lo domains act synergistically enhancing the activity of MP1 suggesting its implication on the inhibitory effect to cancer cell proliferation.

Acknowledgement: DSA FAPESP and PROPE-UNESP; JRN FAPESP and CNPq. E.E.A. is a researcher from CONICET and thanks CONICET (PIP 2013-2015) and FONCyT (PICT 2012-1377) for financial support.

Lipids, Structure and Function (SEB, Spain)**Selection Young Researcher***Chair: Juan Carmelo Gómez Fernández, Gerardo Fidelio***Oxidative Stress on Lipid Membranes as Investigated by Small Angle X-Ray Scattering***Raffaella de Rosa, Rosangela Itri Instituto de Fisica - Universidade de São Paulo**Instituto de Fisica - Universidade de São Paulo*

In this work, we investigate how the photo-oxidation of large unilamellar vesicles (LUVs) in the presence of different photosensitizers (PSs) impacts on plasma mimetic membranes. In this way, LUVs composed of POPC(1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine), POPC:sphingomyelin(SM) (molar ratio 1:1) and POPC:SM:Cholesterol(CO) (molar ratio 1:1:1) were investigated by SAXS, dispersed in four PS-containing solutions: methylene blue (MB), azure A (AA), azure B (AB) and thionin (Ti) upon photo-irradiation ($\lambda = 660$ nm) of 2 hours. The analysis of oxidized membranes performed by means of GENFIT software (1) revealed that the electron density profiles do not change under photo-irradiation in comparison to the non-oxidized membranes. After the inclusion of the PSs all the systems present indicative profiles of coexistence of LUVs and large multilamellar vesicles (MLVs). Vesicles of POPC irradiated in the presence of PSs MB, AB and Ti showed respectively 39%, 43% and

59% of considered oxidized membrane. On the other hand, the vesicles of POPC that were not exposed to radiation and the vesicles irradiated in the presence of photosensitizer AA showed, respectively, 18% and 10% of oxidized membrane. The results for membranes composed of POPC:MS showed no photo-oxidation effect for the membranes irradiated in the presence of MB, AA and AB, in contrast to the photo-oxidation of POPC:SM in the presence of Ti that showed an increase of 17% of oxidized membranes. Vesicles composed of POPC:SM:CO showed no structural changes due to oxidation.

Acknowledgment: The authors thank FAPESP for financial support (thematic project 2012/50680-5). R.R. is a recipient of FAPESP fellowship (project 2014/02511-5). R.I. is recipient of CNPq research fellowship.

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Lipids, Structure and Function (SEB, Spain)**Selection Young Researcher**

Chair: Juan Carmelo Gómez Fernández, Gerardo Fidelio

Triglyceride "lenses" at the air-water interface as a model for studying lipid droplets

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Lipid Droplets (LD) are intracellular structures consisting on an apolar lipid core -composed mainly of triglycerides (TG) and steryl esters- which is surrounded by a phospholipid and protein monolayer. LDs originate in the ER bilayer, where TG synthesis concludes. The mechanisms underlying TG nucleation, size maturation and budding-off from the ER membrane are a matter of current investigations and the role of dewetting from cytosolic-bilayer interface appears to play a critical role [1]. Here, we study phosphatidylcholine (PC) / triglycerides mixtures at the air-water interface as Langmuir and collapsed monolayers in order to gain insight into those factors that may affect the stability of TG bulk structures in contact with interfaces. PC/TG form

stable monolayers which, under lateral compression, collapse at a surface pressure (π) dependent on composition. Observation under Brewster Angle Microscopy (BAM) revealed the appearance of microscopic structures at π beyond collapse, whose size (3 ± 2 microns radius) did not vary with composition. Contrary to dpPC/TG mixtures, pure TG and EPC/TG compression isotherms are reversible after collapse, and BAM confirmed this behavior. Furthermore, identical results were obtained when monolayers were spread on the interface up to a π higher than collapse. The fluorescent probe Nile Red (NR), commonly used to stain lipid droplets, was able to reveal these structures. Comparison of BAM and fluorescence microscopy indicate that these collapsed structures correspond to TG liquid phase which are known as interfacial "lenses". Latest theoretical models propose nanometer-sized structures to dewet and detach from the bilayer. Being in equilibrium and with a microscopic size, these lenses suggest that dewetting of TG adjacent to a PC monolayer (eventually hemilayer) is enough to reach such lateral size.

Acknowledgments

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S03: Membrane Transporters and Channels (Biophys, Chile)

Thursday 24 th November (10:00 - 12:00)

Chairs: Carlos González León, Wendy González

S03: Membrane Transporters and Channels*Chair: Carlos González León, Wendy González***State-dependent solvation within an ion channel pore reported by genetically encoded fluorescent amino acids.***Sebastian Brauchi**Physiology Department, Universidad Austral de Chile*

Abstract: The incorporation of fluorescent non-canonical amino acids (f-ncAA) has been proved useful to report the localization, stoichiometry, and macroscopic activity of membrane proteins expressed in bacteria, *Xenopus laevis* oocytes, and mammalian cells. Optical recordings of ion channel gating events have been reported in the past restricted to purified protein incorporated into artificial bilayers or liposomes. Here, by genetically encoding a coumarin side chain within the pore of TRPV1 channels, we performed direct optical recordings of agonist-induced channel gating events in living mammalian cells. We demonstrate the capacity of this strategy to optically examine local solvation of membrane proteins at their native environment.

FONDECYT 1151430.

S03: Membrane Transporters and Channels*Chair: Carlos González León, Wendy González***Structure-based study of TASK channels blockers**Wendy González*Universidad de Talca, Chile*

Two-pore domain potassium (K2P) channels trigger the background K^+ currents in mammalian cells. K2P channels are separated into six subfamilies based on their structural and functional properties. The acid-sensitive TASK-1 and TASK-3 channels belong to the TASK subfamily. TASK channels contribute to the central respiratory chemosensitivity (Ortega-Sáenz et al., 2010) and are also important for neuronal excitability (Linden et al., 2007). TASK-3 is an oncogenic potassium channel and it is overexpressed in breast and ovarian tumors (Huang and Jan, 2014). Development of compounds that selectively modulate K2P channels such as TASK-1 and TASK-3 is fundamental to assess the efficacy of therapies targeting these interesting proteins.

In the present work some computational methodologies such as homology modeling, molecular dynamics simulations, virtual screening, docking, binding free energy calculation, etc.; as well as experimental techniques such as two electrode voltage clamp (TEVC) and Fluorometric imaging plate reader – Membrane potential assay (FLIPR-MPA) were employed to: 1) understand why potassium voltage-gated channel

(Kv1.5) blockers preferentially inhibit TASK-1 channels. Our results explained how Kv1.5 blockers, like AVE0118 and AVE1231, which are promising drugs against atrial fibrillation or obstructive sleep apnea, are in fact powerful TASK-1 blockers. Accordingly, the TASK-1 channels blockage by these compounds could contribute to the clinical effectiveness of these drugs (Kiper et al., 2014). 2) To study the role of the fenestrations (side-opening facing the membrane) in the binding of A1899 to TASK-1 potassium channel; our results shown that A1899 binds tightly to structures with open fenestrations and demonstrated that A1899 cannot travel from the membrane through the fenestrations to reach the binding site. Finally, 3) to structure-based discovered novel TASK-3 modulators; our results allowed the identification of two lead ligands showing inhibition of 40.6 μM and 43.1 μM against TASK-3. For this motive, the conserved pharmacophore described in this work, and the novel chemical characteristics of this chemical class makes them good candidates for upcoming development into highly potent TASK-3 modulators through medical chemistry optimization.

In this work we present findings about the understanding of the structural mechanism of TASK channels blockage through a theoretical-experimental approach. This gained information will permit us to propose novel modulators that might aid unraveling the physiological roles of TASK channels in their sites of expression in native organs and cells.

Acknowledgements: Fondecyt grant 1140624

S03: Membrane Transporters and Channels**Selection Young Researcher**

Chair: Carlos González León, Wendy González

Hv1 Channels: Cooperativity and blocking

Carlos Gonzalez, Qiu F, Chamberlin A, Watkins BM, Ionescu A, Perez ME, Barro-Soria R, Noskov SY and Larsson HP.

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Voltage-gated proton (Hv1) channels are depolarization-activated channels that are highly selective for protons. These channels belong to the voltage-gated cation channels. However, in contrast to other voltage-gated cation channels, such as Kv channels, which have six transmembrane (TM) segments per subunit and forms tetramers, Hv1 have only four TM segments per subunit and forms dimers. The four TM segments in an Hv1 subunit form the voltage-sensing domain. In contrast to Kv channels, which have a common pore formed by the assembly of the last two TM segments from all four subunits, each subunit of Hv1 contains a pore. After its cloning in 2016 has been possible to explore, several questions at the molecular level, as the cooperative and Zn^{2+} inhibition mechanisms, in this dimeric channel never addressed before. In this study, we use voltage clamp fluorometry technique to identify the cooperativity and the Zn^{2+} inhibition molecular mechanisms of Hv1 channels. Our data showed that there are two types of conformational changes involved in Hv1 channel opening (1). We proposed that, upon

membrane depolarization, the first type of conformational change is the independent, outward movements of the two S4 segments and that the second conformational change is gate opening, which is most likely caused by a concerted conformational change in both subunits in the Hv1 dimer (1). We find that Zn^{2+} inhibits Hv1 in two ways, most likely by binding to two different Zn^{2+} binding sites: at low concentration, Zn^{2+} decreases the proton current by preventing gate opening; at high concentration, Zn^{2+} inhibits Hv1 further by inhibiting S4 movement. Mutating different residues in Hv1 channels affects the two inhibitory effects of Zn^{2+} differentially, suggesting that the two Zn^{2+} binding sites are made up of different residues. In addition, Zn^{2+} inhibits dimeric and monomeric Hv1 in a similar manner, suggesting that each Zn^{2+} binding site is made up of residues from within one Hv1 subunit. (2).

This work was supported by grants NHLBI R01-HL095920 (to HPL) and Fondecyt 1160261 (to CG).

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S03: Membrane Transporters and Channels**Selection Young Researcher**

Chair: *Carlos González León, Wendy González*

TIP1 but not PIP2 from *Beta vulgaris* is regulated by cell mechanics

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Different regulatory mechanisms are known in aquaporins (AQPs). It was demonstrated in the last years that animal and plant aquaporins are regulated by cell membrane mechanics (Ozu et al., 2013; Leitao et al., 2014). However, this mechanism has not been studied in deep. No direct measurements of membrane mechanics and aquaporins function have been reported yet. Moreover, important topics in the study of mechano-sensitivity in aquaporins are not known. For example, it is not clear if this is a general feature shared by all aquaporins or not, and it is not known which is the sensitivity of this regulation. To characterize the biophysical properties of mechanical regulation in aquaporins, we studied the osmotic response of *Xenopus* oocytes expressing the water channels TIP1 or PIP2 from the beet root *Beta vulgaris* by simultaneously

measuring the internal pressure and volume under different osmotic and initial pressure conditions. Our results indicate that PIP2 would not be a mechanosensitive member of the aquaporin family whereas TIP1 would be since the osmotic permeability coefficient (Pf) measured in oocytes expressing TIP1 follows an exponential decay function with the volumetric elastic modulus (E). Filogenetic analysis classifies human AQP1 and PIPs from plants into the same group, whereas TIPs are classified within another group (Soto et al, 2012). In this context, mechanosensitivity seems to be a feature not shared by all aquaporins, and its appearance along evolution will not be easy to determine.

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S03: Membrane Transporters and Channels

Chair: Carlos González León, Wendy González

Molecular determinants underlying the dysfunction elicited by Cx26G12R syndromic deafness mutation

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Several mutations in Cx26 promote keratitis ichthyosis deafness (KID), a rare disease in which patients exhibit deafness, skin and corneal disorders. Some KID mutations are located in the amino terminal seg-

ment of Cx26, which has been proposed as the putative voltage sensor. Most syndromic mutations elicit a gain of hemichannel function, however the mechanism underlying this phenomenon remains unclear. Here we examined the biophysical properties of the mutant G12R at macroscopic and single channel levels. We perform two-electrode voltage clamp to study the deactivation kinetics and apparent affinity for calcium of hemichannel currents. In contrast to wild-type (wt) Cx26, we found that the activation kinetics of the mutant lack saturation of the current upon depolarization pulses above +40 mV. Estimates of the deactivation time constant yield values of 5s and 10s for G12R and wtCx26 hemichannels, respectively; however the apparent affinity for calcium remains similar to wtCx26. Single channel recordings analysis showed that the mutant virtually eliminates the transitions to the subconductance state. Moreover, although the single channel conductance was similar to wtCx26 hemichannels, the open probability and mean open times of G12R hemichannels become larger. In studying the underlying mechanism, molecular dynamic simulations indicate the arginine insertion makes the N-terminus helix displaced to the intracellular side, allowing interactions with the TM2/IL border residues. The changes induced by the mutation affect the dynamics of the gating, prevents the subconductance state to occur and stabilize hemichannels in the open configuration. Moreover, due to the impairments observed for both the slow and fast gating mechanism, these results strongly suggest that both gates works coupled rather than separates entities and that the N-terminus is a key gating particle for the voltage dependence of con-

nexins.

**S04: Protein Oxidation in Biology and Biophysics
(Biophys, Uruguay)**

Thursday 24 th November (15:30 - 17:30)

Chairs: Daniel Peluffo, Silvina Bartesaghi

S04: Protein Oxidation in Biology and Biophysics (Biophys, Uruguay)

Chair: Daniel Peluffo

***Trypanosoma cruzi* hybrid type A heme peroxidase (TcAPx-CcP): enzyme kinetics, subcellular localization in the infective stage and contribution to parasite virulence**

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Trypanosoma cruzi ascorbate peroxidase is, by sequence analysis, a hybrid-type A member of class I heme-peroxidases (TcAPx-CcP) suggesting both ascorbate (Asc) and cytochrome c (Cc) peroxidase activity. Here we show that the enzyme reacts fast with H₂O₂ ($k = 2.9 \times 10^7 M^{-1} s^{-1}$) and catalytically decomposes H₂O₂ using Cc²⁺ as the reducing substrate with higher efficiency than that of ascorbate (kcat/KM = 2.1×10^5 and $5.7 \times 10^4 M^{-1} s^{-1}$, respectively). Visible-absorption spectra studies of purified recombinant TcAPx-CcP after

H₂O₂ reaction denotes the formation of a Compound I-like product, characteristic of the generation of a tryptophanyl-cation radical (Trp233^{+•}). Mutation of Trp233 by phenylalanine (W233F) completely abolishes the Cc²⁺-dependent peroxidase activity of TcAPx-CcP. In addition to Trp233^{+•}, a Cys-derived radical was identified by EPR spin-trapping, immuno-spin trapping and mass spectrometry analysis after equimolar H₂O₂ addition, suggesting an alternative electron transfer (ET) pathway from the heme. Molecular dynamics studies revealed that ET between Trp233 and Cys222 is plausible and likely to be involved in Trp233^{+•} stability. Recognizing the ability of TcAPx-CcP to use both ascorbate and Cc²⁺ as reducing substrates, we searched for its subcellular localization in the infective *T. cruzi* parasite stages (i.e. intracellular amastigotes and extracellular trypomastigotes). TcAPx-CcP was found in close association to mitochondrial membranes of the cristae and most interesting, associated with the outer plasma membrane of the infective stages suggesting a role at the host-parasite interface. *T. cruzi* APx-CcP-overexpressers were significantly more infective to macrophages and cardiomyocytes as well as in the mice model of Chagas Disease, supporting the involvement of TcAPx-CcP in pathogen virulence as part of the parasite antioxidant armamentarium.

S04: Protein Oxidation in Biology and Biophysics (Biophys, Uruguay)*Chair: Daniel Peluffo***Molecular Mechanism Links Auxin and ROS-Controlled Polar Growth in Plant Cells**

Mangano, Silvina[†], Juarez, Silvina P.D.[†], Marzol, Eliana, Estevez, José M.

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Polar-growth present in root hairs is sustained by oscillating levels of Reactive Oxygen Species (ROS). These cells endogenously controlled by auxin are able to grow hundred-folds of their original size toward soil signals (e.g. nutrients and water) important for crucial plant survival. Although their final cell size is of fundamental importance, the molecular mechanisms that control it remain largely unknown. Here, we showed that ROS-production is under the control of the transcription factor RSL4, who in turn is regulated by auxin through the Auxin Responsive Factor 5 (ARF5). In this manner, auxin controls ROS-mediated polar-growth depending on NADPH oxidases (or RBOHs for RESPIRATORY BURST OXIDASE HOMOLOG proteins) and secreted type-III Peroxidases (PER). A novel group of two RBOHs (RBOHH,J) and four PERs (PER1,44,60,73) are then required to modulate *apo*ROS homeostasis. Chemical or genetic interference with the

ROS balance or peroxidase activity affect root hair final cell size. Overall, our findings establish a molecular link between auxin regulated ARF5-RSL4 and ROS-mediated polar root hair growth.

S04: Protein Oxidation in Biology and Biophysics (Biophys,Uruguay)*Chair: Daniel Peluffo***Structural and Functional Changes in Oxidatively-Modified
Glutamine Synthetase: Experimental and Computational Studies***Bartasaghi, Silvina**Facultad de Medicina, Universidad de la República, General Flores 2125, Montevideo
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Glutamine synthetase (GS) is a key metabolic enzyme that catalyzes the ATP-dependent synthesis of glutamine from glutamate and ammonia. In the central nervous system, it is mainly located in the cytosol of astrocytes, playing an important role in ammonia detoxification and prevention of glutamate-dependent excitotoxicity. Alterations in GS activity may lead to astroglial dysfunction, affecting neuronal function and survival.

Several *in vitro* and *in vivo* studies in plants, bacteria and mammals, have shown that GS activity is highly susceptible to biologically-relevant reactive oxygen species, in particular peroxynitrite (ONOO⁻). Peroxynitrite-derived radicals promote tyrosine nitration yielding 3-nitrotyrosine (3-NT). Tyrosine nitration of GS has been identified as one of the main oxidative modifications associated to enzyme inactivation in pathological conditions; however, the critical residues involved and the molecular mechanisms participating in GS inactivation are still undefined.

Herein we have worked with human glutamine synthetase (hGS), which was expressed in *E. coli*. We studied the effect of different oxidants on hGS function and structure, and the oxidative mechanisms of inactivation, by combining classical biochemical assays with molecular dynamic simulations.

Peroxynitrite addition caused a dose-dependent inactivation of hGS, associated to an increase in 3-NT levels. In addition, we were able to detect the formation of high molecular weight aggregates, resistant to the action of reductants, probably due to di-tyrosine crosslinks. Both, tyrosine nitration and aggregate-formation strongly correlated with enzyme inactivation, however, pH-dependent studies suggested that di-tyrosine formation had a larger impact on enzyme activity, compared to nitration. In addition, peptide-mapping MS analysis identified critical modified residues, namely Tyr 185 and 269. In parallel to the experimental studies, molecular dynamic simulations were performed to understand the catalytic mechanisms of the reaction with the goal of defining, with an atomic level of detail, how the oxidative postranslational modifications affect activity, in particular tyrosine residues associated to the substrate binding sites.

S04: Protein Oxidation in Biology and Biophysics (Biophys, Uruguay)*Chair: Daniel Peluffo***Extracellular L-arginine dependence of NOS-mediated reactive oxygen/nitrogen species production in cardiac ventricular myocytes***Ramachandran, Jayalakshmi¹; Peluffo, R. Daniel²*

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L-Arginine (L-Arg) is the substrate for nitric oxide synthase (NOS) to produce nitric oxide (NO), a signaling molecule that is key in cardiovascular physiology and pathology. In cardiac myocytes, L-Arg is incorporated from the circulation through the functioning of system-y+ cationic amino acid transporters (CATs). Depletion of L-Arg leads to NOS uncoupling, with O₂ rather than L-Arg as terminal electron acceptor, resulting in superoxide formation. The reactive oxygen species (ROS) superoxide (O₂^{•-}), combined with NO, may lead to the production of the reactive nitrogen species (RNS) peroxynitrite (ONOO⁻), which is recognized as a major contributor to myocardial depression. To determine the levels of external L-Arg that trigger ROS/RNS production in cardiac myocytes, we used a two-step experimental design in which acutely-isolated cardiomyocytes were loaded with the dye coelenterazine that greatly increases its fluorescence quantum yield in the

presence of ONOO⁻ and O₂^{•-} but not with NO. Cells were then exposed to different concentrations of extracellular L-Arg, and changes in fluorescence were measured spectrofluorometrically following transport of this amino acid mediated by CATs. It was found that below a threshold value of 100 μM, decreasing concentrations of L-Arg progressively increased ONOO⁻/O₂^{•-}-induced fluorescence, an effect that was not mimicked by D-arginine or L-lysine and was fully blocked by the NOS inhibitor L-NAME. These results can be explained by NOS enzymatic activity, which includes O₂^{•-} production at limiting L-Arg, and provide an estimate for the levels of circulating L-Arg that trigger ROS/RNS-mediated harmful effects in cardiac muscle.

S05: Lipid Protein Interaction (SBP, Portugal)

Thursday 24 th November (15:30 - 17:30)
Chairs: Manuel Pietro, Silvia del Valle Alonso

Lipid Protein Interaction (SBP, Portugal)

Chair: Manuel Pietro, Silvia del Valle Alonso

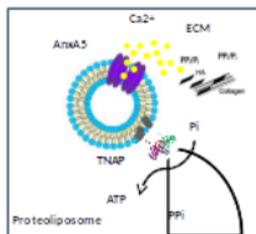
Set up and characterization of matrix vesicles biomimetic systems and their interaction with collagen fibers during biomineralization

Ciancaglini, P.¹; Bolean, M.¹; Simão, A.M.S.¹; Bottini, M.^{2,3}; Hoylaerts, M.F.⁵ and Mill'an, J.L.⁴

1. Departamento de Química, FFCLRP, Universidade de São Paulo, Ribeirão Preto, Brazil, 2. Department of Experimental Medicine and Surgery, University of Rome Tor Vergata, Rome, Italy. 3. Inflammatory and Infectious Disease Center, Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA, USA. 4. Sanford Children's Health Research Center, Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA, USA, 5. Department of Cardiovascular Sciences, Center for Molecular and Vascular Biology, University of Leuven, Leuven, Belgium.

Pathological or ectopic mineralization can occur in many soft tissues, including articular cartilage and vascular smooth muscle cells (VSMCs), most prominently in the kidneys, ligaments and tendons, leading to vascular stiffening and hypertension. Hence, ectopic vascular calcification represents a growing clinical problem associated with cardiovascular diseases in the aging population. VSMCs play an integral role in mediating vessel calcification by undergoing differentiation in chondrocyte/osteoblast-like phenotypes. Chondrocytes and osteoblasts control the deposition of the extracellular matrix (ECM) and the release of matrix vesicles (MVs), which serve as the initial sites for hydroxyapatite formation. Annexin V (AnxA5) is an acidic phospholipid-dependent Ca^{2+} -binding protein, which acts as Ca^{2+} -channel in the

MVs' membrane. Tissue-nonspecific alkaline phosphatase (TNAP) is attached to the MVs' outer membrane and acts as a pyrophosphatase hydrolyzing inorganic pyrophosphate (PPi), a potent mineralization inhibitor, and also as an ATPase producing Pi to help initiate mineralization. Herein, we describe the preparation of DPPC and DPPC:DPPS (9:1) proteoliposomes harboring AnxA5, TNAP or AnxA5⁵⁺TNAP, and their use as MV mimetic systems. Enzymatic activity, Ca²⁺ uptake and phase contrast microscopy of giant proteoliposomes validated the functional incorporation of both proteins in MV membrane. AnxA5 mediated Ca²⁺-influx into both DPPC and DPPC:DPPS (9:1) proteoliposomes at physiological Ca²⁺ concentrations and this process was not affected by TNAP presence. However, the presence of AnxA5 and DPPS significantly affected the hydrolysis of TNAP substrates.



Previous studies have shown that AnxA5 interacts with collagen fibers in the ECM and that this interaction regulates the process of mineralization. Both DPPC and DPPC:DPPS (9:1) proteoliposomes were found to bind type II collagen fibers. Proteoliposomes harboring AnxA5 bound

type II collagen with the highest affinity. The presence of DPPS in MV membrane significantly enhanced the degree of binding up to 74%. DPPC and DPPC:DPPS (9:1) proteoliposomes harboring TNAP poorly bound the collagen matrix (< 20% of binding). Proteoliposomes harboring both proteins showed 30% of binding. Among tested collagen fibers (I, II and III types), the best collagen-proteoliposome affinity was found for type II collagen. The interaction between AnxA5 and type II was Ca²⁺ independent.

These findings suggest that AnxA5 has a double role, i.e., create a Ca²⁺-rich microenvironment inside MVs and anchor MVs to collagen at calcification sites.

Financial supports: CNPq, CAPES and FAPESP.

Lipid Protein Interaction (SBP, Portugal)*Chair: Manuel Pietro, Silvia del Valle Alonso***Protein Aggregation on Membrane Surface investigated by SAXS***Rosangela Itri**Instituto de Fisica, Universidade de Sao Paulo (IFUSP), Brazil*

Several human degenerative diseases are thought to be associated with the deposition in tissues of proteinaceous aggregates known as amyloid fibrils. In addition of sharing the ability to form fibrillar aggregates with a common stacked cross-beta-sheet structure, several amyloidogenic proteins also interact with lipid membranes, particularly those containing negatively charged lipids. Therefore, formation of amyloid fibrils has been proposed to be catalysed by protein interaction with negatively-charged membrane interfaces. On the other hand, it is also recognized nowadays that lipid oxidation perturbs the structure and function of cells having pathological consequences as those found in neurodegenerative diseases. In this presentation, we are particularly interested in comprehending how lipid chemical transformations induced by oxidative stress can alter membrane structural features and, by turn, membrane-protein interaction. In this way, results of Small Angle X-Ray Scattering (SAXS) from liposomes representing model lipid vesicles composed of different amounts of unsaturated, oxidized and anionic charged lipids will be presented and discussed. Furthermore, the

self-assembling of amyloid-like proteins on liposomes surface will be also presented, pointing out the importance of lipid composition playing a role in protein aggregation. Supported by CNPq and FAPESP.

Lipid Protein Interaction (SBP, Portugal)*Chair: Manuel Pietro, Silvia del Valle Alonso***Enzymatic studies on planar supported membranes using a widefield fluorescence LAURDAN Generalized Polarization imaging approach***Luis Bagatolli**Center for Biomembrane Physics, University of Southern Denmark, Denmark*

We introduce a custom-built instrument designed to perform fast LAURDAN Generalized Polarization (GP) imaging on planar supported membranes. It is mounted on a widefield fluorescence microscope and allows kinetic analysis of the GP function in the millisecond time scale, largely improving the temporal resolution previously achieved using laser scanning based microscopes. A dedicated protocol to calibrate LAURDAN GP data obtained with charge-coupled device (CCD) cameras as detectors is also presented, enabling reliable assignment of GP values in the field of view. Using this methodology we studied structural and dynamical transformations induced by Sphingomyelinase D (SM-D) on planar supported membranes composed of N-lauroyl sphingomyelin (C12SM). GP data show the evolution of an initially compositionally homogeneous symmetric bilayer existing in a single liquid disordered phase, to an intermediate configuration showing coexistence of liquid disordered and solid ordered domains, which are not always

in-register across the axial plane of the bilayer. This intermediate state, caused by the transformation of C12SM to C12-ceramide-1-phosphate in the distal leaflet of the bilayer, evolved to a single solid ordered phase at longer time scales. Additionally, we comparatively studied this system using the membrane fluorophore DiIC18. The advantages and limitations of both fluorescent dyes are discussed, emphasizing the adequacy of LAURDAN GP imaging to explore this type of membrane phenomena. Luis

Lipid Protein Interaction (SBP, Portugal)**Selection Young Researcher***Chair: Daniel Peluffo***Membrane anchored metallo- β -lactamase: how to measure activity and stability in a lipid environment?***Giannini, Estefanía; González, Lisandro; Vila, Alejandro**Instituto de Biología Molecular y Celular de Rosario, IBR-CONICET-UNR, Rosario, Argentina*

Carbapenemases are β -lactamases capable of efficiently hydrolyzed almost all β -lactams antibiotics, including the carbapenems considered as "last resort" antibiotics. Among them, Metallo- β -lactamases ($M\beta$ Ls) are an important public issue because there is no clinically useful inhibitor for them. Of particular interest is the newly identified New Delhi $M\beta$ L (NDM-1), which is encoded in mobile genetic elements carrying other resistance genes. This has led to a rapid worldwide dissemination of its 16 alleles among pathogens in more than 70 countries, causing widespread concern [1].

Recently in our group, we have shown that NDM enzymes are particularly fit to endure infectious conditions since, in contrast to other MBLs, are lipidated protein bound to the inner leaflet of the outer membrane in Gram-negative bacteria [2].

With the aim of studying the impact of membrane-anchoring on enzymatic activity and stability, we have recently optimized a protocol

for estimating kinetic parameters in spheroplasts and stability in micelles, i.e., in conditions close to the native environment of the enzyme. Kinetic measurements in spheroplasts followed a Michaelian behavior which allowed us to derive kinetic parameters. The trend in k_{cat}/K_m values correlated with the resistant profile, validating the rationale of our approach, as well as its feasibility. We also optimized a protocol to assay the stability of membrane bound NDM-1, based on pulse proteolysis experiments under the presence of chaotropic agents, followed by Western Blot in micelles. These experiments allowed us to obtain quantitative parameters to evaluate the stability of NDM-1 variants. Overall, this approach will enable to assay the impact of different mutants in the activity and stability of NDM-1 under conditions mimicking the physiological environment.

Acknowledgements: NIH, ANPCyT, CONICET

References

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2. Gonzalez, L. J et al, (2016) *Nat. Chem. Biology*, 12, 51

Lipid Protein Interaction (SBP, Portugal)**Selection Young Researcher**

Chair: Manuel Pietro, Silvia del Valle Alonso

**Interactions between nisin and phosphatidylglycerol membranes.
A vibrational spectroscopy study**

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Nisin is an antibiotic polypeptide naturally synthesized by acid lactic bacteria.¹ Cytoplasmic membrane of Gram positive bacteria is the primary target of its bactericidal action.² This polypeptide has a positive net charge (+5) and an amphiphilic behavior that permit high affinity interaction with anionic lipid membranes.

The aim of this work is to perform a vibrational spectroscopic study (Raman and IR) of pure nisin and of phosphatidylglycerol (PG)/nisin mixtures in different lipid phases. Structural alterations of the anionic model membranes induced by the peptide addition were evaluated. In

turn, conformational changes in the peptide backbone upon its incorporation to the lipid media were also studied.

This is the first complete vibrational characterization of nisin. Spectral analysis was performed by quantum-chemical calculations of a peptide fragment (Ile1-Ala7) and by taking into account published assignments for amino acids and related peptide systems^{3,4}.

Vibrational bands considered as spectral markers of the lipid interchain coupling as well as the hydration degree of membrane polar headgroups evidenced the peptide effects in the different bilayer regions. Changes in the secondary structure of nisin embedded in lipid membranes were evaluated by the Amide I band in both the IR and Raman spectra.

The results allowed to conclude that nisin penetrated anionic bilayers replacing water molecules from the polar region and decreasing the chain coupling in the hydrophobic region. At the same time, an increment in β -turns was observed when nisin interacted with model membranes.

Agradecimientos

We thank to Dr. Eejfan Breukink for purified nisin donation and to CONICET for financial support

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- (3) Tuma, R. J. *Raman Spectrosc.* 36, 307-319 (2005)
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S06: Protein Structure and Function (SAB, Argentina)

Friday 25 th November (10:00 - 12:00)
Chairs: Mario Ermácora, Rodolfo Rasia

Protein Structure and Function (SAB, Argentina)*Chair: Mario Ermácora, Rodolfo Rasia***Use of quantum mechanics methods to study proteins and glycoproteins***Jorge Vila**IMASL-CONICET, Universidad Nacional de San Luis, Ejército de Los Andes 950, 5700-San Luis, Argentina*

Two major techniques have been used to determine the three-dimensional structures of proteins: X-ray diffraction and NMR spectroscopy. Unlike X-ray diffraction, validation of NMR-derived protein structures is one of the most challenging problems in NMR spectroscopy and, hence, a plethora of methods have been proposed to determine the accuracy and reliability of proteins, but not for glycoprotein, structures. Here, we report our efforts to develop a purely physics-based, structure validation method that enables us to characterize the 'quality' of the NMR-derived protein and glycoprotein structures, as a whole, and to unambiguously identify flaws in the sequence, at a residue level.

Protein Structure and Function (SAB, Argentina)*Chair: Mario Ermácora, Rodolfo Rasia***Focusing on the human frataxin, a key mitochondrial protein involved in the iron-sulfur center biosynthesis***Javier Santos**Laboratory 8 IQUIFIB (CONICET) and the School of Pharmacy and Biochemistry, University of Buenos Aires, Paraguay 2155 C.A.B.A.*

The possibility of manipulating biological activity of proteins through delicate modulations of local stabilities and dynamics, open the possibility of designing new variants and specific drugs. Thus, it sounds feasible the specific stabilization of natural variants intrinsically destabilized that take place in a number of diseases, including in Friedreich's Ataxia (FRDA), a rare cardioneurodegenerative disease caused by alterations in the expression of frataxin (FXN), an essential protein in mitochondrial metabolism. The aim of our work is to explore the relationship between stability and the structural dynamics of this protein. We asked how these properties are linked to the biological activity, the interaction with metal ions and the association to proteins of the iron-sulfur cluster assembly protein complex.

In this context, here we explored the effect of large sequence changes of loop-1 on the internal mobility of FXN in different timescales. Our results suggest communication between loop-1 and a region far from the former (including residues from the helix $\alpha 1$ and the beta-sheet)

and involved in protein-protein interactions, important for the biological activity.

Protein Structure and Function (SAB, Argentina)*Chair: Mario Ermácora, Rodolfo Rasia***Intrinsic disorder and linear motifs in host-pathogen interactions***Lucía B. Chemes**Adjunct Researcher, CONICET, Protein Structure Function and Engineering Laboratory, Leloir Institute IIBBA-CONICET, Buenos Aires, Argentina*

Linear motifs are key signaling modules in health and disease which can be defined as short sequences located in intrinsically disordered regions that mediate protein-protein interaction, subcellular targeting, degradation and post translational modification. Linear motif gain or loss can modulate signaling by modifying interaction partners, and changes in binding affinity can alter the strength of individual interactions, leading to strong phenotypic change. Despite their functional relevance, our understanding of linear-motif mediated interactions across organisms is still limited. We are focused on delineating the impact of linear motifs in viruses, where they are known to hijack the cell signaling through mimicry of host linear motifs.

I will present a biophysical and bioinformatics characterization of $L \times C \times E$ motif-mediated interactions, which direct binding of multiple host and viral proteins to the retinoblastoma (Rb) tumor suppressor, a central cell cycle regulator inactivated in human cancers and targeted by oncogenic viruses. The main determinants of binding include the

core L×C×E motif, and also arrangements involving concerted binding of several linear motifs. Specificity is also encoded within flanking regions, and through charge patterning in sites adjacent to the core motif. Sequence and structure-guided analyses of experimentally validated motifs reveal novel features that identify differences between host and viral motifs, and which will improve proteome-wide L×C×E motif discovery. We identify multiple L×C×E motifs in chromatin regulators, suggesting that Rb-mediated chromatin remodeling might be directed by linear motif interactions.

Protein Structure and Function (SAB, Argentina)**Selection Young Researcher**

Chair: Mario Ermácora, Rodolfo Rasia

Using genetically encodable self-assembling GdIII spin labels to make in-cell nanometric distance measurements

1.- Mascali, Florencia C., 2.- Tabares, Leandro C., 3.- Rasia, Rodolfo M.

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(2) *a - Laboratoire hyperfréquences, métalloprotéines et système de spin (LMESS, CEA-Saclay, Francia)*

(3) *a - Instituto de Biología Molecular y Celular de Rosario, IBR-CONICET-UNR, Rosario, Argentina*

In order to understand the function of a protein, it is of great relevance to know its structure, interactions and dynamic in native conditions. An approach to achieve this, is to determine distances between specific sites in nanometric scale. Double electron-electron resonance (DEER) is a technique that allows to measure in a range of 1.5 – 8 nm in a wide variety of environments, even inside cells. In the past, this technique was applied labeling the target proteins *in vitro*, followed by the reintroduction of the marked proteins inside the cells. In this work we used genetically encodable lanthanide-binding tags (LBT) to form self-assembling GdIII metal-based spin labels. The measurements were made between a pair of LBTs encoded one at each end of a 3-helix bundle. The protein was expressed in *E. coli* grown on a media supplemented with

GdIII. Finally, we could determined the nanometric distance in cell, being this the first work to use biosynthetically produced self-assembling metal-containing spin labels for non-disruptive in-cell structural measurements.

Protein Structure and Function (SAB, Argentina)**Selection Young Researcher**

Chair: Mario Ermácora, Rodolfo Rasia

Thermodynamics of the interaction between dengue virus NS3 helicase and single stranded RNA: pH and temperature effects

1.- Cababie, Leila A.; 2.- Incicco, J. Jeremias; 3.- González-Lebrero, Rodolfo M.; 4.- Gebhard, Leopoldo G.; 5.- Gamarnik, Andrea V.; 6.- Kaufman, Sergio B.

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(4) Fundación Instituto Leloir and IIBBA-CONICET

(5) Fundación Instituto Leloir and IIBBA-CONICET

(6) Instituto de Química y Físico-Química Biológicas (CONICET-UBA)

Dengue virus (DENV) NS3 protein is a helicase that catalyzes the hydrolysis of ATP and couples the free energy of this reaction to the translocation on single strands and to unwind double stranded RNA. We have previously presented evidence that the observed equilibrium binding constant, which governs the interaction between DENV NS3 and ssRNA, decreases with increasing salt concentration (salt effect). Additionally, we demonstrated that the formation of NS3-ssRNA complex is predominantly driven by the favorable free energy change from the release of cations from RNA (5-7 monovalent or 3 divalent cations) as a result of the establishment of 10 ionic interactions between protein and ssRNA.

In this work, we studied the effect of pH and temperature on the interaction between NS3 and ssRNA. Using a fluorescent 10 base-RNA oligonucleotide (F-p-R10), we performed spectroscopic titration experiments NS3 / RNA in the presence of different concentrations of mono and divalent cations at different pH and temperatures. We show that pH exerts only a minor and monotonous negative effect on the observed binding constant, which is more pronounced for the unlabeled than for the 5'-labeled RNA. We propose that almost one titratable groups in the protein is directly involved in the interaction with RNA (in the range of pH tested) and that the protonated state of the 5'-fluorescein moiety on the labeled RNA favors the stability of the protein-nucleic acid complex. Finally, from the temperature dependence of the observed binding constant, we obtained the enthalpic and entropic contributions to the Gibbs free energy change due to the salt effect on the equilibrium between NS3 and ssRNA.

Our results indicate that the association of NS3 to F-p-R10 is enthalpically driven under all experimental conditions tested and the effect of salt concentration on $\Delta_r G_{obs}^0$, resides almost entirely in its entropic term $T \cdot \Delta_r S_{obs}^0$, which decreased linearly with $\log[K^+]$.

Funding from: CONICET, and ANPCyT

S07: Biophysics and NanoBiotechnology (SBB, Brazil)

Friday 25 th November (10:00 - 12:00)

Chairs: Rosana Chehín, Rosangela Itri

Biophysics and NanoBiotechnology (SBB, Brazil)*Chair: Rosana Chehín, Rosangela Itri***Nanoparticles activate the immune system through recognition of Toll-like receptors***Jean Marie Ruyschaert**Free University of Brussels- Belgium-jmruyss@ulb.ac.be*

Toll-like receptors are major members of the Pattern Recognition Receptors (PRRs) from the innate immune system, which recognize bacterial or viral components. It was recently shown that these receptors that were considered so far as "detectors" of bacterial components have the capacity to recognize non bacterial motifs (1). These activating motifs are associated to natural nanoparticles (silica particles, asbestos, cholesterol crystals, amyloid aggregates) and engineered nanoparticles (fullerenes, gold nanoparticles, polymers, cationic liposomes). A main conclusion is that Nanomaterials are not as inert as we thought and are able to perturb our immune system. These inflammatory reactions can be desired (for vaccine development), unwanted (for delivery applications) or involved in the induction of non-infectious diseases (cardiovascular, autoimmune, allergic diseases, cancer, diabetes, amyloidoses, prion-related diseases, or pneumoconioses). For that reason, development of new molecules targeting or inhibiting these inflammatory responses may lead to therapeutic perspectives largely unintended

until now. I will be pleased to discuss these aspect and share the data with the meeting attendance. Implications in nanotechnologies (2) and nanomedicine will be briefly discussed.

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Biophysics and NanoBiotechnology (SBB, Brazil)*Chair: Rosana Chehín, Rosângela Itri***Innovative Drug Nanocarriers for Improved Therapy of Leishmaniasis***Frederic Frezard**Universidade Federal de Minas Gerais (UFMG), Brazil*

Pentavalent antimonials are first-line drugs for treatment of the neglected tropical disease leishmaniasis. However, the use of these old drugs is limited by the need for daily parenteral administration, their severe side effects and treatment failures. In light of these limitations, the World Health Organization strongly recommends research into new drugs against leishmaniasis. In this context, several structurally different nanocarriers have been investigated for the targeted delivery of antimonial drugs to infection sites of visceral leishmaniasis, as well as for the oral and topical delivery of antimony in visceral and cutaneous leishmaniasis. This presentation will cover the progress achieved towards pharmaceutically acceptable nanostructured formulations for the improved delivery of antimonial drugs. Special emphasis will be given to innovative nanostructures based on the unique physicochemical properties of antimony(V). The most promising nanosystems comprise liposomes for visceral leishmaniasis and micelle-like nanostructures for the oral delivery of antimony. Supported by CNPq and FAPEMIG.

Biophysics and NanoBiotechnology (SBB, Brazil)*Chair: Rosana Chehín, Rosângela Itri***LIPID-BASED DELIVERY SYSTEMS DESIGNED FOR ALLEVIATING PAIN***Eneida de Paula**Departamento de Bioquímica e Biologia Tecidual, Universidade Estadual de Campinas (Unicamp), Campinas, SP, Brasil*

Local anesthetics (LA) are broadly used for alleviating pain, both in medicine and dentistry procedures. They do that by reversibly blocking nerve conduction, through binding to the voltage-gated sodium channel of excitable membranes. Drug-delivery Systems (DDS) have been proven valuable to prolong the effect, and to decrease the toxicity of LA (de Paula et al., Exp. Op. Drug Delivery 9:1505, 2012). Liposomes and Nanostructured lipid carriers are interesting vehicles for drug delivery. We will present results with different (conventional, elastic, ionic-gradient, and binary) liposomal formulations, designed to extend the duration of action of LA, for infiltrative and/or topical anesthesia. Moreover, results on the encapsulation of the neutral LA species in nanostructured lipid carriers (dibucaine, tetracaine, lidocaine-prilocaine) will also be presented. For both lipid-based DDS, biophysical techniques (Electron/Nuclear Magnetic Resonance, Dynamic Light Scattering/NanoTracking Analysis) have been employed to characterize the structural organization of the nanoparticles, as well as to provide

details on the LA-lipid phase interaction.

S08: Young Initiative Talks

Friday 25 th November (14:00 - 15:00)
Chairs: Macarena Siri, Galo Ezequiel Balatti

Young Initiative Talks

Chair: Macarena Siri, Galo Ezequiel Balatti

An In Silico approach towards a better understanding of GABA_A receptors' structure/function relationship.

Amundarain, María Julia¹; Zamarreño, Fernando¹; Viso, Juan Francisco¹; Costabel, Marcelo¹

Grupo de Biofísica, Departamento de Física, Universidad Nacional del Sur –Instituto de Física del Sur. Bahía Blanca, Argentina

The GABAARs, γ -Aminobutyric acid type A receptors, mediate fast inhibitory transmission in the mammalian central nervous system. They belong to the pentameric ligand gated ion channels family along with serotonin type 3, nicotinic acetylcholine and glycine receptors. These anionic channels are pentameric ensembles of different subunits, and each subtype has specific function and localization. They are the target of many relevant compounds such as GABA, Benzodiazepines, Barbiturates, β -carbolines and Neurosteroids(1). Due to their wide influence in neurological health it is essential to understand their structure and function thoroughly.

We have previously developed a model of the $\alpha 1\beta 2\gamma 2$ subtype using homology modeling with the $\beta 3$ homopentamer as a template (PDBID: 4COF)(2). This structure is hypothesized to be in a closed desensitized state with high affinity for agonists. We evaluated the quality of the model by doing molecular docking with a series of ligands from which

experimental data is known about its binding mode. Molecular dynamics simulations were performed with the aim of analyzing the stability of the receptor modeled and comparing its behavior with and without ligands docked. In order to do so, we simulated the receptor alone, with one GABA molecule inserted in one of the orthosteric sites and with two molecules of GABA and Diazepam in their predicted binding sites. The structures remain stable along the 100 ns simulations, although minor structural changes can be noticed. There are differences among the simulations with and without ligands, possibly related to conformational changes from the transition between the open/closed states. In addition, the behavior of the ligands is evaluated along the simulation, highlighting those interactions important for binding stability.

We would like to thank CONICET and Universidad Nacional del Sur.

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Young Initiative Talks

Chair: Macarena Siri, Galo Ezequiel Balatti

Physiological states of yeast assessed by Zeta potential

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Zeta potential (ZP) measures the electrostatic charge on the solid-liquid interface of suspended particles. In cells, these charges can be related to the ionization of chemical groups, adsorption of ions from the surrounding solution or to ion exchange driven by the metabolic activity.

The aim of this work is evaluate the ZP evolution during the growth of *Saccharomyces cerevisiae* and under different stresses, to find out a correlation between cell surface properties and the physiological state.

Yeast growth was evaluated following the electrophoretic mobility of cells in an electric field, together with CFU, OD_{600nm} and pH variations. Data obtained show a correlation between ZP mean values and OD, and because cells can be classified into subpopulations according to their surface charge it was found that an alteration in these subpopulations coincides with changes in the growth phase.

The decrease in pH during exponential growth phase parallel to a shift of the ZP to less negative values can be related to the partial neutralization of negative charges by the cell acid products. In contrast after the diauxic shift, pH remains relative constant and the ZP changes abruptly

to negative values. This suggests that acid production is not enough to compensate the negative charges, which can be interpreted as a consequence in chemical composition of the cell wall.

ZP measurements were applied on cells exposed at different stresses, and these data were correlated with size information obtained by DLS. The results obtained showed that cells in exponential phase were more sensitive to stresses.

It is concluded that the ZP is a useful technique for monitoring cell culture, and to sense changes in the physiological state of the cell.

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Young Initiative Talks

Chair: Macarena Siri, Galo Ezequiel Balatti

Deconstructing the role of key residues of the iron-binding motif EExxED using unstructured peptides and a grafted protein scaffold

Vazquez, Diego Sebastian¹; Giraudo, Laura Romina¹; Agudelo, William Armando²; Aran, Martín³; González Flecha, F. Luis¹; González Lebrero, Mariano Camilo²; and Santos, Javier¹.

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Proteins belonging frataxin family control intracellular iron homeostasis through a clusters of acidic residues, that are apparently responsible for iron binding. To understand how frataxin works as an iron chaperon, we studied the interaction of metal ions with a putative iron binding site from the bacterial frataxin CyaY. For this purpose, the EExxED motif from the bacterial frataxin was grafted on a 16 residue-long peptide, corresponding to the C-terminal α -helix from *E. coli* thioredoxin [1], giving the GRAP peptide [2]. We found by computational docking experiments that the motif has two mutually exclusive sites, involving only three acidic residues (ExxED or EExxE). In order to study

the effect of each residue separately on the interaction with metal ions of different chemical nature (Mg^{2+} , Al^{3+} , Fe^{3+} and Ga^{3+}), three engineered GRAP-derived peptides were synthesized carrying the sequences ExxED, EExxE and ExxE. Preliminary results obtained by circular dichroism suggest that: (i) In the absence of metal ions all the peptides behave as random-coil peptides at pH 7.0; (ii) Fe^{3+} and Al^{3+} are capable to induce/stabilize the helical structure of GRAP and peptide variants at pH 4.1 and pH 7.0, respectively; (iii) Nevertheless, Ga^{3+} only promote the acquisition of helical structure of GRAP, suggesting a fine-tuning of the helix-coil transition. To gain further insights, the full length *E. coli* TRX was engineered including the EExxED motif in the C-terminal α -helix (TRX_{grap}), which is already structured in the native ensemble. By ^{15}N - 1H HSQC NMR experiments we found a specific interaction of Fe^{3+} with the EExxED motif causing the disappearance of cross-peak signals corresponding to residues located in the surrounding of the iron-binding motif.

This work was supported by grants from UBACyT, ANPCyT, and CONICET

1 Vazquez DS et al., The *E. coli* thioredoxin folding mechanism: The key role of the C-terminal helix, *Biochimica et Biophysica Acta*, 1854(2):127-137, 2015.

2 Vazquez DS et al., A helix-coil transition induced by the metal ion interaction with a grafted iron-binding site of the CyaY protein family, *Dalton Transactions*, 44, 2370-2379, 2015.

S09: Mathematical Modeling and Computational Biophysics (LAFeBS)

Friday 25 th November (15:30 - 17:30)
Chairs: Luis González Flecha, Ari Zeida

Mathematical Modeling and Computational Biophysics (LAFeBS)

Chair: Luis González Flecha, Ari Zeida

Lipid-protein interactions in the plasma membrane: A simulation approach

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Abstract Cell membranes function as physical barriers for the cell and control the exchange of ions, peptides, and small molecules between the interior and the exterior of the cell. The main structural components of cell membranes are lipid molecules, whose hydrocarbon tails provide the barrier-like properties, and membrane proteins, which carry out specific functions depending on their role as receptors, transporters, channels, or enzymes. Computer simulations of membrane models and membrane proteins have been extensively used to study the details of lipid organization. However, these model bilayers usually consist of few lipid types, whereas a typical cell membrane contains hundreds of different lipids. Here, we present computer simulations of ten different membrane proteins embedded in a plasma membrane model. This plasma model consists of 63 different lipid types, and features an asymmetric lipid composition between leaflets [1]. The membrane

proteins include GPCRs, transporters, ion channels, pumps and monotopic membrane proteins. Each simulation system is modelled with the MARTINI force field [2]. Using molecular dynamics simulations, we show how each protein modulates its local lipid environment in terms of thickness, curvature and changes in lipid distribution.

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Mathematical Modeling and Computational Biophysics (LAFeBS)

Chair: Luis González Flecha, Ari Zeida

Affordable viral particle's simulations on desktop computers using SIRAH force field

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The huge advances in technology are leading a breakthrough in the field of molecular dynamics (MD) simulations allowing for larger system sizes and longer time scales to be afforded. The recent use of Graphical Processing Units (GPU) on desktop computers for accelerating computational simulations grants accessibility at good price/benefit to the majority of the scientific community. Yet the simulation of most full viral particles on explicit solvent remains elusive due to processing and memory limits of modern GPUs.

In this work we introduce a new multiscale strategy based on our coarse-grained water model (WT4)^{1–3} and a recently developed mesoscale solvent model (WLS), which permits to considerably reduce the system's size in terms of solvent particles. The viral capsid can be treated atomistically within a shell of water molecules or at coarse-grained level using SIRAH force field⁴. The approach was successfully tested on several viral systems from *Sobemovirus* to *Picornavirus* and *Flavivirus*.

This methodology allows for simulating viral particles as big as *Reovirus* (0.1 mm in diameter) on desktop computers of a few thousand dollars. Even better, the model is included in the SIRAH package [www.sirahff.com] and works as plug and play in free MD engines like GROMACS.

Mathematical Modeling and Computational Biophysics (LAFeBS)

Chair: Luis González Flecha, Ari Zeida

Modeling signaling of proliferation and survival from ligand/receptor systems using *Pathway logic* based in *Rewriting logic*

De Las Rivas, Javier

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The biological pathways in the living systems define sets of complex biochemical reactions and interactions between multiple molecular elements that produce some specific products or cellular effects in response to different signals. These biological systems are dynamic and we need mathematical methods that can analyze symbolic elements and complex interactions between them to produce adequate readouts of such systems. *Rewriting logic* (RLo) is one of the mathematical methodologies and procedures adequate to study dynamic systems that can be applied to explore the behaviour of biological pathways. *Pathway logic* (PLo) is a bioinformatics program recently developed applied to biological pathways, which is based in the *Rewriting logic* language called *Maude*. PLA allows to define transition rules and to set up queries about the flow in the biological pathways. In this communication we describe the use of *Rewriting logic* and *Pathway logic* to model and analyze the dynamics in a well-known signaling transduction pathway: epidermal

growth factor (EGF) pathway. We also use *Pathway logic Assistant* (PLA) tool to browse and query this system.

Santos-García G, Talcott C, De Las Rivas J (2014). A logic computational framework to query dynamics on complex biological pathways. **8th International Conference on Practical Applications of Computational Biology and Bioinformatics (PACBB 2014)**, 207-214.

Santos-García G, Talcott C, De Las Rivas J (2015). Analysis of cellular proliferation and survival signaling by using two ligand/receptor systems modeled by *Pathway logic*. **International Workshop on Hybrid Systems Biology**, 226-245.

Santos-García G, Talcott C, Riesco A, Santos-Buitrago B, De Las Rivas J (2016). Role of Nerve Growth Factor Signaling in Cancer Cell Proliferation and Survival Using a Reachability Analysis Approach. **10th International Conference on Practical Applications of Computational Biology and Bioinformatics**, 173-181.

**Mathematical Modeling and Computational Biophysics (LAFeBS)
Selection Young Researcher**

Chair: Luis González Flecha, Ari Zeida

Cell surface receptors improve their signaling properties when working far from steady-state: a mathematical/computational study

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Instituto de Fisiología, Biología Molecular y Neurociencias

Sensing extracellular changes initiates signal transduction and is the first stage of cellular decision-making. Ligand binding to cell membrane receptors is a key event in those sensing stages. It is rarely certain whether cellular responses are related to initial changes in receptor binding or to the level of receptor binding achieved at some later time, but it is likely that the dynamics of receptor/ligand binding contributes significantly to the dynamics of the response. Particularly, certain properties of the sensing steps are usually characterized in equilibrium, like the value of half-maximal effective concentration, the dynamic range, and the Hill coefficient. However, if the time constant of downstream signal transduction steps is shorter than that of ligand-receptor binding, those properties should be evaluated in pre-equilibrium.

Using a simple monovalent binding model, a two-state binding model, and two limiting cases of this last one in which only one receptor form

can bind or release ligand, we studied the mentioned properties in pre-equilibrium. We combined analytical tools when possible, with computational modeling and parameter space exploration.

Our results imply that pre-equilibrium sensing is possible depending on the relation of binding and activation rates. When binding rates are slower than activation rates, the system can sense high dose concentrations on pre-equilibrium. Conversely, when binding is faster than activation, pre-equilibrium sensing properties remains similar than steady state properties. Moreover, when the time scales are similar, pre-equilibrium sensing is possible but with certain limitations, depending on the time constant and the ligand concentration involved on the downstream process.

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Mathematical Modeling and Computational Biophysics (LAFeBS) Selection Young Researcher

Chair: *Luis González Flecha, Ari Zeida*

Modelling the organization and function of circadian clocks: from cells to tissue

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Predictable environmental changes have been critical to the temporal organization of most living beings. Organisms have developed endogenous circadian clocks, which generate daily variations in biological processes, with periods close to 24 h. At the single cell level, circadian clocks are based on a set of clock genes and proteins expressed in a circadian fashion as a consequence of their mutual interactions based on interconnected feedback loops. Translation of some clock proteins, such as PER, is affected by regulatory proteins and/or microRNAs, but the role of translational regulation in the circadian clock dynamics is not fully understood. We hypothesize that translational regulation produces changes in the kinetics of PER synthesis. Using a mathematical model of the core molecular clock we describe different putative

kinetic mechanisms of PER synthesis and their effects on the molecular clock dynamics. In mammals, a master circadian pacemaker in the brain, the suprachiasmatic nuclei (SCN), is composed of cellular circadian clocks able to communicate with each other, sync their activity and hence become a precise and robust biological clock, able to drive circadian rhythms at physiological and behavioral levels. The expression of clock proteins within a SCN slice displays a specific spatio-temporal pattern, characterized by the heterogeneity of their phase peaking. The mechanisms by which these phase relationships are established are not well understood, but depend on the integration of multiple intercellular signals, which ultimately define the functional connectivity of the SCN. We use a model of circadian cellular oscillators coupled through different network architectures to simulate the dynamical behavior observed in SCN slices. We then present the characterization of the emerging dynamical behavior of our models according to their topology and show how these models can be used to infer the functional connectivity of the SCN.

S10: Calcium Signaling (SOBLA)

in memoriam R. Dipolo

Friday 25 th November (15:30 - 17:30)

Chairs: Ariel Escobar

Calcium Signaling (SOBLA), it in memoriam R. Dipolo*Chair: Ariel Escobar***Is the coupling between the voltage-sensing domains and the pore of high voltage calcium channels allosteric?***Alan Neely**Centro Interdisciplinario de Neurociencias de Valparaíso, Valparaíso, Chile**Division of Molecular Medicine, Department of Anesthesiology, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA**Universidad de Valparaíso, Valparaíso, Chile*

Like other the voltage-activated ion channels, HVA channels are designed as four-fold symmetric structures with four units, each composed of the two well define domains: the voltage-sensing domain (VSD) and the pore domain (PD). These units are daisy-chained in a single polypeptide so that each of the four VSD and PD domains can have different amino acid sequences and thus likely to exhibit distinct voltage-sensing properties and function. Monitoring the activity of individual VSD through changes in the emission of fluorescent probe tethered to strategic position in the pore-forming protein revealed that functional properties of individual VSD are very different from each other and only a subset of them activated within a voltage- time- scales compatible with channel opening. The energy transferred to channel opening varies and appears to be regulated by auxiliary subunits when modelled within an allosteric framework. On the other hand the number

of charges necessary to open the channel was close to the sum of the voltage dependency of the activation for the 2nd and 3rd VSD, suggesting that only these VSDs are strictly coupled to the PD domain despite that all four of these protein domain are covalently linked to the pore domain.

Calcium Signaling (SOBLA), it in memoriam R. Dipolo*Chair: Ariel Escobar***Slow force response to myocardial stretch: Mechanical expression of a redox-triggered increase in intracellular Ca^{2+} transient***Nestor Gustavo Perez**Centro de Investigaciones Cardiovascular –Conicet Facultad de Medicina- Universidad Nacional de La plata*

The stretch of cardiac muscle increases developed force in two phases. The first phase occurs immediately and is expression of the Frank-Starling mechanism. The second one called slow force response (SFR) occurs gradually and is consequence of a stretch-triggered autocrine/paracrine loop of intracellular signals leading to reactive oxygen species (ROS)-promoted increase in intracellular Ca^{2+} transient. The complete chain of events triggered by stretch comprises: 1) release of prohypertrophic factors (angiotensin II-endothelin) and activation of their corresponding receptors, 2) activation of the mineralocorticoid receptor, 3) NADPH oxidase activation and transactivation of the epidermal growth factor receptor, 4) mitochondrial ROS production, 5) activation of redox-sensitive kinases (ERK1/2-p90RSK) upstream Na^+/H^+ exchanger (NHE1), 6) NHE1 hyperactivity, 7) increase in Na^+ concentration, and 8) increase in Ca^{2+} concentration through $\text{Na}^+/\text{Ca}^{2+}$ exchanger. Interestingly, sustained increase in ROS, NHE1 activation, and increased Ca^{2+} concentration appear to be crucial for the development

of pathologic cardiac hypertrophy following an increased mechanical strain. In this context, it seems that mechanical stretch may not only trigger immediate heart mechanisms (Frank-Starling mechanism and SFR) to adapt cardiac output to changes in hemodynamic conditions, but also would constitute the first step toward pathologic cardiac hypertrophy if the mechanical stimulus is maintained over time.

Calcium Signaling (SOBLA), it in memoriam R. Dipolo*Chair: Ariel Escobar***Ionic Bases of Epicardial Ventricular Action Potentials***Ariel L Escobar**School of Engineering, University of California, Merced.*

Assessing the underlying ionic currents during a triggered action potential (AP) in intact perfused hearts offers the opportunity to link molecular mechanisms with pathophysiological problems in cardiovascular research. We developed the Loose Patch Photolysis (LPP) technique that can provide striking new insights into cardiac function at the whole heart level during health and disease. LPP allows the measurement of transmembrane ionic currents in intact hearts. During a triggered AP, a voltage-dependent Ca^{2+} conductance was fractionally activated by rapidly photo-degrading the Ca^{2+} channel blocker nifedipine. The ionic currents during a mouse ventricular AP showed a fast early component and a slower late component. The early component was driven by an influx of Ca^{2+} through the L-type channel, $\text{CaV} 1.2$. The late component was identified as a Na^{+} - Ca^{2+} exchanger (NCX) current mediated by Ca^{2+} released from the sarcoplasmic reticulum (SR). Finally, LPP allowed the dissection of transmembrane ionic currents on the intact heart. We were able to determine that L-Type Ca^{2+} current contributes to phase 1 while the NCX contributes to phase 2. In addition, LPP revealed that the influx of Ca^{2+} through the L-type Ca^{2+}

channels terminates due to voltage-dependent deactivation and not by Ca^{2+} dependent inactivation, as commonly believed.

**Calcium Signaling (SOBLA), it in memoriam R. Dipolo
Selection Young Researcher**

Chair: Ariel Escobar

**Novel CaV2.1-inhibitors with potential to treat Hemiplegic
Migraine**

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Human mutations in the CACNA1A gene, encoding the pore-forming α_{1A} subunit of the voltage-gated CaV2.1 calcium channel, cause most of the familial and sporadic hemiplegic migraine (FHM/SHM) cases. Hemiplegic Migraine (HM) mutations induce a gain of CaV2.1 channel activation that specifically enhances cortical excitatory transmission to

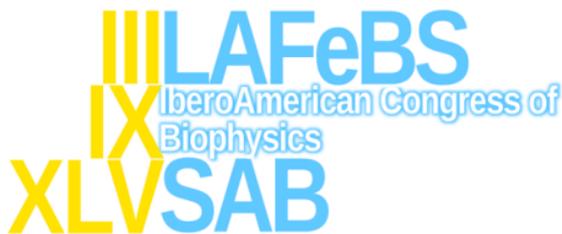
favor initiation and propagation of cortical spreading depression, a key process in migraine pathophysiology¹. Accordingly, there are pharmacological evidences suggesting that reduction of CaV2.1 activity can provide a new therapeutic approach for the treatment of Hemiplegic Migraine (HM) and the relief of common migraine^{2,3}. Currently the only truly CaV2.1-selective inhibitors are peptide toxins of the agatoxin family, which are not suitable therapeutic tools: their mode of inhibition can give rise to undesirable side effects and peptides have limited utility for *in vivo* studies. Here we have employed two strategies to identify novel selective CaV2.1-inhibitors: a) chemical modifications from a start-point-molecule that we have found to be more effective on the Y1245C FHM mutant CaV2.1 channel than on the WT channel; and b) a structure-based design approach to identify ligands that selectively bind to the calmodulin binding (IQ-like) domain of the CaV2.1 channel, whose amino acid sequence differ from the IQ-site of other Cav family members. Indeed, CaV channel blockers with tunable selectivity, kinetics and potency have been previously characterized via targeting of the IQ motif⁴. From an initial set of 80 compounds identified as potential CaV2.1 blockers *in silico*, our results highlight eight novel small organic molecules with higher selectivity for CaV2.1 inhibition (over CaV2.2 and CaV1.2 blockade) as prospective elements to develop HM therapeutic tools.

Calcium Signaling (SOBLA), it in memoriam R. Dipolo**Selection Young Researcher***Chair: Ariel Escobar***Direct quantification of the Ca^{2+} free diffusion coefficient in *Xenopus laevis* oocytes.***Villarruel, Cecilia; Lopez, Lucía; Ponce Dawson, Silvina**Instituto de Física de Buenos Aires (IFIBA), Argentina. Facultad de Ciencias Exactas y Naturales, UBA*

The versatility of calcium as a signaling agent is based on the great diversity of spatio-temporal behaviors that its concentration can display inside cells. In most cell types Ca^{2+} signals not only depend on Ca^{2+} entry from the extracellular medium, but also on Ca^{2+} release from internal stores. *Xenopus laevis* oocytes are a model system in which an enormous variety of calcium signals can be evoked, from localized signals to cellular waves. In particular, in *X. laevis*, the endoplasmic reticulum (ER) is the largest store of releasable calcium. The rate of Ca^{2+} transport is key in shaping the spatio-temporal dynamics of intracellular calcium signal. Diffusion coefficients can be estimated with Fluorescence Correlation Spectroscopy (FCS) experiments. The direct application of this technique to the case of Ca^{2+} transport in intact cells is not completely straightforward because the recorded fluorescence is emitted by the Ca^{2+} -bound dye that diffuses much more slowly than

free Ca^{2+} and because the dye competes for Ca^{2+} with other endogenous trapping mechanisms present in the cell. In this work we perform FCS experiments in the cytosol and the ER lumen of *X. laevis* oocytes, using Fluo-8 and Fluo-5N, respectively as the Ca^{2+} dye. We then apply our previously developed theory to interpret the results and quantify the free diffusion coefficients of Ca^{2+} and some representative coefficient of the endogenous buffers that compete with the dye for Ca^{2+} .

Poster Sessions



Submission ID 151 – Poster
Lipids, Structure and Function

L-cysteine Methyl ester interaction with DPPC multilamellar membranes by means of FTIR, Raman and DSC in anhydrous and hydrated states.

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Introduction: Many important mechanisms for life take place within cellular biological membranes. Since they are very complex, biomimetic systems like lipidic vesicles have been developed to study their properties and structure. This work complements a series of studies carried out for Cys and its esters that present an easily oxidable thiol group. In this case it is for L-Cysteine Methyl ester (MC). The thiol group has numerous biological functions because of its high reactivity.

Objectives: To study the participation of the main functional groups of DPPC lipidic membranes involved in the membrane-solution interaction with MC in gel and liquid crystalline phases by means of FTIR and

Raman microscopy and to determine by DSC how MC affects membrane fluidity and thermodynamic values.

Results: When the transition temperature of DPPC multilamellar vesicles was studied at different MC concentrations following the C-H methyl group symmetric stretching band, there was a transition temperature change with respect to that of the pure liquid. The FTIR spectra of the MC:DPPC systems prepared in D₂O and H₂O were obtained to carry out the corresponding assignments to the C=O and PO₂⁻ polar heads respectively. Changes in the wavenumbers of the stretching modes of the C=O and PO₂ were observed in the liposomes formed by MC:DPPC at different molar ratios with respect to the pure liquid both in hydrated and dehydrated states.

Conclusions: The formation of PO₂⁻-MC hydrogen bonds and a dehydrating effect are inferred by FTIR in the interphasial region of the carbonyl group for both P1 and P2 populations without the later formation of the CO-MC hydrogen bond. The gauche rotamers population of the acyl chains was not modified with the increment of MC concentration.

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Submission ID 157 – Poster
Lipids, Structure and Function

Development of liposomal formulations for the encapsulation of mucolytics: combined treatments against respiratory diseases.

1.- Feas, Daniela Agustina; 2.- Lopes Da Silva, Adriana; 3.- Rocco Macedo Rieken, Patricia; 4.- Morales, Marcelo; 5.- Alonso, Silvia del Valle; 6.- Chiaramoni, Nadia

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Respiratory diseases are very common and affect a large part of the population worldwide. The key for the enhancement of efficiency of treatments is that therapeutic agents reach the target lung tissue. In order to achieve this, barriers such as lung architecture, defense mechanisms and mucus must be overcome [1].

Mucus is a viscoelastic gel that protects the respiratory tract [2]. Mucolytic agents break down the gel structure of mucus and decrease its elasticity and viscosity [3]. Moreover, lungs are covered with a surfactant layer, which main component is DPPC (dipalmitoyl-sn-glycero-3-

phosphatidylcholine); other minor components are PMPC (1-palmitoyl-2-myristoyl-sn-glycero-3-phosphocholine) and POPC (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine) [4].

Based on this, the aim of this work was the development of mucolytic transporters, which encapsulated the mucolytics dornase alpha and L-cysteine. In order to improve interaction of these transporters with lung tissue, liposome formulations were composed of phospholipids present in the surfactant layer.

To carry out this work, liposomes were obtained by the dehydration/rehydration method and were combined with the different mucolytics. Large unilamellar vesicles were obtained by passing the mixture 15 times through an extruder (Transferra Nanosciences Inc.). Particle size of the different formulations was determined in a ZetaPlus Particle Sizing (Brookhaven Instruments Corp).

Currently, citotoxicity of the formulations is being assessed in A549 cells, which are used as an in-vitro model of the alveolar epithelial type II cells, and in primary cell culture of mice lung.

Acknowledgments: ANPCyT-MinCyt (PICT-Cabbio 511/14), UNQ, IMBICE-CONICET.

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Submission ID 188 – **Electronic Poster**
Lipids, Structure and Function

Theoretical studies of the interactions of a new lipid-antimetastatic agent with different membranes.

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An antitumoral lipid named Ohmlin (1-O-hexadecyl-2-O-methyl-sn-glycero-3-lactose) has been shown to reduce metastasis development in a mice model of tumor. The behavior of Ohmlin interacting with four bilayers (with different compositions) was analyzed using an united-atom molecular dynamics simulations. Additionally, a new force field combining the GROMOS FF 43A1-S3 for lipids and GROMOS 56A-carbo for sugars was developed.

The simulated system were: i) Pure 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) bilayer as a model system, ii) Pure sphingomyelin (SGML) bilayer as another model system, iii). A system similar to a cell membrane in composition, formed by 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) in one monolayer and 1-

palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (POPE) and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-L-serine (POPS) in the other monolayer and iv) A system similar to a membrane nanodomain in composition, formed by a mixed of 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine (DSPC), sphingomyelin and cholesterol (CHOL). The results suggest that the Ohmlin molecule has a variable behavior according to the environment where it is located. Nevertheless it is able to decrease the order of the lipid tails specially in the nanodomain system changing also the lipid-lipid interactions among the lipid components. These results could be related with the fact that some membrane receptors involved in the development of cancer cells are located into these domains and probably the Ohmlin molecule affect their activities by changing some properties of the surrounding membrane (e.g. membrane order and lipid organization). Finally this work will help into the understanding of the unknown antimetastatic mode of action of the Ohmlin molecule.

Acknowledgment: Agencia Nacional de Promoción Científica y Tecnológica (PICT 1249).

Submission ID 202 – Poster
Lipids, Structure and Function

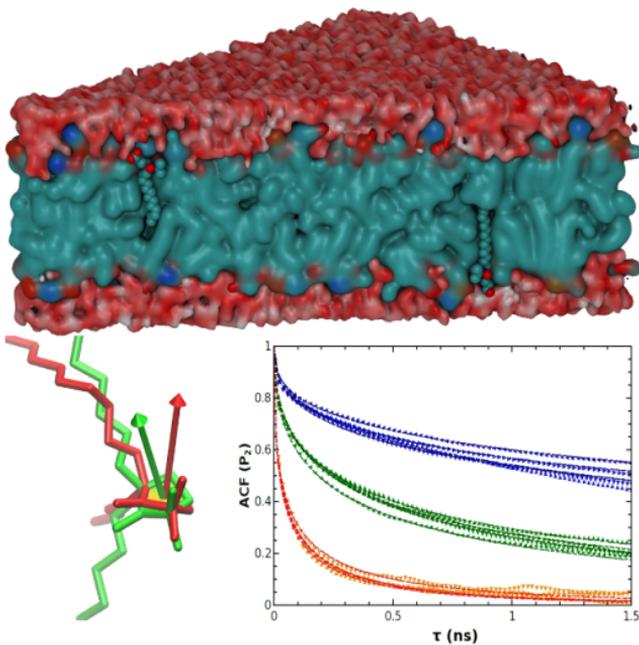
Spin Labels n-SASL (n = 5, 12, 16) Dynamics in Fluid Phase of DPPC Model Membranes: a Molecular Dynamics Study to Understand the EPR spectra features and their relation to membrane properties.

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The interpretation of the Electronic Paramagnetic Resonance (EPR) spectra of spin labels used in biomembrane investigations is still based in heuristic models and assumptions about location, environment and orientational dynamics of the probe. This fact limits the scope of inferences provided by the experimental technique. We simulated, by Molecular Dynamics, hydrated DPPC (Dipalmitoylphosphatidylcholine) bilayers of 480 lipids doped with 5, 12 and 16-SASL (Spin Label Stearic Acid) in fluid phase (LC) to analyze the location and orientational dynamics of the doxyl ring containing the unpaired electron, source of the magnetic properties associated with the EPR spectra. We used molar concentration of the spin labels similar to those employed in experimental conditions (<1%) to avoid for exchange narrowing of the spectra. This condition also assures that the properties of the host

membrane were not modified by the molecules of the probes. Four independent long simulations (>450ns) were performed to overcome the problem of the poor statistical sampling due to the low concentration of the spin label. The results for the three spin label species show that their polar head locate approximately at the same depth inside de membrane. The interactions that determine this behavior were analyzed. The depth interval sampled by each spin label specie were determined. The Rotational Autocorrelation Functions of the normal to the doxyl ring were calculated and the rotational dynamics were identified as anomalous (subdiffusive) with correlation times $\tau_R = 4, 0.54$ and 0.059 ns for 5, 12 and 16-SASL respectively. The one order of magnitude change in the rotational correlation times τ_R among different spin label species was associated with the conformational freedom of the portion of the carrier fatty acid between the polar head and the site of the doxyl ring (5, 12 and 16). The results were in good agreement with the EPR spectra of these spin labels in oriented samples of egg DPPC.



Submission ID 209 – **Electronic Poster**
Lipids, Structure and Function

The interaction of miltefosine with lipid monolayers is modulated by its rheological properties

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Miltefosine (hexadecylphosphocholine) is a synthetic alkylphospholipid, with moderate water solubility and tested surface activity. It is currently used as a selective cytotoxic drug for anticancer treatment or against diseases such as Leishmaniasis. To explore the modulation of the incorporation of miltefosine into membranes, we used lipid monolayers onto air /water interfaces as model membranes. Those monolayers, composed of pure lipids were previously characterized as being in phase states such as; liquid-expanded (LE), liquid-condensed (LC), solid (S) and liquid-ordered (LO), as well as binary mixtures of charged lipids and ternary and quaternary mixtures that mimic the properties of membranes containing cholesterol and stratum corneum.

We found that the insertion of miltefosine induces changes in the rheological properties of the monolayers employed, becoming more easily

compressible. In turn, membranes with different phase states regulated the extent and kinetics of the penetration process. The more fluid monolayers showed greater insertion and faster adsorption / desorption kinetics compared to condensed membranes. Monolayers containing sterols allowed a small but fast insertion and slow desorption of the drug.

We also evaluated the preferential partitioning of the drug into heterogeneous membranes by Brewster angle microscopy. We observed that both mixtures containing cholesterol with coexisting LO / LE and LC / LE-phases allow the insertion of the drug preferentially into the LE phase. A shift of the miscibility phase point towards higher surface pressure and changes in the interfacial texture was found, in some cases showing very small domains at low pressures. This may have important implications for the regulation of biochemical processes of lipid homeostasis by miltefosina in target cells.

Submission ID 210 – **Electronic Poster**
Lipids, Structure and Function

Properties of membranes with different sterols and hopanoids

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It has been proved that liquid-ordered phases may be present in the membrane of organisms of different kingdoms, however there is a lack of systematic studies in which the differences and similitudes between the different sterols and hopanoids are evaluated together. Therefore, we are interested in investigating how the presence of sterols and hopanoids regulate the properties of the membranes using different model systems for lipid bilayers and monolayers.

In this work, as a first approach, we used fluorescence microscopy in order to construct phase diagrams for canonical raft mixtures containing DOPC, DPPC and different sterols (cholesterol, ergosterol or stigmasterol) and diplopterol, an hopanoid. We observed that in Langmuir monolayers at the air-water interface ergosterol presents phase coexistence in a wider range of surface pressures than the rest of the tested compounds. For mixtures containing the phytosterol stigmasterol, the phase coexistence region is very thin, but a second two phase region exists near a surface pressure of 20mNm^{-1} at 10% of the sterol. This

behavior was also observed for cholesterol at 10%. Interestingly, for diplopterol, a two phase region was observed between 0-6mNm⁻¹ and the domains presented an elongated worm-like shape, suggesting that with this particular mixture of phospholipids, the hopanoid form a condensed phase instead of a Lo phase. In planar free-standing bilayers, 10% of the different compounds in the same lipid system led to different phase behaviors.

Submission ID 220 – **Electronic Poster**
Lipids, Structure and Function

A molecular, rheological and thermodynamic study of lipid-vaseline mixtures

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Lipid microemulsions (ME), commonly used to encapsulate molecules of pharmacological interest, are oil in water dispersions stabilized by an interfacial layer of a surfactant. Previously we defined monomolecular layers of phospholipids at Vaseline 80 SSU (VAS)/water interface as an experimental model of ME, and reported data of their composition and thermal behavior using a Langmuir interfacial trough. Results suggested that molecules from oil phase were incorporated in the monolayer transforming it in a VAS/DPPC mixture. In the present work we confirmed this hypothesis by studying the interfacial behavior of VAS/DPPC pseudobinary mixtures at the air/water. According to $^1\text{H-NMR}$ and GC-MS analysis, VAS was a mixture of alkanes with carbon lengths (CL) ranging from 19 to 29 (mean $\text{CL}=25\pm 3$) and a weighted mean molecular mass of 346.89 g/mol. It is noteworthy that VAS was unstable at the air-water interface (it did not form monolayers) so, at $\text{XVAS}=1$, the value for the mean molecular area (Mma) was

0 \AA^2 . Thus, the Mma for all mixtures was proportional to the molecular area of DPPC. At all the assayed compositions the π -A isotherms were shifted to higher molecular areas with respect to pure DPPC. The π value for bidimensional phase transition (π T) increased from 8.5 for pure DPPC to 15.5 mN/m for mixtures containing a molar fraction of VAS (XVAS) within the 0.05-0.6 range. Up on XVAS increased, the phase transition became less cooperative. At XVAS=0.8 it disappeared and the monolayer acquired a smooth liquid-expanded behavior. The Mma vs. XVAS plot revealed that DPPC/VAS mixtures exhibited huge positive deviations from ideality at all compositions, indicating repulsive intermolecular interactions. The π -XVAS phase diagram allowed predicting a phase separation at high π and XVAS This conclusion was supported by BAM images which exhibited the emergence of bright dots at XVAS=0.9, possibly due to collapsed multilayered structures. Acknowledgements: CONICET, FONCYT, SeCyT-UNC.

Submission ID 243 – **Poster**
Lipids, Structure and Function

Conformation of Jojoba oil esters at the air-water interface

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Wax esters (WE) are lipids highly abundant in nature (providing energy storage, buoyancy or preventing dessication) and a constituent of many technological applications (foods, cosmetics, paintings). However, their interfacial behavior has been scarcely described and focusing on those with saturated chains (thus in solid state) which don't form stable monolayers. Previously, we evaluated the configuration of the WE from "jojoba oil" (J) at the air-water interface and we associated the ability of the long chains of WE to form monomolecular layers with its physical state. Thus, we proposed that, in the solid phase, WE exhibited a linear configuration whereas in the fluid state it adopted a hair-pin structure with an amphipathic-driven orientation, being this one the structure organizable like compressible monolayers. Such hypothetical configurations were derived just from molecular area measurements. In the present work we contributed with further experimental evidences by combining Langmuir isotherms, measurements of surface potential, PM-IRRAS analysis of J and atomic-scale molecular dynamic simulations of an ether representative of WE. The surface potential (SP) of the monolayers during compression exhibited a pattern similar to that of

most glycerophospholipids. The maximal SP, derived from a model that considered two populations of oriented water, was very close to the experimental value. The orientation of the ester group that was assumed in that calculation was coherent with the PM-IRRAS behavior of the carbonyl group with the C=O oriented towards the water and the C-O lying along the surface and were in accordance with their orientational angles (45° and 90° , respectively) determined by atomic-scale molecular dynamic simulations. Taken together the present results confirm a hairpin configuration of WE at the air-water interface.

Acknowledgements: CONICET, FONCYT, SeCyT-UNC.

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Submission ID 265 – Poster
Lipids, Structure and Function

Effect of Thymoquinone on DMPC liposomes: Study by FTIR, Raman and SERS spectroscopies.

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A great deal of natural origin quinones has pharmacologically interesting properties [1]. Thymoquinone (TQ) is one of the most studied quinines because of its potential anti-cancer, anti-oxidant and anti-inflammatory effects [2]. However, the molecular pathways involved in these activities are not well understood. Hence, we decided to carry out research on TQ interaction of with model lipid membranes.

In this work, TQ interaction of with dipalmitoylphosphatidylcholine (DMPC) bilayers was studied by FTIR, Raman and SERS spectroscopies. The results showed that TQ interacted to different extents with the phosphate and carbonyl groups of membranes in the gel and the liquid crystalline states.

The absorption band of the symmetric stretching of the methylene groups of fatty acid was studied to determine if TQ interaction with lipidic membranes affected the hydrophobic region bilayer [3]. From

different molar ratios of TQ:DMPC we observed a gradual decrement of the transition temperature that was proportional to TQ concentration until reaching a limit value of 20°C. This suggested that the stability of the DMPC liquid crystalline phase would increase as a result of TQ partition into the membrane because the lipid molecules need energy to carry out the phase change [4].

From the phospholipid Raman signature we could obtain information about the lateral order in the characteristic film of the inter and intra-chain interactions. In particular, the peaks height ratios and have been recognized for a long time as representative of chain packing and mobility. These ratios increased from 0.90 to 0.98 and 1.80 to 3.00, respectively when TQ concentration increased. This behavior is in agreement with the results observed in SERS for this region, where an increase in to be observed.

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Submission ID 275 – **Poster**
Lipids, Structure and Function

Interaction of Proline and Glycine Betaine with DPPC in hydric stress conditions by FTIR-ATR

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Glycine Betaine (GB) and Proline (Pro) are two major organic osmolytes that accumulate in a variety of plant species in response to environmental stresses such as drought, salinity and extreme temperatures. Although their actual roles in plant osmotolerance remain controversial, both compounds are thought to have positive effects on enzyme and membrane integrity along with adaptive roles in mediating osmotic adjustment in plants grown under stress conditions. This work seeks to interpret the mechanism by which each of these amino acids produces its protective effect on the membrane. In this context, evaluation of controlled dehydration of DPPC MLV's in presence of these amino acids by FTIR-ATR was performed. The information of functional groups on IR spectra was complemented by using dipolar potential and surface pressure on DPPC monolayers.

When dehydrated MLV's containing Pro was evaluated, IR spectra showed lower water band intensity concomitant with an increase in the band corresponding to carbonyl associated population, and a shift to

lower wavenumbers of symmetric vibration of phosphate groups. Interesting the same shift in phosphate populations were observed in highly hydrated control MLVs.

These effects added to the decrease of dipolar potential founded may let us think that Pro interacts with DPPC membranes by hydrogen bond at interface level.

On the other hand, dehydrated MLV's containing GB showed higher water band intensity, and no effects on carbonyls and phosphates bands. Also no changes were observed when dipolar potential was assessed.

In addition, when surface pressure were recorded, in the presence of each aminoacid in the subphase no differences were observed. Overall, the mechanism by which each aminoacid interacts with the membrane is visibly different. GB seems helping to maintain water in the membrane but lacking observable direct interaction, while Pro induce a partial dehydration by water molecules replacement.

Submission ID 281 – **Electronic Poster**
Lipids, Structure and Function

Examining water status in membranes by near infrared spectroscopy (NIR)

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Near infrared spectroscopy (NIR) informs about the covalent O-H bonds and H bonds in water systems, being sensitive to perturbations caused by solutes^{1,2}. The absorption band centered at 1450/55 nm is considered the first overtone of water representing signals of different overlapping water and O-H stretching bands³. The less the number and/or the strength of H-bonds the more the strength of the O-H bonds. Thus, they vibrate and absorb radiation at higher frequencies and this shift is employed here to evaluate the water status in different lipid membranes. Multilamellar vesicles of DMPA or DMPC were prepared at 250 y 500 μM in MQ water and heated at 10 temperatures in the range 13-58 °C. Absorbance were registered every 1 nm in the 1100-2300 nm interval with a path length of 1 mm.

As NIR spectra have not a straightforward interpretation, spectroscopic data were subjected to Principal Component Analysis. From the score plot of the first two PCs, significant differences were found in the water status depending on both the type and concentration of phospholipids.

Careful examination of the loading plots reveals that both phospholipids increase the absorbance at shorter wavelengths and decrease it at longer wavelengths of the water main band. The shift to higher frequencies (i.e. higher energy) in the O-H vibration band implies disrupting/ weakening effects on the H-bonds, particularly for DMPC and for the highest concentration. Increasing temperature dramatically debilitates H bonds, more than any of the two phospholipid and/or concentration.

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Submission ID 302 – **Electronic Poster**
Lipids, Structure and Function

Solubility and diffusion of oxygen in phospholipid membranes

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The transport of oxygen and other nonelectrolytes across lipid membranes is known to depend on both diffusion and solubility in the bilayer, and to be affected by changes in the physical state and by the lipid composition, especially the content of cholesterol and unsaturated fatty acids. However, it is not known how these factors affect diffusion and solubility separately. Herein we measured the partition coefficient of oxygen in liposome membranes of dilauroyl-, dimiristoyl- and dipalmitoylphosphatidylcholine in buffer at different temperatures using the equilibrium-shift method with electrochemical detection. The apparent diffusion coefficient was measured following the fluorescence quenching of 1-pyrenedodecanoate inserted in the liposome bilayers under the same conditions. The partition coefficient varied with the temperature and the physical state of the membrane, from below 1 in the gel state to above 2.8 in the liquid-crystalline state in DMPC and DPPC membranes. The partition coefficient was directly proportional to the partial molar volume and was then associated to the increase in

free-volume in the membrane as a function of temperature. The apparent diffusion coefficients were corrected by the partition coefficients and found to be nearly the same, with a null dependence on viscosity and physical state of the membrane, probably because the pyrene is disturbing the surrounding lipids and thus becoming insensitive to changes in membrane viscosity. Combining our results with those of others, it is apparent that both solubility and diffusion increase when increasing the temperature or when comparing a membrane in the gel to one in the fluid state.

Submission ID 148 – Poster
Proteins, Structure and Function

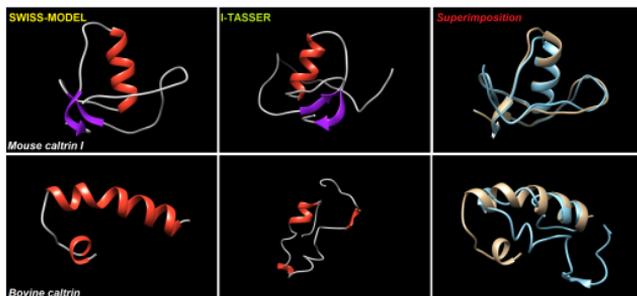
Structural prediction and in silico physicochemical characterization for mouse caltrin I and bovine caltrin proteins.

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It is known that caltrin (calcium transport inhibitor) protein binds to sperm cells during ejaculation and inhibits extracellular Ca^{2+} uptake. Although the sequence and some biological features of mouse caltrin I and bovine caltrin are known, their physicochemical properties and tertiary structure are mainly unknown. We predicted the 3D structures of mouse caltrin I and bovine caltrin by molecular homology modeling and threading. Surface electrostatic potentials and electric fields were calculated using the Poisson-Boltzmann equation. Several different bioinformatics tools and available web servers were used to thoroughly analyze the physicochemical characteristics of both proteins, such as their Kyte and Doolittle Hydropathy scores and helical wheel projections. The results presented in this work significantly aid further understanding of the molecular mechanisms of caltrin proteins modulating physiological processes associated with fertilization.

Keywords: homology modeling; threading; mouse caltrin I; bovine caltrin; hydrophathy plots; electrostatic surface potentials.



Submission ID **150** – **Poster**
Proteins, Structure and Function

Biophysical characterization of human frataxin precursor

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Friedreich's ataxia is a disease caused by a reduced expression of frataxin (FXN), a small mitochondrial protein. FXN is translated in the cytoplasm as a precursor of 210 residues. Our laboratory has studied in detail the human mature form. Here we investigate the conformation, and the stability of a precursor variant (thFXN1-210) that includes in the N-terminal a His tag and a TAT peptide to simplify its purification and to be translocated across the cytoplasmic membranes, in cell cultures. thFXN1-210 was produced in *E. coli* and purified by Ni-NTA affinity. The translocation of the fluorescent labeled protein (FITC-thFXN1-210) was checked by fluorescence microscopy using B104 and N2a cell lines. thFXN1-210 was studied by circular dichroism, static light scattering and Trp and ANS fluorescence. The sensitivity to proteolysis was investigated by SDS-PAGE. Protein stability was explored by urea-induced and thermal unfolding experiments. The N-terminal region (NTR) of thFXN1-210 showed to be mostly unstructured, as judged by the analysis of Far-UV CD spectra. Although thFXN1-210 is mainly monomeric, an increase in the aggregation of this variant was evident at

high temperatures. Near-UV CD spectra suggest subtle changes in the local environment of aromatic residues. However, neither the presence of the NTR alters significantly the stability of the C-terminal domain in urea unfolding experiments, nor the fluorescent probe ANS binds to the purified precursor, in principle excluding the possibility that the N-terminal keeps the conformation of C-terminal domain in a partially folded state.

Submission ID 159 – **Electronic Poster**
Proteins, Structure and Function

Mitochondrial nitric oxide production supported by reverse electron transfer

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The aim of this work was to study mitochondrial NO production using phosphorylating electron transfer particles (ETPH) obtained from bovine heart. In these vesicles, mitochondrial inner membrane has a reverse orientation with the NADH dehydrogenase center of complex I and the F1-ATPase exposed to the solutes in the surrounding medium. In our experimental conditions, 60% of the particles are in reverse status, estimated both through the O₂ consumption rate using NADH as substrate and the NADH-O₂ oxidoreductase activity, in the presence or absence of cytochrome c³⁺. ETPH produced 1.2 ± 0.1 nmol NO \times min⁻¹ \times mg protein⁻¹ through the reaction catalyzed by mitochondrial nitric oxide synthase (mtNOS). These particles showed a succinate-NAD⁺ reductase activity of 64 ± 3 nmol NADH \times min⁻¹ \times mg protein⁻¹ sustained by reverse electron transfer (RET) at expenses of ATP and succinate. The same particles, without NADPH and

in conditions of RET produced 0.97 ± 0.07 nmol NO \times min⁻¹ \times mg protein⁻¹. Rotenone inhibited NO production supported by RET measured in ETPH and in coupled mitochondria, but did not decrease the activity of recombinant nNOS, indicating that the inhibitory effect of rotenone on NO production is due to an electron flow inhibition and not to a direct action on mtNOS structure. NO production sustained by RET corresponds to 20% of the total amount of NO released from heart coupled mitochondria. A mitochondrial fraction enriched in complex I produced 1.7 ± 0.2 nmol NO \times min⁻¹ \times mg protein⁻¹ and reacted with anti-75 kDa complex I subunit and anti-nNOS antibodies, suggesting that complex I and mtNOS are located contiguously. These data show that mitochondrial NO production can be supported by RET, and suggest that mtNOS is next to complex I, reaffirming the idea of a functional association between these proteins.

Submission ID 153 – Poster
Proteins, Structure and Function

Effect of amyloid oligomerization on the α -synuclein curvature-dependent vesicle binding selectivity

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Parkinson's disease (PD) is a neurodegenerative movement disorder associated with axon degeneration of dopaminergic nigral neurons. The cardinal pathological of PD, is the presence of proteinaceous highly organized fibrillar inclusions, termed Lewy bodies, the main component of which is the 140 aa protein α -synuclein (AS). In addition, a number of prefibrillar intermediates have been associated with PD pathology. These oligomers have been pointed as the most toxic species and they are more likely located in axons and presynaptic terminals where

they might damage synapses and dendrites. Functionally, AS assists in the regulation of the distal reserve pool of synaptic vesicles where protein/membrane interactions would help to dock these vesicles in a region distal from the synapsis. Among the several membrane properties that modulate AS binding, curvature plays a key role. The aim of our work is to determine the loss-of-function that might be associated to the conversion of AS from its monomeric functional state to its pathological oligomeric form by evaluating the impact of AS oligomerization on protein membrane-curvature sensitivity. We will present quantitative measurements on the interaction between monomeric and oligomeric AS with vesicles varying in sizes using Fluorescence Correlation Spectroscopy.

Submission ID **163** – **Poster**
Proteins, Structure and Function

ICA512–RESP18HD and insulin interaction in the secretory pathway

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ICA512 (or IA–2/PTPRN) is a transmembrane protein-tyrosine phosphatase enriched in secretory granules of neuroendocrine cells. Previous studies imply its involvement in the biogenesis, cargo storage, traffic, exocytosis and recycling of insulin secretory granules, as well as in β -cell proliferation.

The function and structure of a large portion of its N-terminal extracellular (NT) region remains unknown. This region contains a domain called 'glucocorticoid-responsive protein regulated endocrine-specific protein 18 homologous domain' (RESP18HD). It was demonstrated that this domain regulates sorting of ICA512 to secretory granules in INS-1 cells and binds with high-affinity to insulin and proinsulin.

In this work, RESP18HD and insulin co-aggregation properties were studied in conditions that mimics pH and Zn concentrations in the secretory pathway.

The results are consistent with the hypothesis that the interaction between ICA512–RESP18HD and insulin (and probably others SG pro-

teins) might contribute to phase separation of high-order multiprotein assemblies for sorting and trafficking through the regulated pathway.

Submission ID **164** – **Poster**
Proteins, Structure and Function

Metallo- β -lactamases employ different strategies for improving stability during pathogenesis

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Carbapenems are last-resort β -lactam antibiotics employed for the treatment of multiresistant pathogens. In the last years, the number of carbapenem resistant bacteria has increased exponentially, principally by the acquisition of zinc-dependent metallo- β -lactamases (MBLs) capable of inactivating most types of β -lactam antibiotics. These enzymes carry out their activity in the bacterial periplasm, where they fold and bind Zn(II). During infection the host innate immune response withholds nutrient metal ions from microbial pathogens by releasing metal-chelating proteins such as calprotectin, which limits the Zn(II) necessary for MBL activity.

In previous work, we showed that sequestration of Zn(II) ions provokes a diminution in the periplasmic levels of MBLs, rendering the bacteria susceptible to antibiotics. The nonmetallated forms generated under these conditions are readily degraded.

By means of biochemical, NMR and *in vivo* experiments, in the present work we aim to study how MBLs can evade the action of metal deprivation during the host immune response. On one hand, we show that membrane-anchoring of an unstable MBL, as is the case of the clinically relevant New Delhi metallo- β -lactamase (NDM-1), protects the enzyme from cellular degradation by localizing it in the bacterial outer membrane, in which the accessibility to proteases would be diminished. On the other hand, we demonstrate that BcII, a soluble MBL, circumvents metal limitation by exhibiting a stable and rigid scaffold in the nonmetallated form, resilient to protein degradation. In conclusion, we unravel different evolutionary strategies adopted by MBLs to improve bacterial resistance under challenging conditions encountered during pathogenesis.

Acknowledgements: ANPCyT, NIH and CONICET.

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Submission ID 165 – Poster
Proteins, Structure and Function

Role of Active Site Loop - 3 (ASL-3) in Substrate Recognition of the β -lactamase, NDM-1.

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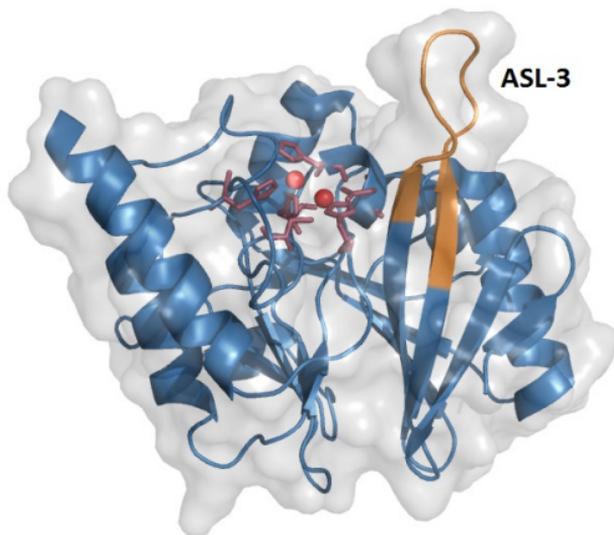
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β -lactams are the most important class of clinically available antibiotics used to treat bacterial infections. The main β -lactam resistance mechanism is the expression of β -lactamases. Metallo- β -Lactamases ($M\beta$ Ls) are Zn(II)-dependent enzymes of particular interest and concern given i) their ability to hydrolyze and provide resistance to virtually all classes of β -lactam antibiotics including carbapenems and ii) to date clinically useful $M\beta$ L inhibitors are not available. Among $M\beta$ Ls, NDM-1 poses an increasing clinical concern since it is one of the most potent and widespread enzymes. The active site loop 3 (ASL-3) of NDM-1 plays

an important role on substrate recognition in $M\beta$ Ls. Comparison of crystal structures of NDM-1 to other clinically relevant $M\beta$ Ls such as VIM-2 and IMP-1 has revealed that in NDM-1 the ASL-3 lacks a conserved Pro residue at its C-terminus leading to a more flexible loop and a wider active site.

To test the hypothesis that these features account for the higher affinity and catalytic activity of this enzyme for several β -lactams, we characterized the substrate profile of three ASL-3 mutants of NDM-1, two in which the ASL-3 was replaced by those of IMP-1 (more charged) and VIM-2 (one residue shorter), and one in which we introduced the conserved Pro residue at the base of the ASL-3. Biochemical characterization of these enzymes included steady state and pre steady state activity, thermal stability and active site coordination changes based on Co(II) derivatives and crystal structures. We also performed minimum inhibitory concentration assays to test their ability to confer resistance against different β -lactams. We found that modifications on the ASL-3 not only affects substrate spectra but also affects the global protein structure and stability. The understanding of structural important features in substrate recognition and protein stability is a crucial step on the desing of a $M\beta$ L clinically useful inhibitor.



Submission ID 173 – Poster
Proteins, Structure and Function

Molecular mechanisms that determine the pathogenicity of human Apolipoprotein A-I natural amyloidogenic Variants.

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Protein aggregation is characterized by a remarkable polymorphism and different conformations have been identified as toxic intermediates in some neurodegenerative diseases¹. Amyloidosis induced by natural mutants of apolipoprotein A-I (apoA-I) depends on the protein variant and affects different organs such as heart, liver and kidney among others. In previous studies we have demonstrated that different apoA-I

mutants show increased tendency to aggregate giving rise to different conformations depending on the incubation conditions 2,3. Here we constructed four natural amyloidogenic variants (Arg173Pro, Gly26-Arg, Leu60Arg and Lys107-0), and compared their behavior with the protein with the wild type sequence (Wt). All the mutants are less stable and with a more flexible structure than Wt at pH 7,4, which could in part explain their aggregation tendency. While Wt does not bind heparin (as a model of Glycosamine Glycane) at this pH, Arg173Pro forms protein-heparin complexes and binds negative lipids with higher efficiency. Mild acidic pH induces a loss of the native structure, which was characterized by Atomic Force Microscopy as oligomer-like conformations. Interestingly, Gly26Arg and Arg173Pro (but not Lys107-0), elicit pro-inflammatory responses from macrophages such as Interleukin 1- beta (IL1) release, and the presence of a pro-oxidant milieu favors the yield of fiber-like species. We strongly suggest that common and specific mechanisms of amyloid cascades determine apoA-I variants' pathogenesis and that a chronic pro-inflammatory environment is either cause or consequence of protein misfolding. The knowledge of the specific pathological pathways is clue in order to apply strategies to avoid organ damage

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Submission ID 177 – Poster
Proteins, Structure and Function

Gene organization and bioinformatic analyses of DefSm1D, a defensin-like protein domain from flowers of *Silybum marianum*.

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Plant defensins and defensin-like (DEFL) proteins are small cationic, cysteine-rich proteins ubiquitously expressed in the plant kingdom and mostly involved in host defence. We have previously cloned, predict the structure and identified the putative function of a DEFL from *S. marianum* flowers (DefSm1D), which revealed a potential antifungal effect.

The aims of this this work were to find promotor motifs of the gene encoding DefSm1 and perform a bioinformatic analysis concerning electrostatic potential of the protein surface in order to confirm our previous results on function-structure of DefSm1D.

To study DefSm1 gene, a BLAST similarity search was performed using the shotgun sequence of *S. marianum* whole genome as database and DefSm1 sequence as query. The promoter analysis revealed the

presence of TATA box and the initiator element with appropriate spacing between them for the synergistic effect that would increase promoter strength. Other regulatory elements were identified using Plant Care all related with biotic stress response (CGTCA-motif involved in the methyl jasmonate responsiveness, TC-rich repeat involved in defence and stress response, elements involved in salicylic and abscisic acid responsiveness). The DefSm1 gene structure, as expected, presented two exons with an intron positioned between the signal peptide and mature protein. The presence of a peptide signal suggests a putative extracellular localization for DefSm1D, which would be consistent with its role in defence.

Electrostatic surface potential of DefSm1D was calculated solving the Poisson-Boltzmann equation through PBEQ-Solver. The surface charge distribution of DefSm1D endows the peptide with amphipathicity, which would allow it to interact with the cell membrane of pathogens and exert its antimicrobial activity.

Both analyses, at gene and protein level, confirm our previous results of DefSm1D role in biotic stress.

Submission ID **180** – **Poster**
Proteins, Structure and Function

Design of a variant of Epidermal Growth Factor resistant to proteases for the treatment of diabetic foot ulcers. Protector role of proteases inhibitors.

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Diabetes mellitus (DM) is the only endocrine-metabolic disorder that expands as a pandemic disease. Ulceration of lower limbs is one of the most serious complications of this disease due to the inability to sustain the growth of granulation tissue, with an appropriate extracellular matrix re-epithelization. The mechanisms by which DM prevents tissue repair are not fully known, evidence suggests a deficit in the production of various growth factors (GF) such as platelet derived growth factor (PDGF), keratinocytes growth factor (KGF), vascular endothelium growth factor (VEGF) and epidermal growth factor (EGF) (1), (2). Despite therapeutic advances, diabetic foot ulcers (DFU) continues being difficult to handle, which is reflected in the high annual incidence of ulceration and amputation. The aim of our work is the design of mutant EGFs that are resistant to proteolysis produced by the majority

proteases in DFUs, in order to encapsulate them in liposomal nanoparticles and apply ectopically in ulcers. We are also studying novel protease inhibitors (PIs) of vegetable origin, in order to test their activity as EGF protectors and eventually encapsulate them with EGF for the same purpose. So far we cloned the EGF generating a construct linked to a signal peptide that allows the expression in *E. coli* BL21, and achieving the recovery of the recombinant protein from the extracellular medium. We have made a meticulous study of the majority proteases in DFUs and, using MEROPS data base, we could identify the presumable cleavage sites in EGF. Based on these results, and considering essential sites for the folding of EGF and its receptor binding, we made proposals for mutable sites, so as to produce a resistant to proteolysis mutant EGF which retains its biological activity.

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Submission ID **186** – **Poster**
Proteins, Structure and Function

Human Telomerase protein/RNA/DNA complex: A Molecular Dynamics study of some conserved residues in the catalytic pocket for the wild-type and mutated systems.

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The Telomerase enzyme adds several nucleotides to the 3' terminal of a DNA strand. It is a protein/RNA complex, formed by the Telomerase protein and a RNA strand. Inside the protein, in a reverse transcriptase catalytic pocket, there are several conserved residues: a triad of aspartates (D712, D868, D869) whose crucial role in the telomerase activity is well understood and other residues (K626, R631, K902) whose role in the activity is not clear. This work aims to understand the role of the latter residues in the activity of the human telomerase protein. Due to the lack of an experimental structure for the telomerase, we build a model for a domain of the telomerase protein. Then, we build a chimeric complex using the protein model, together with a double strand DNA/RNA, a dNTP, and two Mg²⁺ ions (taken from different structures of the PDB). Molecular dynamics simulation was used to obtain a stabilized complex model for the wild-type, in explicit water. Two

configurations were studied: (i) with the dNTP at the reaction site (before the catalytic reaction), and (ii) after the catalytic reaction, where the dNTP was broken in a nucleotide and an inorganic pyrophosphate (PPi). These results highlight the role of the K626, R631 and K902 residues, stabilizing the incoming dNTP to the reaction center before the reaction and accompanying the PPi out of the catalytic site. In order to understand better the role of these residues, we built two mutated complexes: one including the K902N mutation and the other containing the R631Q mutation (both known for dropping to zero the telomerase activity). Then, we have studied by MD these two new complexes, each one in the same two configurations. The results let us understand that the crucial role of the R631 and K902 residues is accomplished after the reaction, because when mutated, they are incapable to accompany the PPi and as a result the PPi stays at the catalytic pocket, stopping the catalytic activity of the telomerase.

Submission ID **190** – **Poster**
Proteins, Structure and Function

Self-aggregation of human apolipoprotein A-I. Studies with pyrenyl-labeled cysteine mutants.

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Apolipoprotein A-I (apo A-I) is the major protein of high density lipoproteins (HDL), to which antiatherogenic properties are attributed for its role in the transport of cholesterol excess from peripheral tissues to the liver for catabolism and disposal.

Apo A-I is composed of several amphipathic alpha-helices. In water solution, they form a bundle with poorly defined tertiary structure. Depending on the concentration, apo A-I is self-aggregated to form dimers and oligomers of different size. It is also interacts with membrane and forms discoidal HDL (dHDL).

The aim was to obtain information on the apo A-I self-aggregation in solution, as well as its interaction with membrane and formation of complex with lipid, since it is important to understand the mechanisms of HDL generation. Six cysteine mutants (K107C, K133C F104C, L137C, K226C and F225C) were specifically labeled with pyrenylmaleimide in these six positions corresponding to the hydrophilic and hydrophobic faces of helices 4, 5 and 10.

The labeled mutants were stable in solution as indicated by tryptophan fluorescence. With the exception of F104C, they were biologically active since they interact with lipids to form dHDL. Fluorescence emission spectra of pyrene in function of the protein concentration showed that pyrene excimer formation occurs only in the case of labeled F225C and K133C mutants, indicating the participation of helices 5 and 10 in the contact regions for oligomerization. Excimer fluorescence was undetectable for all the mutants in the presence of lipid vesicles.

Submission ID 196 – Poster
Proteins, Structure and Function

PMCA conformational changes in phosphorylated intermediaries

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Plasma membrane calcium pump (PMCA) plays a key role in cellular calcium homeostasis by pumping calcium ions outside the cytoplasm. PMCA has a relatively high sequence identity with other P-ATPases like the sarcoplasmic reticulum calcium pump (SERCA). However PMCA structure is less known and has particular properties as presence of an autoinhibitory calmodulin binding site in its C-terminal segment.

In order to understand how calmodulin binding alters PMCA structure, we employed fluoride complexes with beryllium, aluminium or magnesium, which are proposed to stabilize different analogues of the phosphorylated intermediates in P-ATPases. To study the PMCA structure we employed the photoactivable probe 3-(trifluoromethyl)-3-(m-iodophenyl)diazirine (TID) which labels the transmembrane domains of proteins and the fluorescent probe 2',3'-O-(2,4,6-Trinitrophenyl)-adenosine-5'-triphosphate (TNP-ATP) which binds to the nucleotide

binding domain of ATPases. TID labeling show that PMCA surface exposed to the bilayer only decreases in presence of both calcium and calmodulin. However this change also occurs upon fluoride complexes binding. On the other hand upon addition of metal-fluoride complexes, TNP-ATP bound to PMCA decreases its quantum yield by a half without changing the affinity of the probe for the protein. This is consistent with a more exposure to solvent of ATP binding site. The conformational change occurs even when the pump is in presence of calcium and calmodulin.

These results indicate that conformational changes in the transmembrane region of PMCA are restricted in absence of calcium and calmodulin, but even so the nucleotide binding domain is able to hinge upon phosphorylation. By homology modeling of PMCA sequence on SERCA crystallographic structures, we evaluate the implications of these results on PMCA structure model and how the C-terminal domain restricts conformational changes in this protein.

With grants of CONICET, ANPCYT and UBA.

Submission ID **204** – **Poster**
Proteins, Structure and Function

Structural determinants of the interaction of glycosaminoglycans with GAPDH

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Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a glycolytic enzyme that is known as a "moonlight" protein, due to its diverse functions in addition to its role in energy production. It has been associated to neurodegenerative diseases, but its precise role in these pathologies remains unclear. Previously, we have demonstrated that upon

interaction with glycosaminoglycans, such as heparin or heparansulphate, GAPDH is able to reassemble into protofibrils. We have demonstrated the ability of these protofibrils to sequester alpha-synuclein toxic species, a feature that could be exploited for neuroprotection in Parkinson disease. Due to its anticoagulant activity, heparin could not be administered to patients as a mean to increase the formation of GAPDH protofibrils. In this way, understanding the interaction of heparin with GAPDH could aid the search of alternative molecules able to thrust the formation of protofibrils.

In this work, we crystallographic structure of GAPDH bound to fondaparinux, a semisynthetic heparin pentasaccharide. In order to characterize the importance of the interactions established in the crystal, we also study the ability of modified disaccharides to trigger conformational changes and amyloid aggregation of GAPDH. These results unravel the structural determinants necessary to induce the formation of neuroprotective protofibrils and pushes forward the development of a possible therapeutic treatment for Parkinson disease.

Submission ID **208** – **Poster**
Proteins, Structure and Function

Altered lipid content in CHO cells overexpressing the P5-ATPase ATP13A2

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The P-type ion pumps are membrane transporters energized by ATP-hydrolysis. They were classified into five subfamilies termed P1-P5; the substrate specificity of P5 subfamily is still unknown. Five genes named ATP13A1-ATP13A5 that belong to the P5-ATPases are present in humans. Mutations in the ATP13A2 gene (also known as PARK9 or CNL12) underlay a form of Parkinson (1) and a form of Neuronal Ceroid Lipofuscinosis (2). ATP13A2 is localized in lysosomes and late endosomes (LE). Dysfunction of this protein diminishes the lysosomal degradation, the autophagic flux (3) and the exosome externalization (4). We have recently shown that ATP13A2 expression caused a reduction of the iron-induced lysosome membrane permeabilization (5), which suggests that ATP13A2 overexpression improves the lysosome and LE membrane integrity. In this line, we analyzed the accumulation of fluorescent analogs like phosphatidylethanolamine (NBD-PE) and ceramide (NBD-ceramide) in ATP13A2-expressing CHO cells by

fluorescence microscopy. We found that the expression of ATP13A2 increases the NBD-PE fluorescence intensity, reflecting an augmented lipidosis in these cells. Besides, the ceramide-fluorescence intensity was reduced, suggesting that the degradation of membrane components that take place in acidic compartments is being affected. The PE content measured by fluorescence quenching assay in the mitochondrial fraction containing lysosomes and LEs of ATP13A2-expressing CHO cells, was increased in the cytoplasmic leaflet of these acidic vesicles. These results suggest that ATP13A2 may be involved in the lipid homeostasis of these subcellular organelles.

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Submission ID 213 – Poster
Proteins, Structure and Function

Deconstructing the role of key residues of the iron-binding motif EE_{xx}ED using unstructured peptides and a grafted protein scaffold

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Proteins belonging frataxin family control intracellular iron homeostasis through a clusters of acidic residues, that are apparently responsible for iron binding. To understand how frataxin works as an iron chaperon, we studied the interaction of metal ions with a putative iron binding site from the bacterial frataxin CyaY. For this purpose, the EE_{xx}ED

motif from the bacterial frataxin was grafted on a 16 residue-long peptide, corresponding to the C-terminal α -helix from *E. coli* thioredoxin [1], giving the GRAP peptide [2]. We found by computational docking experiments that the motif has two mutually exclusive sites, involving only three acidic residues (ExxED or EExxE). In order to study the effect of each residue separately on the interaction with metal ions of different chemical nature (Mg^{2+} , Al^{3+} , Fe^{3+} and Ga^{3+}), three engineered GRAP-derived peptides were synthesized carrying the sequences ExxED, EExxE and ExxE. Preliminary results obtained by circular dichroism suggest that: (i) In the absence of metal ions all the peptides behave as random-coil peptides at pH 7.0; (ii) Fe^{3+} and Al^{3+} are capable to induce/stabilize the helical structure of GRAP and peptide variants at pH 4.1 and pH 7.0, respectively; (iii) Nevertheless, Ga^{3+} only promote the acquisition of helical structure of GRAP, suggesting a fine-tuning of the helix-coil transition. To gain further insights, the full length *E. coli* TRX was engineered including the EExxED motif in the C-terminal α -helix (TRX_{grap}), which is already structured in the native ensemble. By ^{15}N - 1H HSQC NMR experiments we found a specific interaction of Fe^{3+} with the EExxED motif causing the disappearance of cross-peak signals corresponding to residues located in the surrounding of the iron-binding motif.

Submission ID 214 – Poster
Proteins, Structure and Function

Thermodynamic activation parameters of the ATPase activity of dengue virus NS3 helicase

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Dengue virus NS3 (non-structural protein 3) is a viral helicase that catalyzes the hydrolysis of ATP and couples the free energy of this reaction to drive its translocation along single strands and unwind double strands of RNA. In this work, we characterize the effect of temperature on the enzymatic catalysis of ATP hydrolysis by the NS3 helicase in the absence of RNA.

We obtained the dependence of ATPase activity of NS3 on substrate concentration at different temperatures ranging from 5 °C to 30 °C, by measuring the initial rate of phosphate release from ATP hydrolysis. All reactions were performed in the presence of 10 nM NS3 in media containing 25 mM MOPS; 1.5 mM free Mg^{2+} ; 0.50 mM EDTA; 100 mM (KCl + KOH); 10 mM NaCl; 0.2 mM CHAPS; pH = 6.5 (adjusted with KOH at each temperature tested).

ATPase activity dependence on substrate concentration was well described by simple hyperbolic functions at all temperatures tested, whose

parameters, k_{cat} and K_M , were obtained by least-square non-linear regression analysis. We observed that while k_{cat} and k_{cat}/K_M increased exponentially as a function of temperature, K_M remained approximately constant, at a value of (0.12 ± 0.03) mM.

We analyzed the temperature dependence of k_{cat} by means of transition state theory, employing Eyring equation, which allowed us to estimate thermodynamic activation parameters associated to this kinetic constant. The activation enthalpy and entropy were $\Delta^\ddagger H^\circ = (16 \pm 2)$ kcal mol⁻¹ and $\Delta^\ddagger S^\circ = (1 \pm 56)$ 10⁻⁴ kcal K⁻¹ mol⁻¹ respectively. Therefore, at 25 °C, the free energy barrier, $\Delta^\ddagger G^\circ = (16 \pm 4)$ kcal mol⁻¹, is mostly enthalpic.

Comparison with bibliographic values of $\Delta^\ddagger H^\circ$ and $\Delta^\ddagger S^\circ$ for the nonenzymatic hydrolysis of ATP suggests that, in the range of temperature tested, NS3 enhances the reaction rate mainly by reducing the enthalpic component of the free energy barrier in ~ 10 kcal mol⁻¹.

Funding from: CONICET, and ANPCyT

Submission ID 222 – **Poster**
Proteins, Structure and Function

Structure changes of the enzyme β -Galactosidase induced by the encapsulation in a mesoporous silicate-gel matrix.

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The synthesis of silicate gels by the sol-gel method under mild conditions of pH and temperature, enables the entrapment of proteins with biological activity while allows the diffusion of small molecules through the mesoporous matrix. In previous studies, we determined that the encapsulated enzyme β -Galactosidase ($E\beta$ -Gal) has equal or higher catalytic activity than the soluble enzyme, and we proposed that the structuring of water molecules within the gel nano-pores have significant importance in the differences observed for the hydrolysis of ONPG in fresh and aged gels for different periods of time¹. This observation was supported by ¹H-NMR experiments. In the present work, we studied the changes in the conformational structure of $E\beta$ -Gal confined in the silicate gel employing fluorescence spectroscopy and circular dichroism (DC). The emission spectrum of fluorescence $E\beta$ -Gal evidenced a shift of the center of spectral mass of 3 nm towards longer wavelengths compared to the soluble enzyme. This effect indicates that the

tryptophans of the encapsulated protein are in a less hydrophobic environment (higher dielectric constant) which could be due to a loss of compaction overall the tertiary structure. Furthermore, DC data evidenced changes in the secondary structure of E β -Gal with respect to the soluble β -Gal mainly reflecting an increased proportion of amino acids in α -helix structure. Concluding, the present results suggest that the effects of confinement on E β -Gal activity might be at least partly due to changes in the protein conformation.

Acknowledgements: CONICET, FONCYT, SeCyT-UNC.

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Submission ID 225 – **Poster**
Proteins, Structure and Function

Characterization of a gas binding sensor heme kinase from *Mycobacterium Tuberculosis*

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The two-component system DosST/R controls the entry of *Mycobacterium tuberculosis* and other mycobacteria into a latent, dormant state. Two clinically relevant conditions of the dormant state are its increased resistance to the effect of antibiotics and the relief of the symptoms associated with the acute phase of infection: leading to an early stop of the treatment in affected patients and the consequent increase in the reports of multi-drug resistant strains favored by incomplete treatment regimes. Hypoxia and exposure to NO and CO are stimuli that physiologically induce this dormant states. DosS and DosT are histidin kinases which can sense gases through an heme-binding GAF domain and elicit the biological response through autophosphorylation of a histidine residue and then transfer of this phosphate to the response element DosR. Structurally, these 62 kDa proteins present a modular structure consisting of two consecutive GAF domains, a dimerization domain (which also exposes the phosphorylatable histidine residue) and an ATP binding domain. Only the N terminal GAF domain (GAF-A) binds heme and acts as a

hipoxia and NO and CO sensor. The role of the GAF-B domain remains unknown and no ligand has been reported.

We cloned and expressed DosS and DosT in *Escherichia coli*, and we have been able to purify and generate deletion mutants of DosS. We performed a biophysical characterization and analyzed the binding of gases on full DosS. The autophosphorylation activity of the Kinase Core (dimerization plus ATP binding domain) was also measured. Our objective is being able to characterize the biophysics and biochemistry of this system to gain insight into the allosteric regulation by binding of gases and transduction of the signal from the first GAF domain to the kinase core.

Acknowledgments: CONICET , ANPCyT and UBACyT

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Submission ID 227 – **Poster**
Proteins, Structure and Function

Biophysical characterization of MAPK p38 α interaction with specific targets

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Mitogen activated protein kinases (MAPKs) are serine/threonine kinases that play an important role in regulating various cellular processes including cell growth, differentiation, inflammation and apoptosis. The development of inhibitors of MAPKs is an important research area for various diseases such as cancer, diabetes, arthritis and inflammatory diseases. Much effort is being done in searching new compounds or peptides that could work as inhibitors.

MAPKs utilize a docking strategy to bind its activators, phosphatases, scaffold proteins and its substrates. Linear sequence motifs (the docking motifs) of the MAPKs interacting partners bind the MAPK at the same loci outside the active site of their cognate MAPK (the docking groove). Moreover MAPKs usually share upstream kinases or downstream substrates. There are still basic questions about the mechanisms that guide the binding of substrates and ATP to MAPKs and the specificity of recognition. In addition, it is not clear whether the docking interaction is used only "to fish" its substrate and increase the local

concentration of phosphoacceptor site or if it has additional allosteric effects on the MAPK enzymatic activity.

Focusing on the particular aim of designing specific inhibitors for MAPK p38 we have employed different biophysical techniques to characterize *in vitro* the interaction of this kinase with specific targets. We present here some preliminary results that will be useful not only to understand the mechanisms of protein-protein recognition in p38 signaling network but also to design new and specific inhibitors.

Acknowledgments: CONICET , ANPCyT and UBACyT

Submission ID 228 – Poster
Proteins, Structure and Function

Interaction of apolipoprotein A-I variants with synthetic polymeric matrices that mimic an extracellular matrix

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Specific interactions of apolipoproteins with components of the extracellular matrix, particularly proteoglycans (PGs), have been postulated to play a key role regulating events associated to atherosclerosis. Modification of these interactions seems to be dependent on age, cellular differentiation, and pathological conditions. The mechanism behind these modifications has been reported to be centered on the specific expression of glycosamine glucans (GAGs) with different length, position of sulfate substitution and composition.

Human apolipoprotein A-I (apoA-I) in physiological conditions does not bind GAGs but it is postulated to inhibit the retention of oxidized

LDL in the extracellular matrix and thus the delivery of cholesterol to the macrophages within the artery wall. Our previous results showed that binding of apoA-I to heparin (a model of extracellular component) occurs with wild type apo A-I under acidic pH and with a natural amyloidogenic mutant having a single amino acid substitution (Arg173Pro). These results indicate that electrostatic interactions could play a key role in the interaction of apo A-I with the extracellular matrix.

In order to further study the specific role of the matrix's charge on the interaction of apoA-I with GAG, we synthesized model polymers having different ratios of sulfated-to hydroxylated monomers and studied the binding of apoA-I or Arg173Pro, both fluorescently labeled. Our results showed that binding of both proteins is higher in the polymer containing higher sulfated-to hydroxylated monomers ratio, and Arg173Pro binds with higher affinity to it.

We conclude that charge and chemical composition of the extra cellular matrix could mediate apo A-I binding to GAGs and as a consequence alter the delicate equilibrium of protein and function.

Submission ID 235 – **Electronic Poster**
Proteins, Structure and Function

Glycosaminoglycans may link tau amyloid aggregation and abnormal phosphorylation.

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Tau is a microtubule-associated protein that plays a crucial role in regulating microtubule dynamics, axonal transport and neurite outgrowth. In physiological conditions, these functions are regulated by site-specific phosphorylation. In pathological conditions, abnormal phosphorylation

and amyloid aggregation lead to the formation of neurofibrillary tangles (NFT), a neuropathological hallmark in Alzheimer's disease brain. The relationship between phosphorylation and aggregation is not well understood and could be essential to understand the toxic transition of Tau.

In this work we study extracellular glycosaminoglycans as the link between phosphorylation and amyloid aggregation. In Alzheimer's disease, heparan sulphate (HS) accumulates at the intracellular level in neurons co-localizing with the NFT, while they persist at the neuronal cell membrane in normal brain. It is described that the presence of glycosaminoglycans like heparin or (HS) is needed to achieve the pathological phosphorylation profile. Using fluorescence, infrared and SAXS spectroscopy we analyzed tau conformational changes alone and in the presence of these sugars in order to understand the molecular events leading to aggregation. The protein alone is not prompt to aggregate, remaining stable over the time. However, in the presence of heparin or HS, aggregation as monitored by ThT follows a sigmoidal kinetics. After 24 h, both oligomers and amyloid fibrils are observed by TEM. Surprisingly, SAXS and FTIR data showed significant difference between heparin and HS prefibrillar aggregates. These results strongly suggest that glycosaminoglycans are able to induce conformational changes in Tau leading to abnormal phosphorylation and generation of novel toxic oligomeric species.

Submission ID 237 – Poster
Proteins, Structure and Function

Annonacin-induced α -synuclein fibrils increase ROS production in dopaminergic cell model: possible role in Guadalupe Island's atypical Parkinsonism

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Annonacin is a chemical metabolite present in some fruits of the Annonaceae family, a traditional food in Guadeloupe Island, and has been

indicated as the cause of atypical parkinsonian disorder which affect a notable percentage of the island population. Recent reports have shown that regular consumption in mice caused brain injuries consistent with Parkinson disease in a mechanism involving the inhibition of Complex I of the respiratory chain in mitochondria. Although the pathological aggregation of α -synuclein was signed as the essential molecular event in Parkinson disease, there are not reports on the effect of annonacin on α -synuclein aggregation. In this way, the aim of the present work is to gain knowledge on the effects of annonacin on the kinetics α -synuclein aggregation, as well as the toxicity of fibrillar species on dopaminergic cell model. By using transmission electron microscopy we have found that annonacin induces morphological changes in the fibrillar structure of α -synuclein. Through ThT fluorescence assay and small angle x-ray scattering we confirmed that annonacin is able to affect the kinetics of α -synuclein amyloid pathway. The fibers formed in the presence of annonacin display increased toxicity on dopaminergic cell model and trigger increased reactive oxygen species (ROS) production. Taken together, our results suggest that annonacin induced the production of α -synuclein fibers with increased toxicity on dopaminergic cell model. Acknowledgment: MFGL received Bec.Ar and CONICET fellowship. Institut du Cerveau et de la Moelle Epinière – Paris.

Submission ID 247 – **Poster**
Proteins, Structure and Function

Influence of oxidation state of lipid membranes in conformational changes of Tau and its implication in the pathogenesis of neurodegenerative diseases

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Alzheimer's disease is a progressive neurodegenerative disease that carries to a progressive lack of memory and other cognitive skills, culminating in a total and irreversible disability. Histopathologically, the Alzheimer's disease is characterized by the presence of two distinct types of protein aggregates: extracellular amyloid deposits compound of $A\beta$ peptide ($A\beta_{1-42}$), and neurofibrillary tangles located in the intra- and extracellular spaces respectively. It is now accepted that only neurofibrillary tangles clinically correlate with the degree of dementia and are considered the most important histopathological markers in AD. These tangles are composed mainly of the protein Tau. This protein is an important component of the neuronal cytoskeleton that stabilizes microtubules, maintains the cellular shape and axonal transport. Normally Tau is phosphorylated at specific positions, but may be abnormally phosphorylated by certain kinases, losing its biological function and increasing their propensity to form amyloid aggregates. Kinases

carrying out the abnormal phosphorylation would be the same kinases that produce normal phosphorylation, and the differences in the degree and type of phosphorylation would be due to conformational changes in Tau protein. The literature shows that oxidative stress is the main event during aging, and being Alzheimer closely related to aging, it was postulated that oxidative stress is the cause of neuronal death. In this work we plan to study conformational changes in Tau protein in the presence of lipid membranes submitted to peroxidation processes. For this purpose we will develop Fluorescence and Infrared Spectroscopy Assays, SAXS and Electron and Confocal Microscopy. We will also determine if the conformational changes are able to alter the protein phosphorylation profile. The results obtained will clarify basic aspects of the Alzheimer's disease pathogenesis, constituting them as new targets for future drug development.

Submission ID 248 – **Poster**
Proteins, Structure and Function

Defining the role of a novel protein in the assembly of the human cytochrome c oxidase

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The dinuclear copper center CuA is the electron entry point of the cytochrome c oxidase (COX). It funnels the electrons from reduced cytochrome c to the CuB center where O₂ is reduced to water molecules. The correct assembly of this metal center is essential for the function of the complex and thus for the survival of the cell.

Several proteins are involved in the assembly of the CuA site. Recently, using a model of the human CuA binding domain, we found that two proteins of the Sco family play essential roles in the formation of the CuA center. First Sco2, in its copper bound form, acts as a thiol-disulfide oxidoreductase, and then Sco1 functions as a metallochaperone transferring the copper ions.¹

While the incorporation of the copper ions to the CuA center is well understood, the proteins involved in the metalation of the Sco proteins are still in debate. It is known that the protein Cox17 can transfer copper ions to both Sco proteins but there is not a specific protein that functions a thiol oxidoreductase of the Sco proteins.

Recent results showed that mutations in the protein Coa6 (Cytochrome oxidase assembly factor 6) also affect the formation of a functional CuA site in human COX. Coa6 is a small soluble protein of the mitochondrial intermembrane space with two pair of Cys in Cx9C and Cx10C motifs and *in vitro* results also showed that is able to bind copper ions. For these reasons Coa6 can function as a metallochaperone or as a thiol-disulfide oxidoreductase.²

The specific function and interactions of Coa6 cannot be discriminated by *in vivo* experiments but here we show, *in vitro*, that it can be involved in a disulfide relay system involving Sco1 and Sco2 proteins.

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Submission ID 250 – **Electronic Poster**
Proteins, Structure and Function

Structural Insights into the Catalytic Mechanism of Human Glutamine Synthetase

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Glutamine synthetase (GS) catalyzes the formation of glutamine from glutamate and ammonia, using ATP as a cofactor. Glutamate is the major excitatory neurotransmitter, and in human's brain GS activity prevents glutamate dependent excitotoxicity (as also detoxifying ammonia). Neurodegenerative disorders such as Alzheimer's disease have been associated with the lack of GS activity. Moreover, GS activity has been shown to be affected by oxidative modifications, including tyrosine nitration in specific residues.

The human enzyme has 373 residues per subunit and exists a decamer, formed by two stacked pentamers, with the active sites located at the

interface of two adjacent monomers of the pentamer. The crystallographic structure was determined for several organisms, and mainly from this results a reaction mechanism is proposed. The overall reaction proceeds through a two step mechanism, involving first the ATP γ -phosphate transfer to the lateral chain of glutamate, followed by the nucleophilic attack of ammonia yielding glutamine and inorganic phosphate.

In this work we present a detailed description of the reaction mechanism of human GS (HsGS) at an atomic level, as well as an exhaustive examination of structural properties closely related with the catalysis, using to this purpose molecular dynamics simulations (MD), and combined quantum mechanics and molecular mechanics simulations (QM/MM). Our results suggest that the first step in which the γ -glutamyl phosphate intermediate forms, has a 5 kcal/mol free energy barrier and a ~ 8 kcal/mol reaction free energy, and then the second and rate-limiting step has a free energy barrier of 19 kcal/mol and a reaction free energy of almost zero. Also, these results were essential to achieve atomistic detailed information about how HsGS catalytic mechanism is affected by tyrosine nitration.

Submission ID 253 – **Poster**
Proteins, Structure and Function

The effect of molecular crowding on β -Galactosidase activity revisited.

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Previously we reported that molecular crowding modified β -Galactosidase (β -Gal) enzymatic activity. Using o-nitrophenyl-b-galactopyranoside (ONPG) as substrate and polyethylene glycol (1) as crowding agent we demonstrated that kinetic parameters were affected: V_{max} was slightly diminished, while the affinity of the enzyme-substrate interaction (KM) suffered a significant decrease at growing molecular crowding levels. In the present work, we reanalyzed these results under the Eadie-Hofstee model. We found a biphasic behavior in the presence of PEG with two components, one of them resembling the behavior in dilute media and another one exhibiting an affinity decreasing in a PEG concentration-dependent manner. It is known that in the mechanism of the β -Gal catalyzed ONPG hydrolysis, water participates in the rate limiting step of the reaction. So, our results suggest that in the presence of PEG, the availability of water as a substrate which is partially lowered with respect to PEG-free media (2) is affecting the reaction. The biphasic kinetics of hydrolysis was not observed with the substrate

p-nitrophenyl- β -galactopyranoside (PNPG) whose reaction mechanism did not involve water in the rate limiting step. Similar results were reported for β -Gal entrapped in mesoporous silicate matrix (3). Both environments, the mesoporous matrix and PEG solution have in common the presence of more than one population of water molecules differing in their mobility. Thus, taken together our results strongly suggest that restrictions in the water availability emerges as a generalized phenomenon affecting the rate limiting step of β -Gal catalyzed reaction.

Acknowledgements: CONICET, FONCYT, SeCyT-UNC

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Submission ID 258 – Poster
Proteins, Structure and Function

The influence of Alkyl-Functionalized Ionic Liquids With Human and Bovine Serum Albumins: a structural and spectroscopic study

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Ionic liquids (ILs) are salts that are liquid at temperatures smaller than 100 °C and are gaining prominence in the so-called green chemistry due to the several research areas that these molecules are applied. In this work, we study the influence of three different ILs ([C₁₀mim][Cl], [C₁₂ mim][Cl] and [C₁₄ mim][Cl]) with Human and Bovine Serum Albumins HAS and BSA, respectively, by means of UV-vis absorption, fluorescence, circular dichroism (CD) and small angle X-ray scattering (SAXS). In all studied cases, a fluorescence quenching was observed as the ionic liquid concentration increases. The decrease in the intensity was (55±3)% and (16.1±0.8)% in the presence of 0.6mM [C₁₄ mim][Cl], for both BSA and HAS, respectively. Interestingly, a similar trend were obtained for [C₁₂ mim][Cl] and [C₁₀mim][Cl]. We also noted the shift of the fluorescent peak of BSA and HSA for shorter wavelengths (blue-shift), as the IL content was increased. The maximum shift ($\Delta\lambda$) achieved corresponded to (21±1) nm for both albumins. CD data suggest a slight loss of secondary structure of both albumins (BSA and HSA), from 80 to 65% of α -helix in the absence and

presence of 0.6mM [C_{14} mim][Cl], respectively. SAXS data indicated an increasing in the proteins radius of gyration (R_g) as ILs was added in the solution, indicating an unfolding process. R_g of BSA and HSA in the absence of IL are (29 ± 1) Å, (30 ± 1) Å, respectively, and go to (46 ± 1) Å and (44 ± 1) Å, in the presence of 0.6mM [C_{14} mim][Cl] for both BSA and HAS, respectively. Taking together, our experimental data suggest that the interaction between IL and the proteins is initially driven by electrostatic forces, having also a major hydrophobic contribution. We believe this work provides new information about the interaction of ILs with model proteins, indicating its ability to alter the conformation of the same.

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Submission ID 277 – **Poster**
Proteins, Structure and Function

Free energy barriers for catalysis in the ATP binding domain of the thermophilic Cu(I) transport ATPase from *Archaeoglobus fulgidus*.

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The thermophilic Cu(I) transport ATPase from *Archaeoglobus fulgidus* (Af-CopA) is an membrane protein that couples the energy from ATP hydrolysis to the translocation of Cu(I) across plasma membranes. The ATP hydrolysis takes place within the globular domain of CopA (Af-CopA-ATPBD) which is located in the intracellular portion of the cell. It is composed by two subdomains: a nucleotide binding or N-domain and a phosphorylation or P -domain I.

The aim of this work is to study and characterize the hydrolysis of ATP catalyzed by the A-fCopA-ATPBD using a combined experimental and computational approach.

For this purpose the ATPBD was cloned, expressed in *E. Coli* and purified by Ni-NTA chromatography. The ATPase activity was measured as a function of ATP concentration determining k_{cat} and k_{cat}/k_M at different temperatures. By fitting a Kramers type model to the kinetic

coefficients, it was obtained ΔG^\ddagger , ΔH^\ddagger and ΔS^\ddagger , following the analysis described in previous reports³.

In addition, computational analysis was performed using AMBER and a self-developed code named LIO2 to optimize the selected quantum subsystem calculus. Crystal structure of Af-ATPBD (PDB 3A1C) was used as initial structure. It was solvated with TIP3P water box, energy-minimized and equilibrated with classical molecular dynamics simulation protocol. Free energy calculations were performed using Hybrid QM/MM methods beginning with different initial structures, changing the protonation state of Asp424, or atoms of the sphere of Mg^{2+} . ΔG^\ddagger values for each coordinate were obtained using Jarzynski's equality.

Comparisons between experimental and theoretical kinetic parameters allow us to obtain valuable information to better understand the catalytic mechanism of Af-ATPBD and to compare it with that corresponding to the complete membrane protein⁽³⁾.

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Submission ID **410** – **Poster**
Proteins, Structure and Function

Unveiling the role of surfactants on amyloid-like protein self-assembling

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Some proteins can undergo structural changes that may trigger an aggregation process where they self-assemble into highly ordered aggregates called amyloid fibers. In vivo, these amyloid fibers are related to more than 25 different diseases, some of them are lethal as Creutzfeldt-Jakob disease and others can lead a person to incapacities, as diabetes type II, Alzheimer's and Parkinson's diseases.

With the aim of understanding the conditions and mechanisms by which proteins form amyloid fibers, we mixed bovine serum albumin (BSA) at pH 3.7 with sodium dodecyl sulfate (SDS) and sodium perfluorooctanoate (SPFO) to induce the amyloid fibers formation. BSA conformational changes were followed in order to suggest a possible pathway of aggregation.

Turbidity (UV-VIS spectroscopy) and Thioflavin T fluorescence data revealed, respectively, the presence of large aggregates and the formation of amyloid-like fibers as the surfactant concentration increased, whereas circular dichroism (CD) showed that BSA second structure changes from α -helix to β -sheet. Transmission electron microscopy (TEM) permitted us to obtain images of fibers and aggregates in the micrometers scale. Further, small angle x-ray scattering (SAXS) measurements provided information about the protein's quaternary structure as a function of surfactant concentration and a more detailed analysis allowed us to suggest a pathway of fibrillation process. Lastly, we performed molecular dynamics simulations to obtain an all atom structure of BSA at pH 3.7 and to study the influence of SDS in the BSA conformation.

Submission ID 411 – Poster
Proteins, Structure and Function

Reversible unfolding of the catalytic domain of the Cu (I) transport ATPase from the hyperthermophilic *Archaeoglobus fulgidus*

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The copper transport ATPase from *Archaeoglobus fulgidus* is an integral membrane protein composed of seven transmembrane segments and four globular domains: the catalytic (ATPBD), an actuator and two metal binding domains. The ATPBD is responsible for the hydrolysis of ATP which occurs with a maximum activity at 70 °C. This domain is composed by two sub-domains (N and P); the N-subdomain binds the nucleotidic part of ATP and the P-subdomain is phosphorylated in D424 during the reaction cycle.

The aim of this work is to characterize the folding mechanism of the ATPBD. For this purpose unfolding and refolding experiments were performed at pre-equilibrium and equilibrium conditions using urea as a denaturing agent.

In equilibrium conditions circular dichroism (CD) signals at far UV and the intrinsic fluorescent signal of the unique tryptophan were registered as function of the urea concentrations. Unfolding plots obtained for CD

and intrinsic fluorescence differ one from each other, indicating different unfolding paths for the reversible unfolding of the ATPBD. As consequence it is impossible to postulate a two state mechanism for these process.

Additionally, time courses of unfolding and refolding of ATPBD, by measuring tryptophan fluorescence as a function of time were obtained in the presence of different urea concentration. Even though, the experimental data could be described by a single exponential function of time, the dependence of the kinetic coefficients (k_{obs}) with the urea concentration indicated the existence of, at least, one intermediate state. Finally, equilibrium and time courses experiments were performed at different temperatures. This results, were analysed according to Kramer's theory and thermodynamic activation parameters (ΔG^\ddagger , ΔH^\ddagger y ΔS^\ddagger) were obtained.

Acknowledgments: with grants from UBA, CONICET and ANPCYT.

Submission ID **421** – **Poster**
Proteins, Structure and Function

DEVELOPMENT OF MULTI-ENZYMATIC COMPLEXES FOR THE IMPROVEMENT OF BIOFUEL PRODUCTION

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Lignocellulose is the most abundant renewable resource on the planet and is an excellent substrate for the production of biofuels. Its enzymatic degradation generates sugars that upon fermentation produce bioethanol. The ligno-cellulolytic enzymes currently used in this process are expensive and have low efficiency. For an economically viable production of biofuels it is essential to develop new methods to increase the activity and stability of these enzymes. Cellulosomes are multi-enzymatic complexes that colocalize different cellulolytic enzymes and cellulose binding domains, increasing their degradation activity through enzymatic proximity and substrate targeting effects. However, the industrial production of natural cellulosomes has serious limitations because of the properties of their scaffolding protein. Our goal is to develop artificial cellulosomes using the structure of an oligomeric protein that is highly stable and highly expressed in bacteria, as a scaffold for the colocalization of cellulases, hemicellulases, beta-glucosidases

and cellulose binding domains. For the assembly of these multienzymatic particles we use a non-covalent coupling strategy through high affinity heterodimeric modules (coiled peptides or cohesin/dockerin modules) complementary fused to the structure of the protein targets and the oligomeric scaffold. It is expected that these complexes will help to increase the enzymatic lignocellulose degradation, reducing the cost of bioethanol production and favoring fossil fuels substitution.

Submission ID 423 – Poster
Proteins, Structure and Function

Differences and similarities in the mechanisms of inhibition of aluminium on the plasma membrane and the sarcoplasmic reticulum calcium pumps

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Aluminium (Al^{3+}) is a metal widely distributed in the environment. Al^{3+} is involved with the pathophysiology of neurodegenerative disorders, such as Parkinsonism dementia and Alzheimer's disease. Several mechanisms explain its neurotoxicity, for example, damage to the glycolytic metabolism, lipid peroxidation leading increased free radicals, protein modifications and changes in the cellular calcium homeostasis. The aim of this work was to study the molecular inhibitory mechanism of Al^{3+} on Ca^{2+} -ATPases like the plasma membrane (PMCA) and the sarcoplasmic reticulum (SERCA). The function of these pumps is to reduce the cytoplasmic calcium concentration. For this purpose, we performed enzymatic measurements of the effect of Al^{3+} on purified preparations of PMCA and SERCA. Then, we measured the effect of different concentrations of Al^{3+} on intracellular calcium concentration in HEK293 cells using fluorescent probes.

Our results show that: (1) Al^{3+} inhibits Ca^{2+} -ATPase activity of both enzymes with similar apparent affinity; (2) In the presence of ATP, Al^{3+} dissociates SERCA, showing reversibility of the process. While in PMCA not occur. (3) In the presence of Al^{3+} , the apparent affinity for Ca^{2+} of SERCA decreased, but not for PMCA; (3) Al^{3+} increases the phosphorylated intermediate (EP) of PMCA while it has not effect on SERCA; (5) Al^{3+} inhibits calcium efflux mediated by PMCA in HEK293 cells, suggesting that aluminum inhibits PMCA in vivo; (6) Preliminary studies using fluorescence microscopy and Lumogallion fluorescent probe, suggest that Al^{3+} could be located in the closeness of PMCA.

This work propose for the first time a different inhibitory mechanism of action for Al^{3+} that involves intermediates of the ATP hydrolysis by these two Ca^{2+} -transport ATPases.

With grants of ANPCYT, CONICET, UBACYT

Submission ID 424 – **Poster**
Proteins, Structure and Function

Structure-function of β -galactosidase in inclusion bodies

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From the first studies of beta galactosidase (β -gal) it was established that the active form of the enzyme is a homotetramer but has residual activity as a dimer. In later studies in our laboratory we proposed that the enzyme could present higher oligomerization states when the enzyme interacts with liposomes interfaces and thus safeguard its stability against aggressive environmental conditions like higher temperature, pH and proteolysis. Now we produce in our laboratory β -gal structured in inclusion bodies (IB). We demonstrated that the enzyme still active in this condition. Production of IB was carried out under conditions that favor the specific activity of β -gal (Temperature induction of expression condition, $T_i = 30^\circ\text{C}$ instead of $T_i = 37^\circ\text{C}$). Successive washings of IB proved enzyme IB-desorption with different protein quality (variable specific activity). An heterogeneous structural quality within the aggregate was previously reported for other proteins structured in IB. Our studies also suggest that the enzyme activity measured in IB samples

comes mainly from the enzyme that desorbed from these supramolecular structures during the catalysis. On the other hand, the analysis of the β -gal fluorescence emission spectra showed that λ_{max} of the enzyme that desorbed from IB change towards minor values than the soluble enzyme. Suggesting that it may be still in an oligomeric state or exhibit a new soluble protein conformation.

Submission ID 194 – **Electronic Poster**

Lipid-Protein Interaction

Interaction of antimicrobial peptides with POPC lipid structures modeled by coarse-grained molecular dynamics simulations

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Antimicrobial Peptides (AMPs) are a wide group of small cationic molecules of the innate immune system. They have proven activity against agents among bacteria, fungi, viruses and eukaryotic parasites. It is suggested that they act by binding to the bilayer increasing the permeability of the membrane [1].

Among them, two peptides obtained from Australian tree frogs, the aurein 1.2 and the maculatin 1.2 show structural features typical of helical AMPs with high lytic activity, the key aspect of AMPs [ibid]. Nevertheless, is still under discussion the molecular mechanism by which they damage biomembranes.

In order to shed light about the molecular mechanism of aurein and maculatin interaction with membranes, we carried out extensive Molecular Dynamics (MD) simulations. Taking into account the system size and the time scales required, we have chosen a coarse grain approach within the MARTINI force field [2].

The simulations were carried out starting from three different configurations: the peptides placed in water near to a POPC planar bilayer ("outside the membrane"), the peptides inside the hydrophobic core of a POPC planar bilayer ("inside the membrane"), and the molecules randomly distributed along the space ("self-assembly"). Our results show that both peptides can form pore-like structures, highlighting two different behaviors on the peptide-membrane interactions and membrane leakage of aurein and maculatin, in good agreement with previous experimental observations [1]. While maculatin can form a pore maintaining the structure of the bilayer and can induce membrane curvature, aurein exhibits surfactant properties and this may cause the total membrane destabilization and disintegration.

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Submission ID **169** – **Poster**
Lipid-Protein Interaction

Microvesicles released from erythrocytes treated with alpha hemolysin of E.coli.

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Uropathogenic strains of *E. coli* deliver the toxin alpha-hemolysin (HlyA) to optimize the host environment for the spread of infection. It is synthesized as a protoxin (ProHlyA) and needs to be activated in bacterial cytosol to the active form by acylation at two internal lysine residues. Recently, we have demonstrated that at sublytic concentration HlyA induces the shape transition; discocyte-echinocyte-spherocyte1 and finally erytosis2 or lysis. Although ProHlyA is an inactive protein it also induces early morphologic shape transitions in erythrocytes1. This sequence of morphologic changes observed for HlyA is the same one occurring with amphiphiles that induce exovesicle formation. Within this context, we studied the release of microvesicles from human erythrocytes treated with sublytic concentrations of HlyA.

HlyA and ProHlyA-treated erythrocytes were observed by Transmission Electron Microscopy (TEM). Images show that HlyA-treated erythrocyte present some vesicles around the cell, which has an echinocyte shape, while ProHlyA does not. Then microvesicles were purified by

ultracentrifugation from the supernatant of HlyA-treated erythrocytes at two toxin concentrations. TEM images show that the sizes of the purified microvesicles are different depending on the toxin concentration. At 35 nM the population of microvesicles is heterogenous, showing a diameter ranging from 170 to 250 nm, instead at 70 nM this population is more homogenous presenting a diameter of 200-220 nm.

Results indicate that human erythrocytes treated with sublytic concentration of HlyA induce the release of microvesicles. Actually we are studying if HlyA is delivered in these microvesicles as a mechanism of spread of the toxin in circulation.

Agradecimientos: This work was supported by the ANPCyT [PICT 2657/2013] and UNLP [M/181].

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Submission ID 170 – Poster
Lipid-Protein Interaction

Effect of fatty acylation on the interaction of *E. coli* alpha hemolysin with lipid Langmuir monolayers

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Alpha-hemolysin (HlyA) is considered the prototype member of the RTX toxin family of Gram negative bacteria and constitutes the main virulence factor produced by uropathogenic *E. coli* strains. This protein toxin is synthesized as a protoxin (ProHlyA) that is transformed into the active form in the bacterial cytosol prior to its secretion. The posttranslational modification consists in the amide-linkage of fatty-acyl moieties to the $\hat{\text{I}}\mu$ -amino groups of two internal lysine residues. HlyA and

ProHlyA bind to the same extent to erythrocyte membranes but only HlyA produces cell lysis. In a very recent study, however, we have demonstrated that ProHlyA still induces early morphologic shape transitions in rabbit erythrocytes¹. In the present work, with the aim at further studying the effect of fatty acylation on protein-membrane interactions, we explored the association of the acylated and unacylated forms of HlyA with lipid monolayers mimicking the outer leaflet of red blood cell membranes. Surface pressure measurements were performed that evidenced a faster incorporation of the acylated protein into the lipid films. Polarization-modulation infrared reflection absorption spectroscopy (PM-IRRAS) measurements revealed that the adsorption of the proteins to the lipid monolayers produced an increase in the lipid acyl chains disorder independently of protein-fatty acylation. When pure proteins films were analyzed by PM-IRRAS, different secondary structure elements were found exposed at the air-water interface, being the entire HlyA polypeptide chain more extended than its unacylated counterpart. These differences in protein arrangement at a hydrophobic-hydrophilic interface could be an important factor for biological activity. Acknowledgments: This work was supported by grants from UNLP, ANPCyT and CIC-PBA.

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[1] Vázquez, R.F., et al. *Biochim Biophys Acta* 1858 (8): 1944-53, 2016.

Submission ID 171 – Poster
Lipid-Protein Interaction

Effect of fatty acylation on the interaction of *E. coli* alpha hemolysin with lipid Langmuir monolayers

1.- Vázquez, Romina; 2.- Daza Millone, Ma. Antonieta; 3.- Pavinatto, Felipe; 4.- Herlax, Vanesa; 5.- Bakás, Laura; 6.- Oliveira Jr, Osvaldo; 7.- Vela, Ma. Elena; 8.- Maté, Sabina

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ProHlyA bind to the same extent to erythrocyte membranes but only HlyA produces cell lysis. In a very recent study, however, we have demonstrated that ProHlyA still induces early morphologic shape transitions in rabbit erythrocytes¹. In the present work, with the aim at further studying the effect of fatty acylation on protein-membrane interactions, we explored the association of the acylated and unacylated forms of HlyA with lipid monolayers mimicking the outer leaflet of red blood cell membranes. Surface pressure measurements were performed that evidenced a faster incorporation of the acylated protein into the lipid films. Polarization-modulation infrared reflection absorption spectroscopy (PM-IRRAS) measurements revealed that the adsorption of the proteins to the lipid monolayers produced an increase in the lipid acyl chains disorder independently of protein-fatty acylation. When pure proteins films were analyzed by PM-IRRAS, different secondary structure elements were found exposed at the air-water interface, being the entire HlyA polypeptide chain more extended than its unacylated counterpart. These differences in protein arrangement at a hydrophobic-hydrophilic interface could be an important factor for biological activity. Acknowledgments: This work was supported by grants from UNLP, ANPCyT and CIC-PBA.

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Submission ID 176 – Poster
Lipid-Protein Interaction

MEMBRANE COMPOSITION INFLUENCE ON THE HYPOTONIC HEMOLYSIS PROTECTION INDUCED BY ARGININE-BASED SURFACTANTS

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Bz-Arg-NHC10 and Bz-Arg-NHC12, two novel arginine-based cationic surfactants, were synthesized in our laboratory using papain adsorbed onto polyamide as biocatalyst. Hemolytic and antihemolytic assays

were performed using sheep (SRBC) and human (HRBC) erythrocytes in order to clarify the lytic mechanism of Bz-Arg-NHCn and the possible relationship between their effect and the lipid membrane composition. Results showed that the hemolytic effect of Bz-Arg-NHC10 was almost 50% lower than that of Bz-Arg-NHC12. A biphasic behavior was also observed for both surfactants in hypotonic media, showing a wide range of protective concentrations in the case of HRBC. Moreover, the protective effect on SRBC was 50% lower than that observed on HRBC. Although no general correlation was found between the erythrocyte volume expansion and the antihemolytic potency, a 15% increment in the critical hemolytic volume was evidenced for SBRC treated with Bz-Arg-NHC12. Generalized polarization (GP) measurements using Laurdan were aim to analyze this differential interaction between Bz-Arg-NHCn and the erythrocyte membranes. A GP increment was observed in all cases except for SRBC treated with Bz-Arg-NHC12, revealing that the protective mechanism in this case does not involve changes in the membrane packing density. Inorganic phosphorus assays evidenced that the amount of solubilized phospholipids (PLs) in the supernatant of HRBC incubated with Bz-Arg-NHCn at sublytic concentrations was higher than that for SRBC. In the case of HRBC treated with Bz-Arg-NHCn, the presence of microvesicles in the supernatant was observed using Transmission Electronic Microscopy (TEM), whereas no microvesicles were found for SRBC. Results suggest that Bz-Arg-NHCn showed a differential lytic/protective mechanism depending crucially on the lipid membrane composition, and therefore in

its biophysical properties.

Submission ID 178 – Poster
Lipid-Protein Interaction

MICELLAR ATTACK OF ARGININE-BASED SURFACTANTS TOWARDS THE ERYTHROCYTE MEMBRANE

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Arginine-based surfactants constitute an interesting group of amino acid-based surfactants since they are generally non-toxic and highly

biodegradable cationic compounds. Two novel arginine-based compounds, Bz-Arg-NHC10 and Bz-Arg-NHC12, were characterized in terms of surface properties and interaction with human red blood cells (HRBC) membranes. The CMC values, obtained by surface tension determinations, were 0.23 mM for Bz-Arg-NHC10 and 0.085 mM for Bz-Arg-NHC12. According to the Γ_{max} (maximum surfactant adsorption at the air/liquid interface) and Amin (area per molecule) values calculated for both compounds, Bz-Arg-NHC12 showed better surface properties than Bz-Arg-NHC10. Cylindrical worm-like aggregates were observed in atomic force microscopy (AFM) images for Bz-Arg-NHCn supported the predictions based on the surfactant packing parameter value (SPP). Hemolytic effect of Bz-Arg-NHCn on HRBC at different hematocrits allowed the determination of the effective surfactant/lipid molar ratios for saturation (Rsat), being these ratios of 6 molecules of Bz-Arg-NHC12 and 11 molecules of Bz-Arg-NHC10 per lipid molecule. Erythrocyte membrane solubilization was induced by surfactant aggregates, since cell lysis was only evidenced at surfactant concentrations above CMC. Changes in HRBC shape observed at different surfactants' concentrations allowed to conclude that a slow micellar mechanism, based on the extraction of membrane lipids upon collisions between HRBC and surfactant aggregates or by shedding of microvesicles would be responsible for the hemolysis produced by both surfactants.

Submission ID 179 – Poster
Lipid-Protein Interaction

FUNGICIDAL MECHANISM OF ARGININE-BASED SURFACTANTS AGAINST PHYTOPATHOGENIC FUNGAL SPORES VIA MEMBRANE DISTURBANCE

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The antifungal activity of two novel arginine-based compounds (Bz-Arg-NHC10 and Bz-Arg-NHC12), which were synthesized by an en-

zymatic strategy, was studied against phytopathogenic fungi (*Fusarium oxysporum*, *Fusarium solani*, *Colletotrichum gloeosporioides* and *Colletotrichum lindemuthianum*). A commercial cationic disinfectant, Cetrimide (alkyl trimethyl ammonium bromide mixture), was used for comparison. Critical micelle concentration was determined by tensiometry for both compounds, being 0.23 mM for Bz-Arg-NHC10 and 0.085 mM for Bz-Arg-NHC12. Inhibition of vegetative growth and spore germination was also investigated for phytopathogenic fungi. In general, for the vegetative growth, the most potent arginine-based compound was Bz-Arg-NHC10, showing IC₅₀ values ranging from 45 to 71 μ M after 72 hs of exposure. The three compounds tested (considering Cetrimide for comparison) interfered in the spore's development of the four species studied. Experiments in lipid monolayers composed by dioleoylphosphatidylcholine (PC), phosphatidylethanolamine (PE) and ergosterol (ERG) confirmed that Bz-Arg-NHC10 has higher antifungal potency than Bz-Arg-NHC12. The possible mechanism of antifungal activity of the investigated compounds could involve the penetration of surfactant molecules into the cytoplasm and production of reactive oxygen species (ROS).

Submission ID **195** – **Poster**
Lipid-Protein Interaction

Acetylcholinesterase activity inhibition by epigallocatechin gallate. A Biophysical Study.

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Polyphenols are plant secondary metabolites characterized by the presence of phenolic groups. These compounds display a myriad of different properties, among them the modulation of some enzyme activities was reported. In this regard, we previously demonstrated that epigallocatechin gallate (EGCG) inhibited the erythrocyte acetylcholinesterase activity (AChE) and proposed that red blood cell AChE may serve as a good model of the isoform present in the central nervous system.

The scope of the present work was to study the influence of ionic strength in the inhibition of the membrane-bound AChE by EGCG. Besides, we analyzed how NaCl regulated the interaction of EGCG with erythrocyte membranes and attempted to correlate these findings with the enzyme activities measured in the presence and the absence of EGCG.

Our results reveal that EGCG preferentially acted on the membrane-bound enzyme rather than on its soluble form. Actually, it was shown

that this flavonoid may bind to the red blood cell membrane surface, which might improve the interaction between EGCG and AChE. The EGCG binding to the ghost membrane surface was studied by measuring the steady-state DPH fluorescence anisotropy and analyzing the laurdan generalized polarization. In addition, EGCG binding to ghost surface was estimated by following the quenching of octadecyl rhodamine fluorescence.

We were able to demonstrate that EGCG-membrane interaction was enhanced at higher NaCl concentrations. However, EGCG would not get inserted into the hydrophobic core of the membrane since no significant changes in the fluorescence anisotropy was detected. It can be concluded that EGCG would interact mainly with the membrane surface, which could be correlated with the greater inhibition of AChE in this condition

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Submission ID 205 – Poster
Lipid-Protein Interaction

Characterization of lipid-bound Metallo- β -Lactamase NDM-1

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The clinical efficacy of carbapenems, last resort antibiotics, is threatened by the global dissemination of metallo- β -lactamases (MBLs). The genes encoding these Zn(II)-dependent enzymes are frequently associated with mobile genetic elements, accelerating their transfer among pathogens, and there is no therapeutic inhibitor capable of abolishing their activity. Among MBLs, NDM-1 has shown the fastest and largest geographical spread, having already been detected in more than 70 countries worldwide less than a decade after its discovery.

Unlike all other characterized MBLs, which are soluble periplasmic proteins, NDM-1 is covalently bound to a lipid group that anchors the enzyme to the inner leaflet of the outer membrane of Gram-negative bacteria. We have recently shown that membrane-anchoring stabilizes NDM-1 within the bacterial cell and allows it to endure low-Zn(II) availability conditions, similar to those encountered during pathogenesis. Furthermore, we demonstrated that the membrane localization enhances secretion of NDM-1 within outer membrane vesicles in

Gram-negative bacteria. These vesicles possess carbapenemase activity, and are able to protect nearby populations of otherwise antibiotic-susceptible cells. We propose that membrane anchoring of NDM-1 constitutes an evolutionary adaptation contributing to its clinical success.

Up to date, all *in vitro* biochemical and biophysical characterizations of NDM-1 have been performed with the enzyme in a truncated soluble form, overlooking its native state and cellular localization. Here we report the overexpression, solubilization from membranes and purification of lipidated NDM-1, and the initial characterization of this enzyme in detergent micelles.

Acknowledgements: ANPCyT, NIH, CONICET

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Submission ID 212 – **Electronic Poster****Lipid-Protein Interaction****Interaction of cell penetrating peptides with model membranes***1.- Via, Matías; 2.- Del Pópolo, Mario G; 3.- Wilke, Natalia**(1) a - Facultad de Ciencias Exactas y Naturales-Universidad Nacional de Cuyo**(2) a - Facultad de Ciencias Exactas y Naturales-Universidad Nacional de Cuyo**(3) a - CIQUIBIC, Dto. Química Biológica, Facultad de Ciencias Químicas, CONICET, Universidad Nacional de Córdoba, Córdoba, Argentina.*

Our aim is to gain insight in the internalization mechanism of cell penetrating peptides, namely polyarginines [1-3] by means of both, experimental and in silico methods. In the experiments, we analyzed the penetration of the hydrophilic peptide KR9C into Langmuir monolayers of fatty acids with different dipolar potentials, and also of anionic lipids (DMPG). The experimental results show that an increase in the charge density of the fatty acid monolayers (which is achieved by changing the subphase pH) favored the incorporation of KR9C. This was observed in MA and PA monolayers (with $pK_a \sim 6$), while PFTD ($pK_a \sim 0$) [4] is charged in the whole pH range, showing a high peptide incorporation. Conversely, negative DMPG monolayers did not incorporate, even though the molecules are negatively charged.

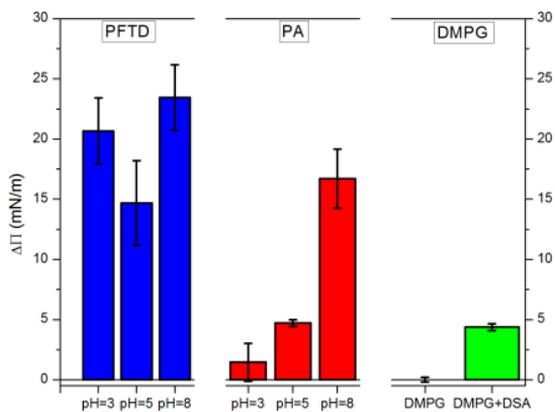
Regarding the influence of the surface potential on KR9C incorporation into the monolayers, we found that the negative value observed in PFTD films is the main factor for the very high peptide incorporation

determined in these monolayers. In this regard, simulation results also put forward that transmembrane potential has a substantial influence on KR9C insertion in the lipid bilayer hydrophobic core.

In summary, our study demonstrates that the presence of fatty acids and a negative surface potential are two important factors in the incorporation of polyarginines into Langmuir monolayers. The negative surface potential would be involved in the first step of the peptide translocation process across cell membranes, triggering the diffusion of the peptide towards the water/lipid interface. Fatty acids incorporate substantially much more peptide than DMPG, suggesting that the carboxyl groups might be relevant for the mechanism.

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Submission ID 218 – **Electronic Poster****Lipid-Protein Interaction****The surface behavior of the HIV-GAG MA domain and its N-terminal peptides is finely-tuned by protein myristoylation and nucleic acid binding***Pérez Socas, Luis Benito, 2.- Ambroggio, Ernesto**Centro de Investigaciones en Química Biológica de Córdoba (CIQUIBIC), Departamento de Química Biológica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Haya de la Torre y Medina Allende, Ciudad Universitaria, X5000HUA, Córdoba, Argentina*

Within the lifecycle of HIV, the capsid protein GAG has a fundamental role in coordinating the assembly process of the virus at the plasmatic membrane. The interaction of this protein with the lipid bilayer is mediated by its N-terminal myristoylated MA domain. In addition, MA possess a highly basic region (HBR) responsible for the interaction with the negative lipids of the plasmatic membrane, especially with PIP2. It is known that this domain also interacts with the viral genome, in what could be a regulatory step of the assembly process. Here we show the modulation of the surface properties of the MA domain and N-terminal peptides (GAGnt) provoked by the presence of DNA. We performed protein Langmuir monolayer experiments using the recombinant MA protein and synthetic GAGnt peptides, in both myristoylated and unmyristoylated variants, and in the presence or absence of the DNA sequence Sel25, which is known to interact

with MA domain with nanomolar affinity. Furthermore, we carried out Brewster angle microscopy (BAM) experiments to visualize the effects of the oligonucleotide on the monolayer lateral structure. Experiments from pure-peptide monolayers show clear-cut differences when the peptide was myristoylated and in the presence of DNA respect to the un-myristoylated peptide monolayer. Notable variations on the film stability was observed, with a noticeable higher collapse pressure for myrGAGnt monolayers than for GAGnt films. This parameter is also affected by the presence of DNA, being the case of myrGAGnt the most drastic change, in which the isotherm almost vanishes when Sel25 is present. On the other hand, MA and myrMA proteins show slight differences on the surface biophysical properties. Lateral-compression isotherms from pure protein monolayer are apparently not affected by the presence of DNA. However, for the case of myrMA it is remarkable the presence of laterally-separated structural domains, observed with BAM, which disappear in the presence of DNA.

Submission ID 219 – Poster
Lipid-Protein Interaction

Lipid selectivity in novel antimicrobial peptides: implication on antimicrobial and hemolytic activity

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For a peptide to be considered for therapeutic development, it needs to not only possess potent antimicrobial activity but also have low toxicity to human cells. Beside many studied has been conducted in order to correlate the antimicrobial activity with the affinity toward model lipid membranes, the use of model membranes in order to explain citotoxicity effects specially hemolytic activity its less explorer. In this context

we designed two novel peptides, peptide 6 (p6) that resulted with high hemolytic values and poor antimicrobial activity and peptide 6.2 (p6.2) derived from the previous one, with lower hemolysis and dramatically increased antimicrobial activity. Using both peptides as models we correlate the affinity toward different lipid composition with antimicrobial and hemolytic activities. Our results from surface pressure and zeta potential data, shown that p6.2, exhibit a higher affinity and faster binding kinetic toward PG containing membranes, while p6 for pure PC membranes. Final position and structure showed for p6.2 an alpha-helix conversion that resulting in parallel alignment with the Trp residues depth insert in the membrane. Meanwhile the inability of p6 to adopt an amphipathic structure plus its lower affinity toward PG containing membranes seems explain the poor antimicrobial activity. Regarding hemolytic activity, p6 showed the highest affinity for erythrocyte membranes that result in high hemolysis values. Overall our data allows us to conclude that affinity toward negatively charged lipid instead zwitterionics ones it seems to be a key factor driving from hemolytic to antimicrobial results.

Submission ID 226 – Poster
Lipid-Protein Interaction

A new Langmuir-Schaefer-based method developed for catalytic studies of acetylcholinesterase in planar films of erythrocyte membranes

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Previously we reported that the catalytic activity of bovine erythrocyte acetylcholinesterase (BEA) located in Langmuir-Blodgett films (LB) of bovine erythrocyte membranes (BEM), LBBEA, depended on the curvature and on the packing of the molecular environment. Moreover, the specific activity of LBBEA was much lower than that of BEA in suspensions of BEM vesicles (SBEA). So, the present work was aimed at maximizing the specific activity of BEA recovered from the transfer of a Langmuir film (LF) from the air-aqueous interface to alkylated solid surfaces and improving the precision of the enzymatic assays. Three main changes were introduced to the previously assayed method. a) Phosphate saline buffer (PBS), pH 7.4, was used instead of H₂O as the subphase over which was spread the BEM to form the LF, assuming that this composition, closer to physiological conditions, would be more effective than water in preserving the BEA protein structure/activity and

the LF organization. b) BEA in LF was transferred from air-PBS interface to hydrophobic flat surfaces by the Langmuir-Schaefer technique (LS) to obtain LSBEA samples. c) A new device was designed to allow performing the whole enzymatic activity assay using a unique LS film as well as the reading of the absorbance values in the same container. The LF of BEM at the air-PBS interface, compared with LF formed over H₂O, showed surface pressure vs area (π -A) isotherms more expanded at low π , more compressible, with a bi-dimensional transition at lower π and lower minimal A. The surface potential reached 250 mV at the collapse point in both conditions (H₂O and PBS). The specific activity resulted SBEA»LSBEA>LBBEA. The use of PBS in the subphase and the transfer of LF at π =35mN/m instead of 10 mN/m improved the recovery of specific activity in LSBEA and LBBEA. The homogeneity of BEA distribution in LSBEA samples highly improved the precision of the kinetic parameters determined in different molecular packing conditions.

Submission ID 229 – **Electronic Poster****Lipid-Protein Interaction****Comparative study of the interaction of melittin and lysine peptides with lipid membranes.**

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Melittin (ML) consisting of 26 amino acids arranged in α -helix, rich in arginine (Arg) and lysine (Lys). The amphipathic helix penetration into the hydrophobic interior of membranes depends on the membrane composition and ML concentration, being involved Arg and Lys residues. However, it is not clear the role of electrostatic forces in the interaction and perturbation in the lipid bilayer. In this context the objective of this study was to evaluate the interactions of ML, penta and tetra peptides of Lys (same total charge of ML and similar characteristic to the C-terminal ML, respectively) through variation of ζ potential ($\Delta\Psi_{\zeta}$) of phosphatidylcholine (PC), phosphatidyletanolamine (PE) and phosphatidylglycerol (PG) unilamellar vesicles. Peptides produce less negative values of Ψ_{ζ} in DMPC, DMPE and DMPC:DMPG liposomes.

However, Lys-peptides saturates at around $2 \mu\text{M}$ in negative Ψ_{ζ} (-10 or -20 mV) while ML saturated at less than $1 \mu\text{M}$ (+10 or +20 mV). The different sign of the Ψ_{ζ} at which ζ stabilizes suggests a different orientation of the peptides at the interphase. The apparent dissociation constants (K_{dapp}) were estimated in order to evaluate the affinity of the peptide interactions with membranes. ML interacted with DMPC with higher affinity than Lys-peptides (K_{dapp} 0,14 and 0,30 μM , respectively). On DMPE, ML and Lys-peptides show a higher affinity than in DMPC without significant differences in K_{dapp} (0,10 and 0,11 μM , respectively). The significant differences in $\Delta\Psi_{\zeta}$ produced by ML in these membranes could be due to a higher binding sites for ML or a better exposition of charges in the final disposition in the membrane due its ability to adopt an α -helix. ML in DMPC:DMPG liposomes exhibited the highest $\Delta\Psi_{\zeta}$ in comparison with the other tested membranes, but lower affinity (K_{dapp} 1,21 μM). In conclusion, in DMPC membranes electrostatic interaction as other hydrophobic forces seems to be involved, whereas in DMPE membranes the lipid electrostatic forces are the dominant ones.

Submission ID 271 – **Electronic Poster**

Lipid-Protein Interaction

Raman spectroscopy study of the effect of Nisin in unilamellar vesicles of DLPG.

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Nisin is a cationic peptide with bactericidal activity, produced by different *Lactococcus lactis* strains. It belongs to a class of antimicrobial polypeptides which is known as Lantibiotics. Nisin contains unusual amino acids, which are formed in posttranslational processes, such as lanthionine (DAla-S-Ala), β -methyllanthionine (DAbu-S-Ala), dehydroalanine (Dha) and dehydrobutyrine (Dhb). (1) Our group is interested in determining the molecular bases of the bacterial activity of Nisin by using spectroscopic techniques such as fluorescence, Raman and FTIR. Previously, by fluorescence studies, we determined the minimum amount of the peptide necessary to produce a significant change in

the membrane (2). Now, we are presenting Raman spectroscopy studies related to the structural modifications induced by the peptide incorporation in critical concentrations to unilamellar vesicles (LUV) of dilauroylphosphatidylglycerol (DLPG). The evaluation and interpretation of the intensity ratios of C-H and C-C stretching bands belonging to the lipid hydrocarbon chains, allowed concluding that the incorporated peptide produces an increase in the rigidity of the membrane, in accordance with our results obtained by fluorescence studies.

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Submission ID 168 – **Electronic Poster**
Membrane Transporters and Channels

Hydrophobic gating in aquaporins: functional study of the participation of Leu206 in channel closure

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PIP aquaporins are transmembrane proteins able to transport water in a strictly regulated manner. The channel closure results from either the protonation of a conserved His residue under cytosolic acidification or by dephosphorylation of conserved serine residues (1). Our group described previously that PIP can be assembled not only as homotetramers but also as heterotetramers of variable stoichiometry combining PIP2 and PIP1 isoforms; pH sensing is shifted to alkaline values favoring the persistence of the close state in all the heterotetramers formed (2).

Despite functional data is abundant both for PIP2 and PIP1 isoforms, only PIP2 structure has been resolved (3). From structural data, a mechanism named 'capping' has been proposed to explain the gating of PIP aquaporins channel. This mechanism implies a large-scale rearrangement of loopD in order to cap the channel in the closed state. Interestingly, this rearrangement involves the conformation of a hydrophobic gate that completes the blocking of the water pore. While the informa-

tion given by the description of this mechanism is highly detailed, up to now PIP mutants haven't been construct to confirm computational data. The Leu206, highly conserved in PIP, is part of the hydrophobic gate and has been proposed to insert into a cavity near the entrance of the channel. We create the mutant protein BvPIP2;2Leu206Ala to evaluate the relevance of this hydrophobic residue in the blocking of the pore under acidic conditions. We study, for the first time, the impact of the hydrophobic gate both in PIP2 homotetramers and PIP2-PIP1 heterotetramers, by means of heterologous expression (*Xenopus* oocyte system) of the mutant alone or in combination with wild type BvPIP1;1.

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Submission ID 175 – **Electronic Poster**
Membrane Transporters and Channels

Structural and functional characterization of human heteromeric 5-HT3 receptors.

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5-HT3 receptors are members of the Cys-loop receptor family that mediate fast excitatory transmission in central and peripheral nervous system. Genes for five different subunits (A-E) have been identified in humans, and most of the subunits have multiple isoforms. The A subunit is capable of forming functional homomeric (5-HT3A), or heteromeric receptors with the B subunit (5-HT3AB). Here we combine single-channel and macroscopic current recordings to determine if other 5-HT3 subunits, Br1, Br2, C, D and E (B-E), can combine with the A subunit to form heteromeric receptors. After co-expression of the A subunit with each of the tested subunits, single-channel events with different conductance and kinetic properties with respect to those of 5-HT3A receptors were detected, except for the Br2 subunit. These results indicate that B-E subunits can assemble into functional heteromeric receptors with the A subunit. From the corresponding recordings, the analysis of the single-channel amplitude of the opening events suggests a possible stoichiometry for each heteromeric receptor, since each subunit (B-E)

that is incorporated into the pentamer increases the conductance of the homomeric 5-HT_{3A} receptor in 14 pS. Conversely, the Br2 subunit does not appear to form functional receptors with A, since macroscopic currents and single-channel recordings did not differ from those of homomeric 5-HT_{3A} receptors. Our results confirm the incorporation of different 5-HT₃ subunits into the receptor, thus leading to a wide variety of receptors showing different functional properties, such as agonist sensitivity, kinetics and single-channel conductance. The functional characterization of different heteromeric 5-HT₃ receptors, which are expressed in different tissues, would contribute to the development of selective therapies targeting this receptor family.

Submission ID 192 – **Electronic Poster**
Membrane Transporters and Channels

Study of ligand binding sites in Plasma Membrane Calcium Pump: An Azido-Ruthenium photoreactive probe as strategy for structural characterization

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The Plasma Membrane Calcium ATPase (PMCA) is a P-type ATPase that maintains the homeostasis of Ca^{2+} in eukaryotic cells. It couples the transport of Ca^{2+} with the hydrolysis of ATP. The structure of PMCA is still not resolved, and only limited information is available of ligand binding sites. The purpose of this work is to identify and

characterize the ligand binding sites of PMCA. We synthesized azido-ruthenium (AzRu) a photoactivable reagent to obtain structural information of PMCA, which binds covalently and specifically to Ca^{2+} -binding proteins after exposure to irradiation at 290 nm. The experiments were performed with purified PMCA from human erythrocytes.

Measurements of phosphoenzyme in presence of AzRu showed an increase of phosphorylated intermediate in experimental conditions that inhibit the ATPase activity. This suggests that AzRu would be affecting the dephosphorylation of the pump.

Studies of ESI-Orbitrap/MALDI-TOF-MS of PMCA with AzRu (after photolysis) showed that some peptides cannot be found, suggesting that these peptides might be related with the interaction sites between AzRu and PMCA. These peptides contain histidine residues and other residues that are targets of covalent binding with the photoactivable azido group. We analyzed the sequence homology model of PMCA on crystallographic structure of SERCA and we found a peptide related with the Mg^{2+} site. Other peptides were analyzed because these are in the C-terminus which is not present in SERCA. Our results suggest that the Mg^{2+} binding site could be involved in the interaction between PMCA and AzRu.

We analyzed the structure of AzRu by MALDI/LDI-TOF and found fragments of reagent that would be photolysis products. This result will be useful to recognize adducts AzRu-PMCA and easily analysis of the results by mass spectrometry.

Submission ID 193 – Poster
Membrane Transporters and Channels

Quercetin and gossypin inhibit the Plasma Membrane Ca^{2+} -ATPase by different mechanisms

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A large number of flavonoids, mostly O-glycosides, are found in foods of plant origin. These compounds are believed to have cancer chemoprotective properties by triggering apoptosis through an increase of cytosolic concentration of calcium ($[\text{Ca}^{2+}]_c$). The increase of $[\text{Ca}^{2+}]_c$ could be due to the fact that some flavonoids inhibit specific Ca^{2+} removing systems, as the sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase and the plasma membrane calcium ATPase (PMCA) [1, 2].

From the screening of Hibiscus flavonoids quercetin and gossypin emerged as the most active compounds on PMCA inhibitors. The aim of this work is to characterize the effect these flavonoids on PMCA. We measured the Ca^{2+} -ATPase activity in purified PMCA obtained from human erythrocytes and the $[\text{Ca}^{2+}]_c$ in HEK293T cells.

Results using purified PMCA showed that (1) PMCA activity was inhibited by quercetin and gossypin with a $K_{0.5}$ of 0.34 ± 0.01 and $5.1 \pm 1.0 \mu\text{M}$, respectively; (2) the inhibition was noncompetitive with Ca^{2+} ;

(3) both quercetin and gossypin affected CaM activation, but quercetin produced a decrease in the apparent affinity for CaM while gossypin did not; (4) quercetin led to the increase of phosphoenzyme level (EP) although gossypin induced a decrease. EP formed in the presence of quercetin was dephosphorylated by ADP, suggesting that it could stabilize the E1P intermediate. These results suggest that gossypin could affect the binding of ATP while quercetin could prevent the conformational change $E1P \rightarrow E2P$. In HEK293T cells quercetin and gossypin lowered the Ca^{2+} removal rate by PMCA compared to the control. These results show that quercetin and gossypin inhibit PMCA by different mechanisms, changing the cellular calcium homeostasis possibly due to their different molecular structures.

With grants of CONICET, ANPCYT and UBACYT

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Submission ID 203 – Poster

Membrane Transporters and Channels**Steps involved in the transport of Na⁺ by the Na,K-ATPase**

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The Na,K-ATPase is a membrane-bound ion pump that generates electrochemical gradients for Na⁺ and K⁺ across plasma membranes of animal cells. The enzyme oscillates between two major conformations, E1 and E2. Under physiological conditions, E1 binds Na⁺, ATP and Mg²⁺ and forms the phosphorylated state E1P containing occluded Na⁺, with the release of ADP. After a conformational transition to E2P, Na⁺ is released and K⁺ binds and becomes occluded. The subsequent release of K⁺ leads to E1 and the cycle begins again.

In this work we report results on the effects of oligomycin, an antibiotic from *Streptomyces diastatochromogenes* and epigallocatechin-3-gallate (EGCg), a polyphenolic compound obtained from green tea, as inhibitory agents to isolate the intermediates involved in the transport of Na⁺. It is proposed that, like oligomycin, EGCg inhibits the enzyme activity by stabilizing the E1 intermediates, thus blocking the E1P – E2P conformational change. We therefore evaluated the effects of these

inhibitors on the ATPase activity, the phosphoenzyme level, and the binding/occlusion of Na^+ in the enzyme.

Results show that: (i) EGCg inhibited the ATPase activity with a $K_{0.5}$ of 1 mM as previously reported, (ii) the phosphoenzyme level (measured using $[\gamma\text{-}^{32}\text{P}]\text{ATP}$) that remains after the addition of K^+ was higher in the presence of EGCg than in its absence, and (iii) in the presence of either oligomycin or EGCg the amount of tightly-bound $^{22}\text{Na}^+$ was 18 times higher than with the enzyme thermally inactivated, and the amount of $^{22}\text{Na}^+$ in the enzyme without inhibitors was 8 times higher than with the inactivated enzyme.

These results suggest that, like oligomycin, EGCc stabilizes the E1 conformation of the Na,K-ATPase and allows the isolation of the intermediates containing occluded Na^+ .

With grants from ANPCYT, CONICET and UBACYT

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Submission ID 215 – Poster
Membrane Transporters and Channels

Detection and characterization of functional heteromeric receptors composed of the $\alpha 7$ nicotinic subunit and its duplicate form

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The $\alpha 7$ nicotinic receptor subunit gene, CHRNA7, codes for a subunit that forms the homomeric $\alpha 7$ receptor, which is involved in learning and memory. Exons 5-10 of CHRNA7 were duplicated and fused to the FAM7A gene. The product of the resulting chimeric gene, dup $\alpha 7$, is a truncated subunit that lacks part of the ACh binding site. We here combine cell expression and electrophysiological recordings in HEK cells to understand the functional role of the dup $\alpha 7$ subunit. By confocal microscopy and flow cytometry we found that cells transfected with dup $\alpha 7$ do not show cell surface binding of α -bungarotoxin, a specific antagonist of $\alpha 7$. The incorporation of dup $\alpha 7$ cDNA during cell transfection with $\alpha 7$ cDNA reduces α -bungarotoxin binding compared to that determined with $\alpha 7$ alone, indicating a negative modulatory role of the duplicated subunit. To determine if dup $\alpha 7$ forms functional pentamers, we recorded single-channel and macroscopic currents elicited by an allosteric agonist that binds to a transmembrane site that is conserved between $\alpha 7$ and dup $\alpha 7$. We found that, in contrast to cells ex-

pressing $\alpha 7$, currents are not detected in those transfected with $\text{dup}\alpha 7$ cDNA. To determine if $\text{dup}\alpha 7$ can co-assemble into functional receptors with $\alpha 7$ we used an $\alpha 7$ subunit carrying mutations in determinants of ion conductance ($\alpha 7\text{LC}$) as a reporter of receptor stoichiometry. Although $\alpha 7\text{LC}$ forms functional receptors, single-channel openings cannot be detected due to their low conductance. Co-expression of $\alpha 7\text{LC}$ with $\text{dup}\alpha 7$, which by itself does not form functional receptors, allows detection of single-channel openings elicited by ACh. This result unequivocally indicates that $\alpha 7$ and $\text{dup}\alpha 7$ subunits assemble into functional heteromeric receptors. The analysis shows that a minimum of two $\alpha 7$ subunits is required for forming functional receptors. Our results contribute to the understanding of the functional significance of the partial duplication of the $\alpha 7$ gene.

Submission ID 216 – **Electronic Poster**
Membrane Transporters and Channels

DUAL EFFECT OF THE ACETYLCHOLINESTERASE INHIBITOR CAFFEINE ON THE MUSCLE NICOTINIC ACETYLCHOLINE RECEPTOR

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Cholinergic deficit is regarded as an important factor responsible for Alzheimer's disease symptoms. One of the molecular targets for the treatment of this disease is acetylcholinesterase (AChE), an enzyme that hydrolyzes acetylcholine at the synaptic cleft. It has been shown that some AChE inhibitors also act at nicotinic receptors (nAChR) potentiating their therapeutic effect. We found that metabolic extracts of *Camellia sinensis* (red tea) exhibit a strong anticholinesterase activity. By chromatography and NMR spectroscopy we found that caffeine was the active compound exerting such effect. We next explored if caffeine has a direct effect on the nAChR. Using the AChR conformational-sensitive probe crystal violet (CrV), an AChR open channel blocker, and AChR-rich membranes from *Torpedo californica*, we observed that increasing concentrations of caffeine (10-300 μM) decreased the KD of CrV in the resting state without changes in the KD in the desensitized

state. In the presence of α -bungarotoxin, a specific AChR competitive antagonist, a dual effect was evident: low concentrations of caffeine did not produce any effect in the KD of CrV in the resting state, whereas higher concentrations produce a great increase of this value compatible with a competition with CrV for its site on the channel pore. To confirm this, we performed single channel recordings in Bosc cells expressing the adult muscle nAChR in the presence of 30 μ M ACh and increasing concentrations of caffeine (150-20000 μ M). We found that the mean open duration decreases, and the relative area of the briefer closed component and the cluster duration increase as a function of caffeine concentration. All these observations are compatible with an open channel blocker. Thus, our results suggest a dual effect of caffeine on the muscle AChR: at low concentrations, in the absence of agonist, induces an AChR conformational change, whereas at high concentrations caffeine acts as an AChR open channel blocker.

Submission ID 217 – **Electronic Poster**
Membrane Transporters and Channels

The natural polyphenol, epigallocatechin-3-gallate, inhibits the Plasma Membrane Ca^{2+} -ATPase by favoring the phosphoenzyme conformation

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Epigallocatechin 3-gallate (EGCG) is the major polyphenol component of green tea. This compound is believed to be the main responsible for many of the health benefits associated with green tea. EGCG beneficial effects include antioxidant, anti-inflammatory and neuroprotective effects. EGCG produces alterations in intracellular Ca^{2+} homeostasis which could be linked to the function of the Sarcoplasmic Reticulum Calcium Pump (SERCA). The maintenance of intracellular calcium levels is fine-tuned by the Plasma Membrane Ca^{2+} -ATPase (PMCA). Therefore, we investigated the effect of different flavan-3-ols and went into detail about the EGCG effect on PMCA.

We performed measurements of the Ca^{2+} -ATPase activity on PMCA purified from human red blood cells and of Ca^{2+} flux on HEK293T cells that overexpress PMCA4. We evaluated PMCA ATPase activity in the presence of catechin (C), epicatechin (EC) and epigallocatechin.

C and EC showed no effect up to 100 μM . However, EGCG showed a strong inhibition with a $K_{0.5}$ of $0.032 \pm 0.003 \mu\text{M}$. Under similar conditions, EGCG showed an increase of the phosphorylated intermediate which was found to be ADP sensitive, suggesting that EGCG could stabilize the E1P conformation on the reaction cycle of hydrolysis of ATP by PMCA.

We performed docking assays of EGCG as ligand and PMCA structure models as the receptor. PMCA models were obtained by homology modelling on SERCA crystallographic structures in different conformations. Our results showed that EGCG binds mainly to the closed conformations of the enzyme. Particularly the E1P conformation forms the lowest free energy binding complexes with the ligand establishing hydrogen bonds with both the P and N domains of the pump.

On the other hand, Ca^{2+} efflux in HEK293 cells is inhibited by EGCG *in vivo* showing its physiological relevance on PMCA activity.

With grants of CONICET, ANPCYT and UBACYT.

Submission ID 231 – Poster

Membrane Transporters and Channels

Intracellular sodium concentration can regulate epithelial sodium channel (ENaC) sensitivity to osmotic stimuli

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Epithelial sodium channel (ENaC) function is affected by changes of the intracellular sodium concentration, in a process called feedback inhibition, which is dependent of ENaC intracellular C-terminal regions and affects open probability (P_o) and number of channels. Although its osmosensitivity is a controversial issue we previously investigated ENaC response to mild hypotonic stimuli in the *Xenopus* oocyte expression system. On this basis we suggested that mild hypotonicity (25%) causes an ENaC inhibition that is principally mediated through an effect on the open probability of channels in the membrane (Galizia et al, 2013). We then attempted to study the possible the role of intracellular sodium concentration in the hypotonic dependent inhibition of ENaC. Our recent results suggest that hypotonicity-dependent ENaC inhibition, could be mediated by an intracellular sodium dependent mechanism, involving a voltage dependent effect. In order to continue the study on the

role of intracellular sodium on the ENaC regulation mediated by osmotic challenges we used the voltage clamp technique with the injection of the three subunits of the mouse ENaC (mENaC) to measure the amiloride-sensitive Na⁺ currents (I_{Na(amil)}). We performed experiments increasing the osmotic gradients applied. Results indicates that at low intracellular sodium conditions (20 mM) the inhibition of the inward I_{Na(amil)} is dependent on the osmotic gradient magnitude (33% hypotonicity). In addition, we performed experiments of acute sodium load during 5 minutes and we measure the I_{Na(amil)} in response to hypotonic stimuli. Results suggest that although intracellular sodium can modify the ENaC sensitivity to osmotic stimuli, a rapid increase in intracellular sodium is not an enough condition to elicit the hypotonic ENaC inhibition.

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Submission ID 254 – Poster
Membrane Transporters and Channels

Analysis of α helices, extracellular loops and pore region in human aquaporin-1 by molecular dynamics

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Human aquaporin-1 (hAQP1) is an integral membrane protein that transports water. In previous work, we demonstrated that the permeability of hAQP1 is modulated by the tension of the plasma membrane. To investigate whether that modulation is related to the dynamic conformation of the pore, the extracellular loops and/or the bending of α helices, the information of two crystalline hAQP1 structures (1FQY and 4CSK) was used. We carried out molecular dynamics (MD) in three different conditions: a) tetramer with restriction in those α -carbons ($C\alpha$) that would be in contact with the lipid bilayer (TR); b) tetramer without any further restrictions that those provided by the tetramerization itself (TL); and c) unrestricted monomer (ML). Several descriptors were used for analysis, such as RMSD, the number of water molecules in

the pore, modifications in estimated diameter along pore sections, the Solvent Accessible Surface Area (SASA) in residues of the NPA and ar/R sites, the geometry of helices and loops, harmonic analysis using Fourier transform and radial distribution function ($g(r)$) of water molecules measured in residues influenced by movements of loop A. Principal Component Analysis (PCA) was performed. The results observed were: 1) the contribution of restricting $C\alpha$ differs from the contribution of tetramerization itself; 2) the tetramerization does not prevent displacement of hydrophilic loops to regions that would not be accessible in the presence of the lipid bilayer; 3) individual monomers behave differently within TL and TR; 4) At level of the pore, no descriptor per se explains the differences observed between TL and TR; 5) localized restrictions in TR cause modifications in far and not restricted sites inside the pore; 6) the distinct behavior shown by H1, H3 and H6 helices becomes the subject of future studies, in search for a possible sensor of the membrane tension that could influence the permeability of the pore.

Submission ID 262 – Poster

Membrane Transporters and Channels

Cortical Cytoskeleton Dynamics Regulates PMCA2 activity

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We have previously shown that purified actin can directly bind to hPMCA4b and exert a dual modulation on its Ca^{2+} -ATPase activity: on one side F-actin inhibits PMCA [1] while short actin oligomers may be responsible for PMCA activation [2]. These studies required to be performed using purified proteins given the nature of the biophysical and

biochemical approaches used. In order to assess whether this functional interaction may be of physiological relevance, we decided to characterize this phenomenon in the context of a living cell by monitoring in real-time the changes in the cytosolic calcium levels ($[Ca^{2+}]_{CYT}$). We tested the influence of drugs that change the actin and microtubules polymerization state on the activity and membrane expression of the PMCA transiently expressed in human embryonic kidney (HEK293) cells. We found that disrupting the actin cytoskeleton with cytochalasin D significantly increased PMCA-mediated Ca^{2+} extrusion ($\sim 50\text{-}100\%$) at the time that pre-treatment with the F-actin stabilizing agent jasplakinolide caused its full inhibition. These results are in full agreement with our previous *in vitro* observations. When the microtubule network was disrupted by pretreatment of the cells with colchicine, we observed a significant decrease in PMCA activity ($\sim 40\text{-}60\%$ inhibition) in agreement with the previously reported role of acetylated tubulin on the calcium pump [3]. In none of these cases we observe a difference in the level of expression of the pump at the cell surface. Taken together, these data demonstrate that our and other's previous observations on the *in vitro* effect of the actin and tubulin cytoskeleton on PMCA activity is also evident in a living cell model.

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Submission ID 272 – **Electronic Poster**
Membrane Transporters and Channels

SOCE is modulated by AQP2 and TRPV4 affecting both Ca^{2+} influx and clearance in renal cells

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Store-operated Ca^{2+} entry (SOCE) is a homeostatic process regulated by the filling state of the intracellular Ca^{2+} stores. This mechanism is strongly influenced by changes of the driving force for Ca^{2+} influx. In addition, SOCE is modulated through the plasma membrane Ca^{2+} ATPase (PMCA)-mediated cytosolic Ca^{2+} [Ca^{2+}]_i clearance with important consequences in downstream signaling pathways¹. In a previous communication we described that AQP2 can modulate SOCE by interacting with TRPV4, affecting K^+ channels and in consequence membrane potential (V_m). The aim of the present work was to further characterize if SOCE modulation by AQP2 depends on changes of TRPV4 localization, the driving force for Ca^{2+} entry and Ca^{2+} clearance rates. We used two renal cell lines; one not expressing AQPs (WT-RCCD1) and another one transfected with AQP2 (AQP2-RCCD1). We performed immunofluorescence studies to determine if Ca^{2+} store depletion with thapsigargin (1 μM) can change subcellular TRPV4 local-

ization. Also, FURA-2 and DIBAC4(3) dyes were used to monitor $[Ca^{2+}]_i$ and V_m changes respectively. We found that after Ca^{2+} store depletion, TRPV4 was enriched only in the membrane of cells expressing AQP2. In addition, SOCE sensitivity to changes in the driving force was evaluated by varying sequentially either the electrical (high extracellular K^+ medium, HK) or the chemical (low extracellular Ca^{2+} medium, LC) driving force for short periods in the same cells. WT cells responded strongly reducing $[Ca^{2+}]_i$ but surprisingly, AQP2 cells showed a minor reduction on $[Ca^{2+}]_i$ upon depolarization following introduction of HK medium. Contrarily, both cell lines respond similarly to LC medium. Experiments designed to evaluate Ca^{2+} clearance revealed that AQP2 expressing cells remove Ca^{2+} from cytosol slower than WT cells. These results suggest that AQP2 can modulate SOCE by influencing either Ca^{2+} influx and Ca^{2+} clearance from cytosol.

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Submission ID 276 – Poster
Membrane Transporters and Channels

Analysis of α helices, extracellular loops and pore region in human aquaporin-1 by molecular dynamics

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Human aquaporin-1 (hAQP1) is an integral membrane protein that transports water. In previous work, we demonstrated that the permeability of hAQP1 is modulated by the tension of the plasma membrane. To investigate whether that modulation is related to the dynamic conformation of the pore, the extracellular loops and/or the bending of α helices, the information of two crystalline hAQP1 structures (1FQY and 4CSK) was used. We carried out molecular dynamics (MD) in three different conditions: a) tetramer with restriction in those α -carbons ($C\alpha$) that would be in contact with the lipid bilayer (TR); b) tetramer without any further restrictions that those provided by the tetramerization itself (TL); and c) unrestricted monomer (ML). Several descriptors were used for analysis, such as RMSD, the number of water molecules in the pore, modifications in estimated diameter along pore sections, the

Solvent Accessible Surface Area (SASA) in residues of the NPA and ar/R sites, the geometry of helices and loops, harmonic analysis using Fourier transform and radial distribution function ($g(r)$) of water molecules measured in residues influenced by movements of loop A. Principal Component Analysis (PCA) was performed. The results observed were: 1) the contribution of restricting $C\alpha$ differs from the contribution of tetramerization itself; 2) the tetramerization does not prevent displacement of hydrophilic loops to regions that would not be accessible in the presence of the lipid bilayer; 3) individual monomers behave differently within TL and TR; 4) At level of the pore, no descriptor per se explains the differences observed between TL and TR; 5) localized restrictions in TR cause modifications in far and not restricted sites inside the pore; 6) the distinct behavior shown by H1, H3 and H6 helices becomes the subject of future studies, in search for a possible sensor of the membrane tension that could influence the permeability of the pore.

Submission ID 412 – **Electronic Poster**
Membrane Transporters and Channels

Gating Currents of Voltage-gated Proton Channel

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Voltage-gated ion channels are able to sense voltage through a charged alpha helix in the voltage-sensing domain. The movement of this voltage sensor across the electric field produces a small transient current known as gating currents, which appears as a non-linear component of the capacitive currents. Although voltage-gated proton channel (Hv1) has a voltage sensor homologous to other voltage-gated channels, and a movement of it in response to depolarizing pulses have been demonstrated by solvent accessibility assays and fluorescence (Gonzalez et al., 2010; Qiu et al., 2013), measurements of its gating currents have been difficult. One of the main problems to perform these measurements is to decrease proton currents to analyze gating currents in isolation. The understanding of some properties of Hv1 as the coupling between voltage-sensor domain and permeation pathway, and its pH dependence of gating could be achieved measuring gating currents of Hv1. To study isolated gating currents of Hv1, we used

a low conducting mutant and *Xenopus* oocytes as heterologous expression system. We have been able to measure asymmetrical capacitive currents of this mutant in inside-out macropatches. These non-linear capacitive currents were recorded only when depolarizing pulses were applied. Although proton currents were not completely removed, they were present as a small stationary component at depolarizing voltages, allowing a better isolation of gating currents. Kinetics of the on and off gating currents were clearly different, with the on slower than the off currents. Using this mutant, Hv1 gating currents can be measured almost isolated from proton currents, which will allow characterizing them in a better way to improve understanding of how Hv1 works.

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Submission ID 300 – **Electronic Poster**
Membrane Transporters and Channels

Hydroxychloroquine inhibits Calcium dependent Potassium channels in THP-1 macrophages.

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The inflammasome is a multiprotein oligomeric assembly with a critical role in the activation of inflammatory responses. Its exact composition depends on the activator that initiates inflammasome assembly. NLRP3 inflammasomes are those that contain the NALP proteins encoded by the NLRP3 gene, involved in the innate immune response. We have used THP-1 human macrophages stimulated with ATP, to explore the role of calcium dependent potassium currents in the formation of those inflammasomes. Whole-cell patch clamp was performed on THP-1 cultured macrophages with an intracellular solution containing aprox. 1 μM free Calcium (calculated with winmaxchelator). Outward Currents with a half activation potential of -10 mV were recorded in these conditions. The outward currents were blocked or inhibited by the presence of either TEA 20-50 mM, BaCl_2 10-30 mM. In addition they almost disappeared when 1 μM BAPTA-AM was added to the bath. Iberiotoxin 50-100 nM blocked most of these currents. These currents were

also blocked almost completely by hydroxychloroquine (HCQ) 30 μ M, an antimalarial drug widely used in the treatment of autoimmune diseases and for the prevention of malaria in endemic regions. Incubation of THP-1 macrophages with HCQ in the same concentration range, acutely inhibited the formation of the inflammasome. Taken altogether, our results suggest that one of the mechanisms involved in the prevention of the formation of the inflammasome by HCQ, is through direct or indirect inhibition of calcium-activated potassium channels in THP-1 macrophages.

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Submission ID 301 – Poster
Membrane Transporters and Channels

Coupling between voltage and calcium sensors in high conductance voltage- and calcium-activated K⁺ (BK) channels

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High conductance calcium- and voltage-activated potassium channels (BK) channels are ubiquitously expressed, but its activity is modulated by β -subunits ($\beta1$ - $\beta4$) in a tissue-specific manner, conferring specific features to channel activity for particular physiological demands. The $\beta1$ subunit increase the apparent Ca²⁺ sensitivity of BK by the stabilization of the voltage sensor domain (VSD) equilibrium in its active configuration. The purpose of this study is to determine the effects of β -subunits on the gating charge in the presence of Ca²⁺, and to evaluate the coupling between Ca²⁺-binding and VSD equilibrium. The number of gating charges per channel was measured in BK channels formed by α subunit alone and with the different β -subunits in Ca²⁺-free internal solutions. The maximum gating charge displaced was obtained from the charge-voltage (Q-V) curve and the total number of channels in the patch was determined using noise analysis. Furthermore, we evaluated the effect of β -subunits on the interaction between Ca²⁺ sensors and

VSD in different Ca^{2+} concentrations. We found that the total number of charges per channel was 4.4, 3.0 and 4.2 e_0 for $\text{BK}\alpha$, $\text{BK}\alpha/\beta 1$ and $\text{BK}\alpha/\beta 3b$ channels respectively. Increasing intracellular $[\text{Ca}^{2+}]$ in $\text{BK}\alpha$ (100 μM) promotes a significant leftward shift (~ -140 mV) of the Q-V curve. The calcium effect on voltage sensor in $\text{BK}\alpha$ becomes apparent at $[\text{Ca}^{2+}] \geq 1$ μM ($\sim \Delta V -30$ mV). However, the leftward shift of the Q-V curve in $\text{BK}\alpha/\beta 1$ channels becomes evident in the nanomolar $[\text{Ca}^{2+}]$ range (100-500 nM). $\text{BK}\alpha/\beta 3b$ channels behave as $\text{BK}\alpha$ channels. We conclude that $\beta 1$ -subunit in addition to modify the resting-active equilibrium of the voltage sensor, also decreases the total number of apparent gating charges needed for channel activation; and that there is a strong coupling between voltage and Ca^{2+} sensors, which increased in the presence of $\beta 1$ -subunit.

Submission ID 422 – Poster
Membrane Transporters and Channels

BK channel role in the modulation of mesenchymal stem cells by platelet lysate

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The use of Mesenchymal Stem Cells (MSC) in cell therapy has been explored because of their properties of proliferation, migration and differentiation to diverse lineages¹. It is known that platelet lysate (PL) modulates those processes¹. However, there is scarce information about the role of ion channels in that effect. In this study, we analyzed the role of the big conductance potassium channel, BK, in the modulation of rat MSC (rMSC) by PL. rMSC treated with PL 5% v/v showed an increase in cell migration, that was avoided by treatment with the BK channel antagonist, Iberiotoxin (IBTX, 10nM). Changes in cellular viability were not observed after treatment with IBTX. The BK channel agonist NS1619 (20µM) did not prompt any modification in the effect on cell migration induced by PL. Activity of BK channel was analyzed using patch-clamp technique in whole cell configuration. We found

cells that do not expressed BK channels and cells with BK channels currents which showed an increase in current density after 24 hour of treatment with PL. Although there were currents different to evocated by BK channel, there was a relation between the increase in current density and the rise in BK channel currents. Flow cytometry assays showed that the increase in BK channel activity induced by PL was not associated with changes in the expression of BK channel subunits analyzed (α , $\beta 1$, $\beta 2$ y $\beta 4$). Results suggest that BK channel activity is related to the effect induced by PL in rMSC. Those effects would be associated with changes in biophysical properties of the channel that induce an increase in open probability of the BK channel. However, it is necessary to do more electrophysiological recordings to improve the characterization of biophysical properties of the BK channel after treatment with PL.

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Submission ID 152 – **Electronic Poster**
New and Notable

Effects of the manipulation of ligand mobility and geometry on the cytotoxic synapse

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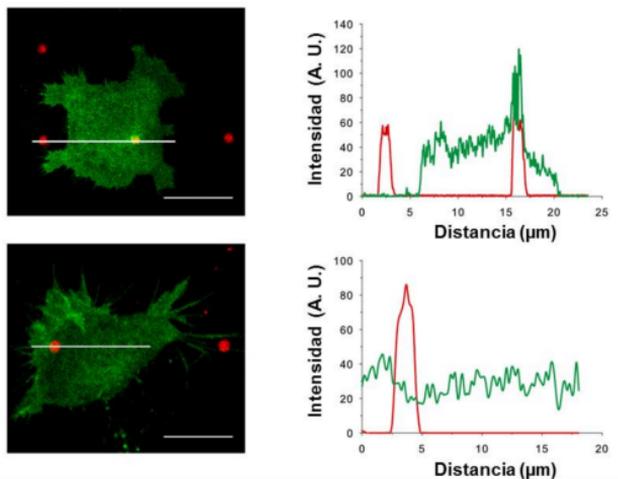
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Cells respond to many external stimuli through membrane receptors. Among the major programmed cell death pathways, Fas mediated apoptosis (extrinsic apoptosis) is one of the most important. The signaling events mediated by Fas receptor have been extensively studied by adding a soluble agonist to the cells culture medium. That is to say, by stimulating the cells in a way that is spatially isotropic. In this work, we utilize receptor Fas activation as a tool to approach a more general question: which is the response of a cell to stimuli that are chemically identical but spatially/geometrically/kinetically diverse?

To tackle this question we generated functionalized surfaces that simulate the plasma membrane of a cytotoxic T lymphocyte with controlled geometry and fluidity.

Our results show that there is a remarkable dependence of the cells apoptotic response with the mobility of the ligand. The response is also dose-dependent. Thus, the spatial distribution and mobility of the stimulus can modulate the apoptotic cell response. Also, the activation of Fas receptor occurs in an anisotropic way, (initially) only in the point of contact between the cell and the artificial membrane, showing a significant difference in both receptor clustering and receptor diffusion in that point in comparison with other parts of the cell membrane.



Submission ID 166 – **Electronic Poster**
Biological Fluorescence and Microscopy

Modifications in membrane potential and permeability by expression of suicide probes EtpM-bacteriocin and its immunity protein on *E. coli*.

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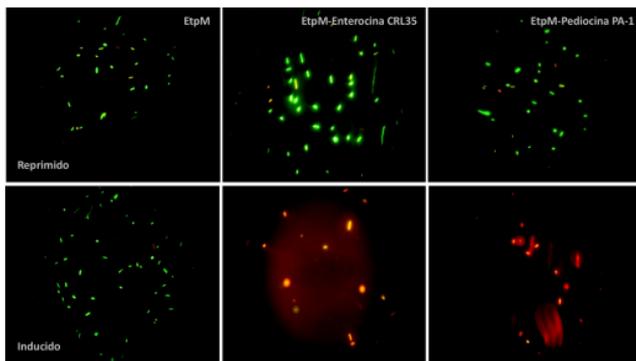
Enterocin CRL35 and Pediocin PA-1 are pediocin-like bacteriocins that act on Gram-positive bacteria membrane. In order to study the mechanism by which they induce loss of membrane integrity, in previous work we constructed "suicide probes": fused genes of bacteriocins with EtpM, a bitopic membrane protein. We demonstrated that these bacteriocins are able to exert a bactericidal effect on Gram-negative bacteria when anchored in the membrane, regardless of their specific receptor (Man-PTS system).

The aim of this study was to evaluate the effect of these bacteriocins on cell membrane potential and permeability, when expressed as suicide probes in Gram-negative bacteria such as *E. coli*.

On the one hand, we employed the potentiometric indicator DiSC3(5), a fluorophore that exhibits changes in fluorescence intensity. These

changes are dependent on transmembrane potential, and they are feasibly to be measured over time. The results showed that the expression of fusions EtpM-bacteriocins generates potential dissipation by membrane depolarization. In contrast, neither the control strain that expressed only EtpM (membrane anchor), nor the strain that co-expresses the suicide probe with Enterocin CRL35 immunity protein (MunC) showed a significant change in membrane potential.

On the other hand, we used LIVE/DEAD BacLight kit, which includes two fluorophores that penetrate the membrane according to its integrity. This allows to discriminate between live and dead cells. The images obtained by fluorescence microscopy clearly evinced that suicide probes expression disturbs membrane permeability, so bacteria emitted red fluorescence (dead cells), while control and co-expressing EtpM-bacteriocin / MunC strains conserve membrane integrity, so they emitted green fluorescence (live cells).



Submission ID 244 – Poster
Biological Fluorescence and Microscopy

Transcription factors dynamics in stem cells using fluorescence correlation spectroscopy (FCS)

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Embryonic stem cells (ESC) can differentiate to cells derived from all three germ layers and have an unlimited possibility of self renewal. These properties determine the relevance of ESC studies for both, understanding embryo development and applications in regenerative medicine. Pluripotency depends on specific transcription factors (TFs), such as Oct4, Sox2 and Nanog. They induce genes necessary to preserve undifferentiated state and repress genes related to differentiation.

The transcription machinery and regulatory components are recruited dynamically on their target genes. This means that the dynamics of interaction plays a fundamental role in the different stages involved in gene expression. Moreover, these interactions are modulated by the local plasticity of the chromatin.

In this work we explore the dynamics of TFs relevant for pluripotency in ESC. With this aim we performed fluorescence correlation spectroscopy

(FCS) in ESC transfected with vectors encoding TFs fused to either enhanced or photoactivatable green fluorescent protein (GFP). To correlate the dynamics of the TFs with the chromatin organization, we co-transfected the cells with chromatin associated proteins fused to red fluorescent proteins.

Submission ID 245 – **Electronic Poster**
Biological Fluorescence and Microscopy

Intracellular dynamics of kinesin-1 molecular motors engaged in mitochondrial transport

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Intracellular transport mediated by molecular motors is essential for most biological functions. These proteins bind to a wide variety of cellular cargoes and step along cytoskeletal filaments using energy provided by ATP hydrolysis. Two families of molecular motors drive transport along microtubules in a bidirectional manner: dyneins walk towards the minus ends located near the nucleus, whereas kinesins walk towards the plus ends at the periphery of the cell. These opposite-polarity motors compete with each other to determine the direction of motion. Recent theoretical works showed that key aspects of the transport are finely tuned by spatial distribution of motors on the organelle membrane. However, there are not experimental evidences of how motors organize on organelles in living cells.

In this work, we explored this issue in *Drosophila* S2 cells expressing a EGFP-labeled kinesin-1 variant. Using confocal laser scanning microscopy, we registered line-scans to recover simultaneously the movement of fluorescent mitochondria along cell processes and the fluorescence of kinesin motors at the organelle with high-temporal resolution. A combined single particle tracking and fluorescence correlation analyses allowed us to determine the dynamics of motors in different regions of the organelle. According to our analysis, motors engaged in organelle transport display different dynamics depending on: 1) the directionality of the organelle being transported (minus-end or plus-end directed), and 2) the position within the organelle where the motors are located (rear-end or leading-end).

Submission ID 246 – Poster
Biological Fluorescence and Microscopy

Active Forces in Living Cells Shape the Microtubule Network

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Microtubules (MT) are involved in relevant cellular tasks including active transport, cell division and maintaining cell shape. These filaments are the stiffest in the cytoskeletal family with persistence length of the order of millimeters. The MT network is highly dynamic, undergoing constant remodeling through polymerization and depolymerization of filaments, translation and bending by the action of molecular motors. All these active processes generate forces that result in an apparent smaller rigidity in comparison to that measured *in vitro*. In this work we study how MT associated forces and cellular environment affect filament shape and rigidity.

Using confocal microscopy and computational tools we were able to observe and track individual, EGFP-labeled MTs in living *Xenopus laevis* cells. A Fourier analysis of the microtubule shapes showed that the curvatures followed a thermal-like distribution as previously reported im-

plying a balance of active forces. This behavior was conserved even after the disruption of actin or vimentin filaments suggesting that these filaments are not involved in balancing the forces on the filaments. We inhibited MT growing/shrinkage by treating the cells with vinblastine and observed that the curvatures did not follow the apparent thermal equilibrium. Also, MTs bend over longer distances in the treated cells. We conclude that polymerization/depolymerization are required to achieve a thermal-like balance of forces on the microtubule network and the elimination of these forces results in filaments with a built-up tension prone to buckle and present higher curvatures.

Submission ID 251 – **Electronic Poster**
Biological Fluorescence and Microscopy

Mechanical tension across focal adhesion protein vinculin in normal and tumoral human breast cells

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Mechanical forces play an important role in the organization, growth, maturation, and function of living tissues. Cells sense the extracellular environment using transient macromolecular assemblies, called focal adhesions (FAs) that physically connect the extracellular matrix to the actin cytoskeleton through integrin receptors. FAs exhibit mechanosensitive properties, they respond to internal and external mechanical stresses. In particular, it has been seen, that the FA protein vinculin is crucial for the ability of cells to transmit forces and to generate cytoskeletal tension¹, and is involved in regulating cell adhesion and motility².

In this work we use a FRET (Förster resonance energy transfer)-based force/tension sensor that reports changes in tension-induced strain within the FA protein vinculin³. Employing this sensor we investigated the tension across vinculin within individual FA in two different human breast epithelial cell lines: MCF10A, normal-like breast

cells and MCF7 breast tumor cells. In order to quantify and characterize FA tension, we developed and implemented a FA segmentation routine combining OTSU threshold method, with the watershed method to optimize automatic single FA individualization. Statistical analysis reveals the existence of FA subpopulations exhibiting high and low levels of vinculin tension in both cell lines. We found that non tumoral cell line, MCF10A, presents a larger subpopulation of low tension FA, while the high tension FA subpopulation is greater in MCF7, tumorigenic cell line.

Acknowledgements: CONICET, ANPCyT and UBA.

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Submission ID **166** – **Poster**
Biological Fluorescence and Microscopy

New fluorescent biosensors for dissecting major signaling pathways

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apoptosis involve control mechanisms mediated by molecular transport and/or the transduction of chemical energy, like redox and nucleotides phosphate molecules. Recent reports indicate a crosstalk between different signals that operate in a highly dynamic and coordinated fashion to achieve a fine-tuning of cellular responses. For instance, cAMP and cGMP are major second messengers that regulate a diversity of cellular processes by acting on kinases with opposing activities. Interestingly, components of different signaling pathways are redox controlled by the glutathione/glutaredoxin system. Developing tools for the *in vivo* detection of these signals on real time and by non-invasive methods is necessary to pinpoint molecular targets and design strategies for therapeutic intervention. In this context, genetically engineered fluorescent sensors are amongst the most powerful tools to quantify *in vivo* the spatiotemporal dynamics of biochemical events. In addition, computational tools together with advanced simulation techniques allow for a quantitative

prediction of the mechanistic details of the proteins used as biosensors. In our work, we developed a novel biosensor for the measurement of intracellular cNMP levels based on the computer-aided design of a new protein architecture, which was characterized biochemically and biologically in cellular systems of biomedical relevance. The new biosensor is a single polypeptide with the cNMP binding domain coupled to two fluorescent proteins acting as FRET pair and was study in detail by spectrofluorimetry. We also generated transgenic reporter cell lines, useful to study host-pathogen interaction and to unravel the mode of action of drugs that were characterized by multiparametric flow cytometry. This biosensors and reporter cell lines promise to become valuable tools for high-content analysis on different fields of fundamental and applied research.

Submission ID 420 – Poster
Biological Fluorescence and Microscopy

Imaging biological nanostructures with super-resolution fluorescence microscopy

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Biological nanostructures that are indistinguishable with conventional fluorescence microscopes become observable by means of methods known as super-resolution fluorescence microscopy or optical nanoscopy [1], which enable imaging with theoretically unlimited spatial resolution. In practice, the achieved resolution is limited to 20 to 50 nm due to experimental factors. Here, we present two nanoscopes designed and constructed at CIBION that deliver super-resolved images of biological specimens through Stochastic Optical Reconstruction Microscopy (STORM) and Stimulated Emission Depletion (STED), respectively.

We explain the working principle of the nanoscopes and illustrate their performance with super-resolved images of i) periodic nanometric structures of actin and spectrin in the subcortical skeleton of neuronal axons and ii) domains of mucins and trans-sialidase on the membrane of *T. cruzi*.

Acknowledgments: This project was funded with the support of CON-ICET and ANCYPT grants, and a Partner Group of the Max-Planck-Society.

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Submission ID 182 – **Electronic Poster**

Mathematical Modeling and Computational Biophysics

GABERGIC PHENOLS ARE CAPABLE TO MODULATE LIPID PHASE TRANSITION RESEMBLING PROPOFOL BEHAVIOR.

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GABA_A receptor (GABA-R) is the main inhibitory receptor of the central nervous system. Its ligands include drugs other than the GABA neurotransmitter, such as benzodiazepines, barbiturates, anesthetics, ethanol and the convulsant picrotoxinin, which behave as allosteric modulators or channel blockers. There is still considerable debate about molecular mechanisms by which general anesthetics induce sedation. Since many lipophilic compounds that regulate GABA-R function can change the physical properties of the lipid bilayer, it is expected that the receptor could be modulated not only by the specific ligand recognition, but also by changes in the physical state of the membrane. Previous experimental results from our group have demonstrated that the intravenous anesthetic propofol, and other derived phenols with gabaergic activity, are able to interact with membranes. NMR, fluorescence anisotropy and Langmuir films studies indicated that they locate in the region between the polar group and the first atoms of the acyl chains

of phospholipid membranes (1). In the present work, we obtained spatially resolved free energy profiles of propofol and GABAergic phenol's partition into DPPC bilayers. These profiles allowed us to determine the most probable phenols-DPPC interaction site. Also, free diffusion Molecular Dynamics simulations of DPPC in presence of the different phenols were used to analyze their interaction with a bilayer. These studies revealed a tighter packing in the hydrocarbon chains of the DPPC in presence of these compounds. The simulations revealed that GABAergic phenols have a cholesterol-like ordering effect on DPPC in the fluid phase, as proved before for Propofol (2).

Acknowledgements: This work was supported by grants from SECYT-UNC, FONCYT-MinCyT and CONICET.

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Submission ID 183 – Poster

Mathematical Modeling and Computational Biophysics

**PARTITION INTO DPPC BILAYERS OF
DIHYDROPYRIMIDINE ANALOGUES WITH LARVICIDAL
ACTIVITY.**

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Two recently synthesized dihydropyrimidines (DHPMs) analogues have demonstrated larvicide and repellent activity against *Anopheles arabiensis*. DHPMs high lipophilicity suggests that these compounds may interact directly with the membrane and modify their biophysical properties. Experimental results indicate that their presence between lipid molecules would induce an increasing intermolecular interaction, diminishing the bilayer fluidity mainly at the polar region (Sanchez-Borzone et al. unpublished results). Spatially resolved free energy profiles of DHPMs partition into a DPPC bilayer in the liquid-crystalline phase were obtained through PMF calculations using an umbrella sampling technique as a function of the distance to the center of the bilayer along its normal axis z [$\Delta G(z)$]. In addition, we performed free diffusion MD simulations to gain insight into the specific interactions of each compound with the bilayer, and analyzed the chemical groups that

interact when DHPM enters the bilayer following the variation of the minimum distance among these groups. In agreement with the experimental assays, PMF profiles and MD simulations showed that DHPMs are able to partition into DPPC bilayers; penetrating into the membrane and establishing hydrogen bonds with the carbonyl moiety and interacting in less extent with phosphate groups. Although the increase in order parameter values was mild, the presence of DHPMs molecules induced a more order state in hydrocarbonate chains.

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Submission ID **224 – Poster**

Mathematical Modeling and Computational Biophysics

Function and coiled-coil interactions in the cytoplasmic domain of Tsr chemoreceptor and their variants

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When swimming in a chemoattractant gradient, bacteria perform a temporal comparison of the molecular concentration via its membrane-bound chemoreceptors (e.g. Tar, Tsr) that act on the Che pathway modulating the switching frequency of the flagellar motors, which in turn sets the tumbling frequency. Therefore, runs are in average longer in the favorable direction and the resulting biased random walk drives the bacterium up the chemoattractant gradient. Beyond the periplasmic ligand-binding domain, the cytoplasmic region consist of a HAMP domain followed by an α -helical hairpin that forms in the dimer a coiled-coil four helix bundle. The signal transmission through this structure is

an open question. Experiments to analyze the activity of different variants ("constructs") of the cytoplasmic domain of this chemoreceptor (e.g. mutations, heptad deletions, insertions) had been performed[1] to clarify the mechanism of signal transmission. We have obtained one hundred 3D structures of each of the WT dimeric helical hairpin Tsr and several constructs by comparative modeling. We analyzed over each of these sets the physicochemical effects of the modifications of the wild sequence using the knob-into-hole paradigm of coiled-coil structures, the comparison among the root mean square fluctuations of the structures and the contributions to the desolvation and electrostatic free energy terms of pairs of residues in each construct. The sampling over a set of 100 structures for each construct allows for the calculation of the likelihood of existence of knobs making the analysis more robust. We found that some constructs that loses their kinase activation ability lack the Q298, L302, V316 and N319 conserved knobs. Concomitantly the likelihood of knobs in the protein interaction region is enhanced. The V347M mutation that restores the kinase activation ability on one construct, shows a lower probability of knobs in the protein interaction zone.

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Submission ID 230 – **Electronic Poster**

Mathematical Modeling and Computational Biophysics

Potential of Mean Forces of a Mg^{2+} along a possible pore in the 5-fold symmetry axis of Triatoma Virus.

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Triatoma Virus (TrV), is an insect virus belonging to the Dicistroviridae family which infects several species of triatomine insects. Since these insects are vectors for human trypanosomiasis, commonly known as Chagas disease, TrV is proposed as a biological control against this vector. In our research we study the capsid of TrV, since viral capsid plays an intrinsic role in the process of viral infection. Particularly we study the role played by the 5-fold symmetry axis in this process.

In previous works we observed that the hydration process of the pore present in the 5-fold symmetry axis was accomplished thanks to the

presence of a magnesium ion inside the pore. In this work, we calculated the potential of mean forces (PMF) of a Magnesium ion traversing the pore. To accomplish this result we used the Umbrella Sampling method.

The results we obtained agrees well with our previous results were we studied the water solvation of the pore in the presence of a Mg^{+2} ion, as well as under different mutations that changed the properties of the aminoacids lining the pore. It also agrees well with the measure radius along the pore. Taking all in account, the obtained result agrees well with our hypothesis on how and why a magnesium ion could enter the pore and its relevance in the process of hydration.

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Submission ID 232 – **Electronic Poster**

Mathematical Modeling and Computational Biophysics

Molecular dynamics simulations study of the conformational changes involved in vinculin activation

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Vinculin is an important protein component of focal adhesion, where it plays crucial roles in the regulation of cell adhesion and migration. The crystal structure of vinculin shows a globular protein with 5 helical domains, the 1 to 4 domains (D1-D4) form the vinculin head domains (Vh) and the fifth is the tail domain (Vt/D5). Domains Vh and Vt are connected by a proline-rich linker region.[1] Among vinculin's various binding partners onto all three regions of the protein we can find: talin, $\hat{I}\pm$ -actinin, actin, paxillin, phosphatidylinositol 4,5-bisphosphate (PIP2) and vasodilator-stimulated phosphoprotein (VASP). [2] In solution, vinculin presents a closed conformation with Vt bound to Vh domains. The activation of vinculin requires conformational changes, from a closed conformation to an activated one, in which the occluded binding sites of its different ligands become exposed. It has also been demonstrated that for these changes to occur vinculin must be bound to two or more of its ligands.

Several mechanisms of activation were proposed; binding to only one ligand; simultaneous binding with different ligands; vinculin phosphorylation and force-dependent mechanism, but the details of this conformational change are not yet established.

In this work, we implemented molecular dynamics simulations to study the mechanism of vinculin activation. In particular, we analysed conformational changes involved in vinculin activation and necessary to allow the interactions between D1 vinculin domain with vinculin binding site (VBS) of talin.

Acknowledgements

CONICET

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Submission ID 234 – **Electronic Poster**

Mathematical Modeling and Computational Biophysics

Interactomic analysis of pro-apoptotic members of Bcl-2 protein family

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There are two main ways in which the cell can undergo apoptosis; the extrinsic apoptotic pathway which starts after activation of death receptors expressed on the cell surface; and the intrinsic pathway, which is activated by a signal of cellular stress and is regulated primarily in mitochondria by the family of Bcl-2 proteins. Interactions between members of the Bcl-2 family include both protein-protein interaction and protein-membrane interaction. Some of the proteins belonging to the Bcl-2 family undergo conformational changes and exposure of hidden domains, allowing activation, binding to the mitochondrial outer membrane and its subsequent permeabilization. These processes allow apoptogenic factors release and caspase activation, thus coordinating the destruction of the cell.

Bax and Bid, pro-apoptotic members of the Bcl-2 protein family, have a pivotal role in the process of MOMP (mitochondrial outer membrane permeabilization). However the mechanisms involved require a better

understanding of the complex behavior of the members of this important protein family.

To determine whether electrostatic interactions can influence the way Bax and Bid interact with each other and with membranes, we computationally modeled the interaction of Bax and Bid with both anionic and neutral membranes. Free Electrostatic Energy of Binding, was computed using FDPB method as implemented in software APBS (Adaptive Poisson Boltzmann Solver). Based on the computational analysis, it was found that recruitment to membranes is facilitated by non-specific electrostatic interactions. This type of calculations provided a starting point for further computational analysis through molecular dynamics simulations (MD). To this end, we used GROMACS simulation package. Molecular dynamics simulations showed the possible relative orientation and which amino acid residues are important for protein-protein and protein-membrane interactions, providing information for experimental analysis.

Submission ID 236 – **Electronic Poster**

Mathematical Modeling and Computational Biophysics

Martini as a tool to study molecular steering in coarse grain simulations

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The kinetics of macromolecular interactions, specially between proteins or proteins and DNA, usually overcome the diffusion (Smoluchowski) limit through several mechanisms (1). One of these, known as electrostatic enhancement or molecular steering, postulate that proteins evolve their surrounding electrostatic fields well beyond their solvent accessible surfaces orienting the approaching macromolecular partner, facilitating in a long range the occurrence of effective encounters. Due to the difficulty to study this mechanism *in vitro*, several computational approaches have been developed. Since the space of orientations encounter is so huge, coarse graining of the simulations is mandatory, and in many of them macromolecules are treated simply as Brownian rigid bodies. The Martini forcefield has proved very valuable to study large systems involving membranes, proteins and coarse-grain water models (2). But since it was not developed to study protein interactions, recent modifications of the forcefield have been developed to reproduce characteristic constants associated to the diffusion of proteins and their

interactions (3). Our aim is to extend this effort to include electrostatic long range interaction and thus reproduce electrostatic enhancement in protein interactions. Our results demonstrate that when introducing Particle Mesh Ewald (PME) summation to simulate long range interaction, electrostatic enhancement can be observed in well studied model systems as barnase-barstar. Thus the advantages of Martini as a molecular toolkit can be extended to the study more realistic protein interactions.

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Submission ID **240** – **Poster**

Mathematical Modeling and Computational Biophysics

The water to solute permeability ratio governs the osmotic volume dynamics in beetroot vacuoles

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Plant cell vacuoles occupy up to 90% of the cell volume and are constantly subjected to water and solute exchange. The osmotic flow and vacuole volume dynamics relies on the vacuole membrane -the tonoplast- and its capacity to regulate its permeability to both water and solutes. The osmotic permeability coefficient (Pf) is the parameter that better characterizes the water transport when submitted to an osmotic gradient. Usually, Pf determinations are made *in vitro* from the initial rate of volume change, when a fast (almost instantaneous) osmolality change occurs. When aquaporins are present, it is accepted that initial

volume changes are only due to water movements. However, in living cells osmotic changes are not necessarily abrupt but gradually imposed. Under these conditions, water flux might not be the only relevant driving force shaping the vacuole volume response. In this study, we quantitatively investigated volume dynamics of isolated *Beta vulgaris* root vacuoles under progressively applied osmotic gradients at different pH, a condition that modifies the tonoplast Pf. We followed the vacuole volume changes while simultaneously determining the external osmolality time-courses and analyzing these data with mathematical modelling. Our findings indicate that in these conditions, vacuole volume changes would not depend on the membrane elastic properties, nor on the non-osmotic volume of the vacuole, but on water and solute fluxes across the tonoplast. We found that the volume of the vacuole at the steady state is determined by the ratio of water to solute permeabilities (P_f/P_s), which in turn is ruled by pH. The dependence of the permeability ratio on pH can be interpreted in terms of the degree of aquaporin inhibition and the consequently solute transport modulation. This is relevant in plant organs such as root, leaves, cotyledons or stems that perform extensive rhythmic growth movements, which involve considerable cell volume changes within seconds to hours.

Submission ID 241 – Poster

Mathematical Modeling and Computational Biophysics

**PATHOLOGIC RELEVANCE OF MOLECULAR STRUCTURE
ANALYSIS OF ISOFORMS OF GLYCEROL 3- POSPHATE
DEHYDROGENASE**

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Glycerol 3-phosphate dehydrogenase catalyzes the reversible conversion reaction dihydroxyacetone phosphate to glycerol 3-phosphate (G3P). Both compounds are intermediates of important metabolic pathways such as glycolysis and triacylglycerol synthesis. They exist in invertebrates and vertebrates with two predominant subcellular locations: cytosolic (GPD1-L) and mitochondrial. Both have a growing number of isoforms involved in the crossroads in the metabolism of lipids and carbohydrates and with providing reducing equivalents from glycolysis to the electron transport chain. This is a report of the in silico modelling and analysis of the structures of normal cytosolic isoforms GPD1 (wt-crystal) Transient Children Hyperlipidemia (substitution R229P, liver enzyme FASTA sequences) GPD1L (wt-crystal) and related Brugada syndrome (substitution A280V- FASTA sequences) obtained from epidemiological and experimental papers. The structures and oligomerization behavior from Swiss Model, I Tasser and Phyre 2,

GROMOS 43 and 96 force fields, allowed to determine the interaction distances of monomers with SCN5A sodium channel (GPD1L wt: 15.0 to 3.3 Å, A280V mutant: 7.3 to 4.5 Å) and binding free energies with NAD (GPD1L wt: ~ -7 , A280V ~ -12 , R229P: ~ -14 kcal / mol) with Autodock Vina, Patch Dock, Swissdock and Dock Blaster. The results support the hypothesis that GPD1 A280V reduces SCN5A sodium currents since it would be located closer and with less binding free energy than wt preventing the interaction with proteins such as calmodulin activator and R229P GPD1 mutant showed higher affinity for NAD than wt and consequently it should improve the formation of G3P and the synthesis of triacylglycerides contrary to the leading interpretations which assume that the mutant isoform present minor affinity.

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Submission ID 249 – Poster

Mathematical Modeling and Computational Biophysics

On the misuse of the Jarzynski equality in biomolecular simulations

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We derive the maximum-likelihood free energy estimator ΔFFD from the Jarzynski equality. Considering Gaussian work distributions from both numerical and molecular dynamics simulations of biomolecules, we illustrate the striking inaccuracy of the ΔFJ estimator. Our results are consistent with recent findings that highlight the exponential increase in the number of trials needed to converge the ΔFJ estimator with an increase variance of the work distribution, or the dissipated work. We conclude that the maximum-likelihood free energy estimator ΔFFD ,

though both accurate and efficient, is currently under-appreciated in routine free energy calculations in simulations of biomolecules.

Submission ID **500** – **Poster**

Mathematical Modeling and Computational Biophysics

**SMALL PEPTIDES DERIVED FROM PENETRATIN AS
ANTIBACTERIAL AGENTS**

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Penetratin is a synthetic small cationic peptide possessing 16 amino acids which might penetrate cell membrane. Our research group was the first to report the antimicrobial activity of penetratin and some structurally related peptides [1-3]. We report here the antibacterial activity of new peptides structurally related to penetratin and an exhaustive conformational analysis as physicochemical properties of these peptides may play a key role in producing their antibacterial effects.

Minimal inhibitory concentration values were determined using the broth microdilution method according to the protocols of the CLSI. Conformational analysis was performed using the GROMACS programs package and circular dichroism spectroscopic measurements. Two different media (water and trifluoroethanol/water) were employed.

In vitro antibacterial effects and conformational study of 13 small-size peptides were accomplished. These were found to be very active antibacterial compounds, considering their small molecular size. Theoretical simulations showed that peptide helical structure is destroyed using the matrix-mimetic environment, resulting in a mixture of β -turn, bend and coil. In contrast, residues adopted a helix-like conformation using the membrane-mimetic environment, being α -helix the predominant form. Initial and final amino acids appear to have a random coil structure. Analysis based on circular dichroism measurements were in agreement with theoretical results.

The antibacterial activity within the series is mainly dominated by amino acid composition adopting a definite spatial ordering. Some of these compounds are the most active small peptides reported until now and constitute interesting structures for the design of new small-size peptides possessing antibacterial activity.

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Submission ID 256 – Poster

Mathematical Modeling and Computational Biophysics

6-OH-2- carboxanilide derivatives, a new series of inhibitors of BRAFV600E obtained from virtual screening. Theoretical and experimental study

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Around a 50% of patients with melanoma expressing the protein kinase mutant BRAFV600E, induces proliferation through activation of ERK [1].

Vemurafenib is an inhibitor of BRAF that is used clinically to treat patients with metastatic melanoma expressing BRAFV600E. The main objective of this work is to find new compounds with potential inhibitory activity of BRAF. The new compounds were obtained from a virtual screening.

We obtained 19 compounds as potential BRAF inhibitors, from which 6 showed significant inhibitory activities against this enzyme.

For the bioassays we use as a model, Lu1205 melanoma cells, which express BRAFV600E, and we analyze ERK phosphorylation as a measure of the activity of BRAF. Several compounds decreased the ERK

phosphorylation at concentrations of 50 and 10 μM . More interesting, derivatives 6-OH-2- carboxanilide obtained by chemical synthesis were the most active compounds presenting activity at a concentration of 1 μM . Preliminary results with the MTT technique suggest that drugs used to 10 μM decrease cell viability.

To better understand these experimental results, we conducted a study of molecular modeling, in which studies docking and molecular dynamics simulations were used. We also carried out the analysis per residue for the most active compounds of the series and these results were compared with those obtained for Vemurafenib and dabrafenib. Our molecular modeling study indicates that this new series of inhibitors might interact in the same active site which interact these two well- known inhibitors of BRAF [2]. The main interactions that stabilize the various ligand-receptor complexes are: ALA481, LYS483, LEU514, THR529, TRP531, LYS532, PHE583, ASP594 and PHE595.

Acknowledgment

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Submission ID **267** – **Poster**

Mathematical Modeling and Computational Biophysics

Singular Value Decomposition filtering, of vehicle component present in actograms recorded over moving platforms

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Driving a ground bus is a complex task. Drivers drowsiness and fatigue impair travel safety. Buses are driven by couples of bus-drivers, which alternate each between driving and non-driving stages 2 hours length.

The actigraphy quantifies body kinetic activity in an actogram, in times supplemented with a Sleep Log compiled by the subject; actographs attached to the subject are used. In order to identify patterns of resting / activity on the studied subjects, actograms can be analyzed manually by a blind expert or by computed algorithms.

Currently, actograms are recorded on motionless platforms, because of contamination by vehicular signals (as is the case for actigraphy on bus-drivers during travelling). In a previous work (1) this situation was present.

In this work, attenuation of the vehicle component was obtained by filtering actograms by Singular Value Decomposition. Further, we developed a method to automatically establish the intensity of kynetic activity developed for each driver, specially during the non-driving period, to obtain the "effective" resting period during the non-driving stages.

We performed a retrospective study on 20 actograms recorded (1) from bus-drivers during 10 long distance travels. Concordance between resting / activity periods obtained by actigraphy and driving / non-driving stages informed in their Sleep Logs, was analyzed by Bland-Altman. Effective rest durations during non-driving periods were computed and averaged over the 20 drivers, and results compared with previous reported by an blind expert (1) by using the Z statistic. Non significant differences (NS) were considered when $p \geq 0,05$.

Submission ID 274 – Poster

Mathematical Modeling and Computational Biophysics

**Dopaminergic isoquinolines with
hexahydrocyclopenta[*ij*]-isoquinolines as D2-like selective ligands**

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Dopamine receptors (DR) ligands are potential drug candidates for treating neurological disorders including schizophrenia or Parkinson's disease. Three series of isoquinolines: (E)-1-styryl-1,2,3,4-tetrahydroisoquinolines (series 1), 7-phenyl 1,2,3,7,8,8a-hexahydrocyclopenta [*ij*]-IQs (HCPIQs) (series 2) and (E)-1-(prop-1-en-1-yl)-1,2,3,4- tetrahydroisoquinolines (series 3), were prepared to determine their affinity for both D1 and D2 -like DR. The effect of different substituents on the nitrogen atom (methyl or allyl), the dioxygenated function (methoxyl or catechol), the substituent at the b -position of the THIQ skeleton, and the presence or absence of the cyclopentane motif, were studied. We observed that the most active compounds in the three series (2c, 2e, 3a, 3c, 3e, 5c and 5e) possessed a high affinity for D2-like DR and these remarkable features: a catechol group in the IQ-ring and the N-substitution (methyl or allyl). The series showed the following trend to D2 -RD affinity: HCPIQs > 1-styryl > 1-propenyl. Therefore,

the substituent at the b -position of the THIQ and the cyclopentane ring also modulated this affinity. Among these dopaminergic isoquinolines, HCPIQs stood out for unexpected selectivity to D2-DR since the Ki D1/D2 ratio reached values of 2465, 1010 and 382 for compounds 3a, 3c and 3e, respectively. Finally, and in agreement with the experimental data, molecular modeling studies on DRs of the most characteristic ligands of the three series revealed stronger molecular interactions with D2 DR than with D1 DR, which further supports to the encountered enhanced selectivity to D2 DR1.

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Submission ID 279 – Poster

Mathematical Modeling and Computational Biophysics

SEARCH OF NEW STRUCTURAL SCAFFOLDS FOR SPHINGOSINE KINASE 1 INHIBITORS.

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Sphingosine kinase 1 (SphK1), the enzyme that produces the bioactive sphingolipid metabolite, sphingosine-1-phosphate, is a promising new molecular target for therapeutic intervention in cancer and inflammatory diseases[1,2]. In view of its importance, the main objective of this work was to find new and more potent inhibitors for this enzyme possessing different structural scaffolds than those of the known inhibitors. Our theoretical and experimental study has allowed us to find two new structural scaffolds (three new compounds), which could be used as starting structures for the design and then the development of new inhibitors of SphK1. Our study was carried out in different steps: virtual screening, synthesis, bioassays and molecular modeling. From our results, we propose new dihydrobenzo[b]pyrimido[5,4-f]azepines, and two pyridinyl-piperazinyl-ethyl-phenyl-O-alkyl-carbamate derivatives as initial structures for the development of new inhibitors for SphK1. In addition,

our molecular modeling study using QTAIM calculations, allowed us to describe in detail the molecular interactions that stabilize the different Ligand-Receptor complexes. Such analyses indicate that the cationic head of the different compounds must be refined in order to obtain an increase in the binding affinity of these ligands.

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Submission ID 414 – **Electronic Poster**

Mathematical Modeling and Computational Biophysics

Artificial Intelligence and Molecular Docking applied on the Prediction of natural substrate of HIV-1 protease with subtypes B, C and F

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Viral diseases affect populations over the globe. The use of computational methods linked to protein information for patterns' discovery in biological systems allows the screening and detection of new drug prototypes from compound data banks. The present work uses natural substrate of HIV-1 protease and computational methods to find compounds which show the interaction probability of HIV-protease and the potential to inhibit it. The linear sequences of the natural substrates were numerically represented by physicochemical properties (AAINDEX) and classified by the SVM algorithm in WEKA tool such as the potential to inhibit the protease of HIV-1. The three-dimensional structures of these substrates were obtained by Modeller software. The molecular docking with the Lamarckian algorithm simulated in AutoDockTools package. The peptides of the gag-pol polyproteins were represented

by 31 physicochemical properties which were fractionated on 8, 6 and 4 amino acids and used as positive and negative sets corresponding to the cleavage sites that do and do not interact with HIV protease. Subsequently, a subset of peptides, classified as positive and negative underwent a molecular docking simulations and analyzed using docking energy values. After that, the peptides of the subtypes B, C and F of HIV-1 protease formed by 4 amino acids were subjected to analysis of molecular docking. The results of the cross-validation of the type 10-k-fold, with SVM, showed that 66% of the correct classification, had precision and that the recall ranging were from 0.63%-0.67% for peptides of different sizes. The machine learning results showed no significant differences in the classification of different size peptides, but the literature assumes that smaller substrates have great potential to inhibit the enzyme HIV-1 protease. The docking results between the different size peptides showed that the peptide size, structure, position and amino acids that compose it are essential to find better interaction with the enzyme. The docking results between the different subtypes of the enzyme with the peptides showed that this interaction is influenced by the peptide composition, probably due to the polarity difference. However, we can conclude that the peptide does not influence the size of the recognition by the enzyme, but the same composition is essential for the interaction. This analysis can be useful to find ligands that interact with different subtypes of the HIV virus. The use of machine learning tools' coupled with molecular docking techniques allowed the data analysis in their linear and three-dimensional shapes and has provided a greater

reliability in the prediction method.

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Submission ID 154 – **Electronic Poster**
Biophysics and NanoBiotechnology

About a γ – irradiated albumin nanoparticle: Structure, Decoration and Immune Toxicity

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A hypothesis on the structure of albumin-based gamma irradiated nanoparticles (BSA NPs) is presented based on methodologies such as microscopy, D.L.S. and spectroscopy techniques. The nanoparticle have a structure that can be compared to that one of an icosahedral viral capsid, with a molecular weight of 4.5 MDa.

The nanoparticles were decorated with folic acid before its gamma irradiation (FA BSA NPs), preserving their interaction with hydrophobic drugs, as well as their cytotoxicity in MCF - 7 cell lines.

BSA NPs were lyophilised (lyo – BSA NP) in order to obtain bigger aggregates. FA BSA NPs, BSA NPs and lyo- BSA NP were tested for their immune activity in murine macrophages and compared to albumin. All samples activated NF- κ B and led to TNF- α secretion, nevertheless this immune response was strongly enhanced for lyo-BSA NPs suggesting a dependence of albumin immunostimulatory properties on particle size.

The gamma irradiated albumin – based nanoparticle has 250 albumin molecules exposed to the surface. It was also possible to modify the

nanoparticles enhancing its specificity or modifying its immune activity by changing its size.

Acknowledgements

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Submission ID 155 – Poster
Biophysics and NanoBiotechnology

Characterization of DG4.0/DG4.5-Tacrine/Carbamazepine Complexes for Alzheimer's Treatment

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Alzheimer's disease is a multifactorial neurodegenerative disease. The most accepted etiological hypotheses are related to a deficiency of acetylcholine and the formation of senile plaques of β -amyloid protein ($A\beta$) and neurofibrillary of hyperphosphorylated TAU protein (p-TAU). Tacrine (TAC) is an inhibitor of the enzyme acetylcholinesterase, which was approved by FDA for Alzheimer's treatment. Due to their hepatotoxicity, TAC was removed from the market. In contrast, carbamazepine (CBZ) is an antiepileptic drug currently on use that was shown to reduce levels of $A\beta$ and p-TAU [1]. However, it has low solubility in aqueous media and inefficient pharmacokinetic profiles. Dendrimers (D) PAMAM DG4.0 and DG4.5 are three-dimensional polymers that bring unique properties to the field of drugs delivery systems [2]. The complexed drugs assimilate D properties, which would increase significantly their solubility and brain arrival [3].

Therefore, our aim was to obtain and characterize complexes of DG4.0 or DG4.5 with TAC or CBZ. *In vitro* drug release was studied using

micro-dialysis and interactions D-drug was analyzed by FTIR. Additionally, toxicity in cell cultured was studied.

Hydrophobic and ionic interactions between TAC and D were observed, but controlled release did not exist; no complexes between TAC and D were formed. Opposite to this, not only hydrophobic and ionic interactions between CBZ and D were observed, but 80% of the CBZ was released after 24 h of dialysis. Complexes between CBZ and D were achieved in a ratio 20:1. In assessing cell toxicity, the co-administration D-TAC failed to reduce the toxicity caused by free drug, whereas the D-CBZ complexes reduce this toxicity.

Acknowledgements: UNQ, IMBICE and CONICET.

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Submission ID 158 – Poster
Biophysics and NanoBiotechnology

Design and biophysical characterization of 5-fluorouracil-loaded nanosystem for topical skin cancer therapy: *in vitro*, *ex vivo* and *in vivo* studies

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Ultradeformable liposomes (UL) are a drug delivery nanosystem with an elastic modulus lower than conventional liposomes. UL are capable to penetrate the stratum corneum (SC) of the skin and release their content into the viable epidermis, where neoplastic events occur in skin cancer. 5-Fluorouracil (5FU) is an antineoplastic drug, administered parenterally, with severe side effects. Therefore, the incorporation of 5FU in UL improves specific-site delivery and aims to reduce side effects.

UL were prepared by resuspension with a 5FU solution of a thin lipid film (soy phosphatidylcholine and sodium cholate as border activator), formed by vacuum rotary evaporation. Vesicles were sonicated to reduce in size and lamellarity. Non-encapsulated 5FU was removed by molecular exclusion chromatography.

Size was determined by DLS and NTA and the stability by ζ potential. The interaction drug-lipid was assessed by DSC, using Laurdan and

MC540 probes. Drug-lipid ratio, encapsulation efficiency and release in the time were determined. Size and lamellarity were also corroborated by AFM and TEM. In order to study the elastic properties, force distance measurements by AFM and a deformability test in an automated extruder were performed. Free radical scavenging activity was evaluated using DPPH free radical.

In vitro cytotoxicity studies were carried out in HaCaT and SK-Mel-28 human cell-lines by MTT, Crystal Violet and Neutral Red. Cellular uptake at 4°C and 37°C was assessed by fluorescent microscopy.

Ex vivo penetration studies were performed on a Saarbrücken Penetration Model device with human skin explants. Intact skin and transversal sections were studied by confocal microscopy and penetration profile was quantified in the SC after tape stripping.

Finally, *in vivo* nanotoxicological and teratogenic studies were carried out in the animal model zebrafish (*Danio rerio*).

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Submission ID 172 – **Poster**
Biophysics and NanoBiotechnology

α -synuclein oligomers detection by FIAsh split-tetracysteine motifs

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α -synuclein (AS) is a protein of 140 amino acids whose aggregation is a prominent feature of Parkinson's disease pathogenesis[1]. As oligomeric precursors are considered cytotoxic and they are involved in the initiation of cell death[2], methods for early detection are required. A recent development for the fluorescent detection of structural changes during aggregation consists in the specific binding of FIAsh to a split tetracysteine motif in two bicysteine proteins[3,4]. Binding of the fluorogenic biarsenical requires and links two bicysteine motifs, thus imposing severe limits of the molecular geometry and distance between them. The emission of the FIAsh biarsenical fluorescence thus becomes

a sensitive indicator of intramolecular conformational changes and intermolecular assembly. In this work, we investigated new recombinant AS mutants bearing split tetracysteine motifs inserted at different positions of the primary sequence. These constructs permitted the detection by sensitive fluorescence spectroscopy of transient oligomers during the early stages of AS aggregation *in vitro*.

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Submission ID 174 – **Poster**
Biophysics and NanoBiotechnology

Interaction of Dextran Sulphate with cationic and anionic membranes

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Abstract

Polysaccharides have been associated with various biological functions through their binding to membranes, but their specific role is still under debate. The aim of this work was to study the interaction of cationic and anionic lipids with Dextran Sulphate, an anionic polymer, and the influence of this interaction on the membrane mechanical stability. The interaction was studied by determinations of the zeta potential using LUVs and of the interface reflectivity with Brewster Angle Microscopy using Langmuir monolayers. The membrane stability was studied in LUVs by following the response upon osmotic shocks or the addition of detergent. The evolution of the vesicles was followed with neferometry, Dynamic Light Scattering and by the fluorecence increment due to the release of carboxyfluorescein. In addition, GUVs were observed in the

absence and in the presence of the polymer and the shape fluctuations were analyzed.

The results indicate that the polymer interacts with the cationic membrane, and also with the anionic membrane but in this case, in a Ca^{2+} -dependent manner. The influence of the polymer on the stability upon detergent and osmotic shocks, as well as on the shape fluctuations depends on the membrane composition.

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Submission ID 181 – **Electronic Poster**
Biophysics and NanoBiotechnology

The interaction of nano-archeosomes with lung surfactant in monolayer biomimetic systems

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The archaeosomes (ARC) are lipid vesicles made of total polar archaeolipids (TPA) extracted from hyperhalophile archaeobacteria (1). Previous studies have shown that ARC are more extensively captured by alveolar macrophages and lung epithelial cells *in vitro* than conventional liposomes (2). This makes ARC excellent candidates for delivering drugs to the lungs. Although, in an *in vivo* context inhaled ARC must first interact with the pulmonary surfactant (PS) lining layer that covers the internal surface of the alveolus and provides the low surface tension at the air-liquid interface that is necessary to prevent collapse during expiration. Interactions with the PS film determine the subsequent retention

or translocation of the inhaled ARC and hence their potential activity on target cells (3). In order to evaluate the effect of the interaction of ARC, the changes in surface pressure (π) and compressibility produced in lung surfactant monolayers by ARC addition were studied. The results show that ARC increases the π , indicating its incorporation which is dependent of the membrane packing because the $\Delta\pi$ observed at high surface pressure (40 mN/m) are negligible in comparison to those obtained at low surface pressure (10 mN/m), ($\Delta\pi=4.5$ mN/m). Besides, the ARC concentration is crucial to produce changes in pressure monolayers, at low concentration (0.3 $\mu\text{g}/\text{mL}$) no significant changes were observed. Moreover, the ARC incorporation rates into the membrane was substantially affected if the addition done onto the monolayer in comparison to that in the subphase being the first much higher than the second.

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Submission ID **184** – **Poster**
Biophysics and NanoBiotechnology

Physiological states of yeast assessed by Zeta potential

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Zeta potential (ZP) measures the electrostatic charge on the solid-liquid interface of suspended particles. In cells, these charges can be related to the ionization of chemical groups, adsorption of ions from the surrounding solution or to ion exchange driven by the metabolic activity. The aim of this work is evaluate the ZP evolution during the growth of *Saccharomyces cerevisiae* and under different stresses, to find out a correlation between cell surface properties and the physiological state. Yeast growth was evaluated following the electrophoretic mobility of cells in an electric field, together with CFU, OD600nm and pH variations. Data obtained show a correlation between ZP mean values and OD, and because cells can be classified into subpopulations according to their surface charge it was found that an alteration in these subpopulations coincides with changes in the growth phase. The decrease in pH during exponential growth phase parallel to a shift of the ZP to less negative values can be related to the partial neutralization of negative charges by the cell acid products. In contrast after the

diauxic shift, pH remains relative constant and the ZP changes abruptly to negative values. This suggests that acid production is not enough to compensate the negative charges, which can be interpreted as a consequence in chemical composition of the cell wall.

ZP measurements were applied on cells exposed at different stresses, and these data were correlated with size information obtained by DLS. The results obtained showed that cells in exponential phase were more sensitive to stresses.

It is concluded that the ZP is a useful technique for monitoring cell culture, and to sense changes in the physiological state of the cell.

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Submission ID 185 – Poster
Biophysics and NanoBiotechnology

Role of lipid membrane on way of action of ”green” silver nanoparticles as unconventional antibiotics.

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Silver nanoparticles (AgNPs) are clusters of zero-valent silver with a size between 1 nm and 100 nm, which are intriguing for showing their great ability in terms of numerous functionalities. In this sense, there is a rising demand to develop ”green” synthetic strategies for AgNPs that provide biocompatible nanomaterials for pharmaceutical applications. Even, nowadays AgNPs are being considered as potential novel nanoantibiotics because of their outstanding antimicrobial activity, however the exact mechanism of action remains to be elucidated.³ In a previous work, we demonstrated that green AgNPs were able to interact with

lipid membranes, and a correlation was found between membranotropic behavior and antimicrobial activity. In this context, the aim of this work was contribute to a better understanding of the role of lipid membrane in the antimicrobial activity of these novel AgNPs. By using dynamic light scattering, we found a significant increase from 100 nm to around 220 nm on the average diameter of small unilamellar liposomes after be incubated with green AgNPs. This result allows us to indicate an interfacial adsorption of the AgNPs (with an average diameter of 45 nm) onto the liposomes. Furthermore, the ability of AgNPs to interact with bacterial membrane was studied by zeta potential using *Escherichia coli* as model. After be treated with green AgNPs, an increase in the net negative charge of the bacteria was determined confirming an interfacial adsorption. In addition, leakage experiments with carboxyfluorescein-loaded liposomes were conducted to evaluate if AgNPs were able to affect the membrane permeability. However, it is noteworthy that green AgNPs are not able to disrupt the lipid membrane.

In conclusion, we could hypothesize that membranotropic properties of green AgNPs would allow to increase their local concentration at the membrane level enhancing the antibacterial effectiveness of them *vía* localized release of biologically active Ag^+ ions.

Submission ID 189 – Poster
Biophysics and NanoBiotechnology

Design and characterization of liposomes containing stimuli-responsive molecules.

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We have design stable redox and optical active liposomes where the optical moiety was a metalloporphyrin and the redox part was ferrocene. Through the combination of these stimuli-responsive molecules and phospholipid assemblies, local control over the payload release based on an endogenous or exogenous trigger confers a spatial and temporal specificity [1].

We studied two different modified molecules (ferrocene and protoporphyrin IX esterified with octadecylamine, namely FC-ODA and PPIX-ODA) and compare their performance with unmodified molecules [2]. Liposomes were synthesized as describes in [3]. A solution of PPIX and PPIX-ODA 3.3 μM were added to the liposome containing samples (in ratio 1:5) and were mixed on a shaker for 6 h to reach the full

binding equilibrium. The Fc and Fc-ODA were added during liposome synthesis.

In the present work we analysed the behaviour of liposomes binding of different molecules. The response was measured by electrochemical and fluorescence signal, electrophoretic mobility and UV spectroscopy. Different behaviours were found for different systems evaluated.

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Submission ID 221 – **Electronic Poster**
Biophysics and NanoBiotechnology

Synthesis and characterization of mesoporous silicate nanoparticles to be used as nano-carriers.

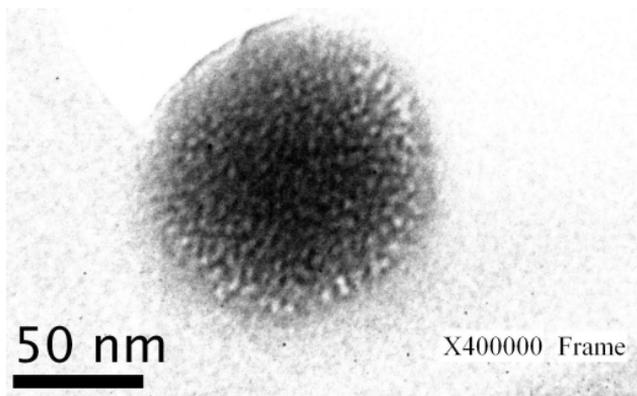
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Nano-carriers are defined as those nanoparticles (NPs) capable of trapping and transporting bioactive components and release them in a controlled manner at the target site. NPs have the ability to interact with cells and tissues at the molecular level. It has been proposed that the encapsulation within nano-carriers protects the bioactive components from unfavorable environmental conditions and also increases its solubility and bioavailability. In the present work we synthesized mesoporous silicate nanoparticles by a template method 1 in an oil in water emulsion (O/W) using heptane as the hydrophobic phase and cetyltrimethylammonium bromide (CTAB) surfactant as the emulsifier. The method involves the simultaneous hydrolytic condensation of tetraorthosilicate to form silica and polymerization of styrene into polystyrene. Both reactions occur within the heptane phase. Polystyrene behaves as the template for the mesoporous structure, and a CTAB and the hydrocarbon solvent as size particle controlling. Various synthesis conditions were tested. The synthesized NP were concentrated

by evaporation and the polystyrene template was completely removed by heat treatment at 500 °C under atmospheric conditions. The structure characteristics, e.g. NP diameter and pore sizes distribution, were analyzed by TEM and SEM microscopies. We obtained NPs with diameters ranging from 30 to 200 nm. The largest NPs showed larger pore sizes (about 6 nm) while NPs with diameters smaller than 100 nm exhibited pores of 3 nm diameter. Experiments of Energy Dispersive X-ray spectroscopy (EDS) performed in a SEM showed that the material obtained consisted mainly of SO₂.

Acknowledgements: CONICET, FONCYT, SeCyT-UNC.



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Submission ID 223 – **Electronic Poster**
Biophysics and NanoBiotechnology

Photo-immobilization of invertase on amyloid nanofibers for the design of a solid nanocatalyst for the production of inverted syrup

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The external invertase enzyme (β -fructofuranosid fructohydrolase, EC3.2.1.26) obtained from yeast *Saccharomyces cerevisiae* is of great importance in the food and beverage industry. This enzyme is used to catalyse the conversion of sucrose to an equimolar mixture of glucose and fructose. The aim of this study was to design a solid nanocatalyst throughout photo-induced immobilization of the enzyme invertase on the amyloid nanofibrils for technological applications.

Lysozyme was used to produce the support applying the protocol of the patent WO-2014006560-A2. Spectroscopic fluorescence and transmission electron microscopy confirmed the success in formation of amyloids nanofibers.

The enzymatic immobilization into this support was attained through photocrosslinking. Several experimental conditions were assayed (temperature, pH, irradiation time, concentration of reagent, presence of detergents, etc) to optimize the reaction. The performance of the immo-

bilization protocol was increased from 0.006 to 4.24 UE/ml once the optimal conditions were reached. The kinetic parameters and stability were determined for the free and immobilized invertase, showing that the stability, the optimal pH and temperature of the enzyme were not altered upon immobilization. The immobilized biocatalyst also showed high operational stability, capable to be reused up to 10 batches with only 40% decrease in activity.

Results indicate that fibrils obtained by low cost modifications can be applied as a suitable support for the immobilization of invertase. This nanobiocatalyst could be efficiently engaged in sucrose hydrolysis in batch reactor

Submission ID 257 – **Electronic Poster**
Biophysics and NanoBiotechnology

Membrane elastometry by magnetic field-cycling NMR relaxometry: a new tool for the non-invasive characterization of drug-delivery nano-device formulations

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The elastic properties of lipid membranes can be conveniently characterized through the bending elastic modulus κ [1,2]. Reliable and non-invasive methods to characterize the elastic properties of membranes are attractive for both fundamental research and industrial applications. Elasticity directly affects the deformability of a membrane, morphological and shape transitions, fusion, lipid-protein interactions, etc. It is also a critical property for the formulation of ultradeformable liposomes, and of interest for the design of theranostic liposomes for efficient drug delivery systems and/or different imaging contrast agents. A new method for the measurement of κ in liposome membranes is currently under consideration using the fast field-cycling nuclear magnetic relaxometry technique (FFC) [3]. Main advantages of this technology are the absolutely non-invasiveness and the possibility to test nano-devices like liposomes, niosomes, polymeric vesicles, etc. of any size between just a few nanometers in diameter up to several hundreds of nanometers.

This technique can also provide valuable information on the local and collective dynamics of the assembling molecules, and through them, on the mesoscopic properties of the membrane. An example is the first experimental determination of the quantity of lipids directly affected by a cholesterol molecule in a DOPC-cholesterol mixture [4]. In this presentation we will comment on these aspects and show selected examples.

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Submission ID **259** – **Poster**
Biophysics and NanoBiotechnology

Characterization of unilamellar DPPC membrane over Ag nanoparticles by SERS

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Surface enhanced Raman spectroscopy (SERS) is a technique that enhances in several orders of magnitude the Raman signals of molecules adsorbed on nanostructured metallic substrates, allowing the detection of low concentrations of analytes and even traces of them. In the last decades, this technique has been amply extended to the detection of biomolecules. Rapid label-free identification of small target analytes by the "fingerprint" type spectra makes SERS adequate for the detection of a broad range of biomolecules of interest, as phospholipids. Even though Raman spectroscopy has been extensively used for the study of lipids, this area is relatively new and emergent for SERS (1) .

We studied unilamellar lipid membranes by SERS, using a suspension of Ag nanoparticles (AgNPs), which were covered with a bilayer of dipalmitoylphosphatidylcholine (DPPC) (2) . To obtain hot spots for SERS we deposited the DPPC-AgNPs over a filter means according to

an already published protocol (3) , but introducing some modifications. The SERS spectrum of DPPC unilamellar membrane shows a decrease on its fluidity compared to multilamellar systems, and a notorious enhancement of vibrational modes of the head polar groups relative to those of hydrocarbon chain.

AgNPs covered with DPPC were characterized, before and after deposition over the filter means, by TEM and SEM respectively. Also AgNPs were characterized by UV-Vis spectroscopy. The importance of studying lipids disposed in this kind of nano-liposome lies on its potential use for biosensing applications

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Submission ID 261 – Poster
Biophysics and NanoBiotechnology

Nanosystems made from amphiphilic antimony(V) complex incorporating amphotericin B for the oral treatment leishmaniasis

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Pentavalent antimonial drugs have been used to date in the treatment of leishmaniasis, besides the need for parenteral administration for long periods. As an attempt to promote the oral delivery of pentavalent antimonials, we recently introduced an innovative approach based on amphiphilic antimony(V) complexes [1,2]. Such complexes were obtained from N-alkyl-N-methylglucamide surfactant and self-associate in aqueous solution forming nanoassemblies. The resulting nanostructures showed kinetic stability upon dilution below the CMC, suggesting that they may be used as carrier systems of other lipophilic antileishmanial drugs, such as amphotericin B (AmB). This work aimed to investigate the incorporation and aggregation state of AmB in Sb-decanoyl-N-methylglucamine (SbL10) nanoassemblies and the antileishmanial activity of the resulting nanosystem in *in vitro* and *in vivo* models of visceral leishmaniasis. HPLC analysis showed 75% incorporation of

AmB in SbL10. Characterization of SbL10-AmB by circular dichroism and UV-visible absorption spectroscopies indicated that AmB is present predominantly under the monomeric form, which is the least toxic form to the host. SbL10-AmB showed *in vitro* activity against *Leishmania infantum* promastigotes at a slightly higher level than the commercial drug (Anforicin B®). Balb/c mice infected with *Leishmania infantum* and treated orally with SbL10-AmB nanosystem (150 mg Sb/kg and 10 mg AmB/kg per day) showed significant reduction of parasite loads in the spleen and liver, when compared to the untreated control, to a similar level as animals treated parenterally with Anforicin B® (1 mg/kg/day). This study established for the first time the potential of SbL10-AmB nanosystem as an oral nanodrug for the treatment of visceral leishmaniasis.

Acknowledgments: Brazilian agencies CNPq and FAPEMIG for financial support.

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Submission ID 263 – **Electronic Poster****Biophysics and NanoBiotechnology****Ionic-gradient liposomes as drug-delivery systems for tetracaine and etidocaine***Juliana Damasceno Oliveira, Munazza Tamkeen Fatima, Bruna Renata Casadei, Lúgia Nunes Moraes Ribeiro, Eneida de Paula**Departamento de Bioquímica e Biologia Tecidual, Instituto de Biologia / Universidade Estadual de Campinas (Unicamp)*

Liposomes are carriers for drug delivery, and a number of liposome-based products have been approved for clinical application. Local anesthetics interact with liposomes, distributing themselves in the lipid bilayer region and in the aqueous core, prolonging anesthesia time [1]. To improve drug upload, ionic gradient liposomes [2-4] have been proposed. We have preprepared ionic-gradient (sulfate, citrate and pH) liposomes, composed by egg phosphatidylcholine-cholesterol (4:3 mole %) for the upload of tetracaine (TTC) and etidocaine (EDC). Dynamic light scattering, transmission electron microscopy (TEM) and electron paramagnetic resonance (EPR), were used to characterize the liposomes in terms of average diameter, polydispersity, zeta potential, morphology, and membrane fluidity. Large multilamellar (LMV), multivesicular (MLVV) and unilamellar (LUV) vesicles were prepared. TEM images showed clear differences between the vesicles. As expected, LMVV

were larger and less spherical than LUV, probably due to the freeze-thaw process used in their preparation; TTC and EDC did not affect the vesicles' morphology. Encapsulation of TTC, but not EDC, significantly decreased the size of (LMV and LUV) liposomes, but EPR experiments did not show changes in the bilayer packing to explain that. In MLVV, the profile of encapsulation efficiency (%EE) of TTC was: sulfate gradient (41 %) > citrate (35.9 %) > pH (29.5 %). %EE of EDC was measured only in LUV with sulfate gradient (31.5 %), since its low water solubility curbed the use of citrate / pH gradient liposomes. Higher amounts of TTC and EDC were uploaded, showing that gradient liposomes are good drug-delivery carriers.

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Submission ID 266 – Poster
Biophysics and NanoBiotechnology

FTIR and Raman characterization of DMPC lipid vesicles with different biomolecules

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This work aims at gaining insight into the characteristics of the membrane-biomolecule interaction in order to understand the action mechanisms of the biomolecules, their effectivity and physicochemical properties.

Vitamin C (ascorbic acid) is an essential cofactor for certain enzymes that are widely used in medicine and in food industry, annonacin is a mono-THF acetogenin a neurotoxic chemical compound found in some fruits with potential applicability as insecticide, acaricide, fungicide, as well as antiparasitic and antitumor agents, finally, valproic acid is an antiepileptic drug and mood stabilizer with action on different channels of the central nervous system.

1,2-Dimyristoyl-sn-glycero-3-phosphocholine (DMPC) multilamellar vesicles were prepared in the presence of different concentrations of the above-mentioned biomolecules. FTIR and Raman spectroscopies were used to analyse the participation of specific functional groups in the biomolecule-membrane interaction.

Spectra recorded for the complexes Biomolecule:DMPC were analysed in comparison with those of the pure liquid, studying spectral bands related to the inner regions of the lipid bilayer (hydrophobic regions) and the interphasial region (hydrophilic region). Low and high-frequency shifts were observed depending on the group and the temperature. The effect of each biomolecule on the transition temperature (T_m) the phase gel transition (L_β) – liquid crystalline (L_α) of the phospholipids exhibits a feature transition. Raman spectra show variations according to the presence of ascorbic acid, valproic acid or annonacin.

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Submission ID 270 – Poster
Biophysics and NanoBiotechnology

Raman micro-Spectroscopy application for studying of the components of Cytoplasm bovine oocytes matured *in vitro*.

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he process of *in vitro* oocyte maturation, both at nuclear and cytoplasmic level, is a crucial stage to the success of subsequent events, such as the *in vitro* fertilization and the early embryonic development. During this process an increase and redistribution of organelles occurs which leads to higher lipid and glutation contents. In addition, the biochemical profile of the cytoplasm experiences significant changes, mainly due to the increase in transcription and the consequent protein synthesis. The necessary energy for these events is provided by an increased glycolysis (1).

The aim of this work is to characterize the chemical composition of the cytoplasm of *in vitro* matured bovine oocytes (IVM) by using Raman microscopy. We focus on the spectral behavior of characteristic bands of DNA, RNA, proteins, carbohydrates and lipids.

The Raman spectra were acquired from five different points of the cytoplasm of each whole oocyte ($n = 16$). In order to minimize the contribution from the molecules of the Zona Pellucida (ZP), the ZP spectrum

was subtracted from the cytoplasm spectra. The vibrational characterization of the ZP glycoproteins has been previously performed by our group (2). We used the band corresponding to the vibration of sialic acid, component of the ZP glycoproteins, as a reference for the spectral subtraction. The resulting spectra show high phospholipid content and a significant absence of sugar bands in the IVM cytoplasm. These results are in accordance with the vesicle formation and the expenditure of energy reserves that led to the matured oocyte status.

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Submission ID 278 –

Poster

Biophysics and NanoBiotechnology

Rational design and characterization of a novel multivalent bifunctional protein scaffold based on Brucella Lumazine Synthase (BLS)

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The Brucella Lumazine Synthase (BLS) is a homodecameric protein formed by the dimerization of homopentamers. We have previously demonstrated its high quaternary stability and immunogenicity, allowing it to be used as antigen carrier. In this work we aimed to mutate the

homodimer interface in order to interrupt the association between homopentamers and simultaneously promote the association between mutant heteropentamers. In this way, we will be able to produce a BLS with different fusion proteins on each side of the scaffold. The mutations have been rationally designed based on its crystallographic structure using the bioinformatic softwares FoldX and Pymol. The BLS mutants have been named as BLSa and BLSb. Structural analyses demonstrate that BLSa and BLSb form pentamers in solution and when incubated together they are able to form a dimer of heteropentamers (BLSab). Biophysical properties of the complex (such as thermal stability and dissociation conditions), do not differ from the wild type BLS. In addition, studies in mice demonstrated that the heteropentamer complex immunogenicity is not different from wild type BLS. However, both pentamers are less immunogenic than BLS. As a concept proof, we have functionalized BLSab with a sialic acid binding domain on one side of the protein and a fluorophore on the other side, in order to label mammalian cells *in vitro*.

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Submission ID 257 – **Electronic Poster**
Biophysics and NanoBiotechnology

Membrane elastometry by magnetic field-cycling NMR relaxometry: a new tool for the non-invasive characterization of drug-delivery nano-device formulations

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The elastic properties of lipid membranes can be conveniently characterized through the bending elastic modulus κ [1,2]. Reliable and non-invasive methods to characterize the elastic properties of membranes are attractive for both fundamental research and industrial applications. Elasticity directly affects the deformability of a membrane, morphological and shape transitions, fusion, lipid-protein interactions, etc. It is also a critical property for the formulation of ultradeformable liposomes, and of interest for the design of theranostic liposomes for efficient drug delivery systems and/or different imaging contrast agents. A new method for the measurement of κ in liposome membranes is currently under consideration using the fast field-cycling nuclear magnetic relaxometry technique (FFC) [3]. Main advantages of this technology are the absolutely non-invasiveness and the possibility to test nano-devices like liposomes, niosomes, polymeric vesicles, etc. of any size between just a few nanometers in diameter up to several hundreds of nanometers.

This technique can also provide valuable information on the local and collective dynamics of the assembling molecules, and through them, on the mesoscopic properties of the membrane. An example is the first experimental determination of the quantity of lipids directly affected by a cholesterol molecule in a DOPC-cholesterol mixture [4]. In this presentation we will comment on these aspects and show selected examples.

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Submission ID **160** – **Poster**

Protein Oxidation in Biology and Biophysics

Study of the lasso peptide microcin J25 effect on the *E. coli* terminal oxidases

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The antimicrobial lasso peptide microcin J25 (MccJ25) displays a bacteriostatic activity by inhibiting RNA polymerase. This peptide is secreted by *Escherichia coli* and it can also target the enzymes of the respiratory chain on the bacterial membrane. *E. coli* has two type terminal oxidoreductases, the cytochrome bd (bdI and bdII) and cytochrome bo₃. Previously, it was demonstrated that the MccJ25 inhibits the cell respiration and increases the superoxide production. In this work, the effect of this peptide on *E. coli* C43 cytochrome deficient strains was studied. We evaluated the NADH dehydrogenase activity and the oxygen consumption rate. MccJ25-GA1 and Y9F2 are two modified peptides obtained in our laboratory. The ability of these peptides to inhibit the ubiquinol oxidase activity and to produce a superoxide overproduction in the purified cytochromes was also analyzed. Our results indicate that both, cytochrome bdI and bo₃, are necessary for MccJ25 inhibitory effect. Because of its extreme resistance to proteolytic degradation and

high temperatures, MccJ25 is a potential candidate for a number of applications including food preservation and treatment of food-borne diseases.

Acknowledgements: We thanks to PhD Gennis, R. and Schurig-Briccio L. from Department of Biochemistry, University of Illinois for give us the *E. coli* C43 strains and cytochromes overexpressing plasmids.

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Submission ID 264 – **Electronic Poster****Protein Oxidation in Biology and Biophysics****Stability and structural insights of U-Omp19, a *Brucella abortus* broad spectrum protease inhibitor with immune adjuvant activity**

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In our laboratory we study U-Omp19, a *Brucella abortus* protein with immune adjuvant properties. Bioinformatic analysis indicated that U-Omp19 has significant identity (30%) with bacterial protease inhibitors of the I38 family. Enzymatic kinetic assays demonstrated that it is broad spectrum protease inhibitor, which may play a role in its adjuvant activity by increasing the half-life of co-delivered antigens [1, 2]. In order

to further characterize this new protein adjuvant, we performed stability studies of U-Omp19 samples stored for different periods at different temperatures and/or subjected to stress conditions. SEC, CD, DLS, SDS-PAGE and enzymatic assays demonstrated that after 9 months of storage at -20 and -80 °C both the physicochemical and protease inhibitory properties of U-Omp19 remained unaltered. Furthermore, the inhibitory activity and protein integrity of U-Omp19 were stable after lyophilizing and repeated freeze-thaw cycles. Interestingly, although a progressive degradation of the N-terminal of the protein is observed after 1 month of storage at 20 or 4 °C, its CD and UV spectra together with its protease inhibitory activity were similar to those of the -20 and -80 °C samples. Initial NMR studies (PLABEM service) showed that U-Omp19 bears a disordered N-terminal region (residues 1-64) and a C-terminal compact β -barrel (residues 70-158), which suggests that the U-Omp19 inhibitory activity is located at the C-terminal β -barrel. Ongoing X-ray crystallographic studies with N-terminally truncated forms of U-Omp19 will shed light on the protease inhibitory properties of this oral adjuvant vaccine candidate.

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Submission ID 273 – **Poster**

Protein Oxidation in Biology and Biophysics

Towards new chemistry by redesign of electron transfer copper proteins

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Copper-containing proteins that contain type 1 (T1) and CuA centers conform a group that shares a cupredoxin fold and whose only function in biological systems is related to the exceptionally important electron transfer (ET) process(1).

The structural similarity between proteins bearing CuA and T1 centers, and the fact that most of the ligands in these metallic sites are in a single loop, has allowed the design of mutants with new functionalities by exchanging the ligand loop between these copper proteins. A number of these redesigns have been reported(2), particularly those where CuA loops are inserted in T1 scaffolds. These chimeras showed spectroscopic and functional features similar to those from which the loop was obtained.

In order to gain more insight into these mutants, and taking advantage of our expertise in loop engineering over the *Thermus thermophilus* CuA scaffold (TtCuA)(3), we generated and characterized two chimeras

(amicyanin-TtCuA and azurin-TtCuA) bearing two different T1 loops in the TtCuA scaffold, and found that they showed perturbed T1 sites with interesting features regarding redox catalysis: binding of exogenous ligands and higher redox potentials. As these features could be further optimized to develop new chemistry, our current aim is to evaluate them in a greater number of cases. For that purpose, we have generated six more mutants of the same class, inserting a variety of T1 loops in the TtCuA scaffold. These new mutants showed T1 centers with different degrees of perturbation according to their UV-vis spectra and can also bind exogenous ligands in a reversible manner. This is a promising feature that will allow us to explore catalytic functions and develop new chemistry not observed in native cupredoxins.

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Submission ID 280 – Poster

Protein Oxidation in Biology and Biophysics**Evolution of NDM Metallo- β -lactamase enhances antibiotic resistance under Zn(II) depletion by improving protein stability***1.- Vitor Horen, Luisina, 2.- Bahr, Guillermo, 3.- Vila, Alejandro J**(1) a - Instituto de Biología Molecular y Celular de Rosario, IBR-CONICET-UNR, Rosario, Argentina**(2) a - Instituto de Biología Molecular y Celular de Rosario, IBR-CONICET-UNR, Rosario, Argentina b - Área Biofísica, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario.**(3) a - Instituto de Biología Molecular y Celular de Rosario, IBR-CONICET-UNR, Rosario, Argentina b - Área Biofísica, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario.*

β -lactam antibiotics remain the most important class of clinically used antibiotics. Unfortunately, bacteria are gaining resistance to these drugs, limiting treatment options. The main mechanism of resistance is the expression of β -lactamases, enzymes which hydrolyze and inactivate β -lactam antibiotics. New Delhi Metallo- β -lactamase (NDM), a Zn(II)-dependent enzyme produced by Enterobacteriaceae, has been found to confer resistance to nearly all β -lactam antibiotics, including last resort carbapenems. Sixteen natural variants of NDM have been reported in clinical settings since the discovery of NDM-1 in 2008. Reported *in vivo* and *in vitro* characterizations have not detected major differences in the structures, catalytic efficiencies or substrate spectrums

among NDM variants. Therefore, it remains unclear whether these alleles possess evolutionary advantages over the original NDM-1 enzyme. Since previous studies on NDM variants were performed under different experimental conditions, we cloned all NDM alleles in the same expression plasmid for comparative studies. We performed an *in vivo* characterization of the variants in *E. coli* cells, evaluating protein levels and MICs values of different β -lactam antibiotics. Although we could not observe significant differences among alleles in these measurements, when we acquired the MICs values under zinc deprivation conditions by addition of a metal chelating agent, we observed that the resistance conferred by most variants was less susceptible to metal depletion than NDM-1. Protein stability measurements revealed that these variants are in fact more resilient to protein degradation under metal limitation. As these conditions resemble those encountered *in vivo* during infection, our results suggest that optimization of protein stability is the main driving force during evolution of NDM in clinical pathogens.

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