



XLIX Reunión Anual SAB

1 al 3 de diciembre 2021





Sociedad Argentina de Biofísica

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XLIX Reunión Anual SAB

1-3 de diciembre de 2021

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1-3 December 2021

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Welcoming words

Dear colleagues,

It is our pleasure to welcome you to this new virtual event of our society! Feeling encouraged by the success of last year's *Primeras Jornadas Virtuales*, early on we decided to resume our annual meeting program. However, we must confess that the organization of the past event implied many uncertainties and demanded strenuous secretarial efforts. These trying times have forced everyone to experience the need of exploiting the electronic means of communication at their maximum. We felt confident that the past virtual experience could be effectively reproduced and expanded. However, we do not overlook the fact that we are still in the process of exiting these tragic times of Covid-19 pandemic. With the sanitary situation in steady improvement through worldwide vaccination efforts, we decided to move forward and materialize the ongoing plans for the cancelled 2020 Iguazú meeting.

As for the future, let us hope for the best, in the anticipation that next year we will be able to celebrate the 50th anniversary of our society! Plans are already moving forward for L Rosario 2022!

In regard to this year's annual meeting, I feel very fortunate to rely on a very enthusiastic and capable team of coworkers who devoted their best efforts to the undertaking. Along these lines, we strove to maintain the registration fee at a minimum to allow ample participation. We appreciate and thank you all for your encouragement and your continuous support in the form of scientific contributions. Our Executive Council undertook the task of putting together an outstanding program of international and local speakers. After the start on Tuesday with a full day organized by our young colleagues (*Young Initiative in Biophysics*), we will enjoy a three-day gathering, featuring three plenary lectures, five thematic symposia and



numerous contributed presentations. The interactive virtual platform will allow delivery of outstanding scientific material and will provide assistance in discussion and networking opportunities.

We sincerely look forward to your lively participation in the planned activities!

The Organizing Committee

Scientific programme



Wednesday, December 1

9:30 – 10:00 h *Entry to virtual platform*

10:00 – 10:30 h *Opening Ceremony*

José María Delfino, IQUIFIB-CONICET, FFyB-UBA, Buenos Aires, Argentina.

10:30 – 12:30 h *Symposium Biomembranes and Interactions*

Chairs: Nadia Chiaramoni & Axel Hollmann

Relevance of the protein macrodipole in the membrane binding and orientation of fatty acid binding protein. Guillermo Montich. Facultad de Ciencias Químicas, Universidad Nacional de Córdoba. CIQUIBIC-CONICET. Argentina.

Atomic force microscopy-based assessment of cardiovascular risk. Nuno Santos. Instituto de Medicina Molecular, Facultad de Medicina, Universidad de Lisboa. Portugal.

Increasing Complexity in Membrane Computer Biological Simulations. Peter Tieleman. Calgary University. Canada

Transtegumental electrical potentials of Protoscoleces of the cestode Echinococcus granulosus from bovine origin. Mónica P. Antonella Carabajal. Instituto Multidisciplinario de Salud Tecnología y Desarrollo (IMSATeD). Argentina.

Arginine based cationic surfactants interaction with stratum corneum model membranes. Melisa Hermet. Centro de Investigación de Proteínas Vegetales (CIPROVE), Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas, Universidad Nacional de La Plata. Argentina.



12:30 – 14:00 h *Lunch*

14:00 – 15:00 h *Gregorio Weber Lecture*

Chair: Lía Pietrasanta

Division of Labor and Mechanism of Translocation in a RING ATPase.
Carlos Bustamante. University of California, Berkeley. USA.

15:00 – 17:00 h *Symposium Protein Structure. In memoriam Prof. Dr. Mario Amzel*

Chair. Santiago Di Lella

How immunotherapies catch oncdrivers to treat solid tumors.
Sandra Gabelli. Department of Medicine, Oncology, Biophysics and Biophysical Chemistry, Johns Hopkins University School of Medicine. USA.

A tale of two pandemics, the role of cryo-EM in the fight against enveloped viruses. Mario Borgnia. Molecular Microscopy Group, Genome Integrity and Structural Biology Laboratory, National Institute of Environmental Health Sciences, NIH. USA.

Role of conformational entropy in building allosteric connections in a family of transcriptional regulators. Daiana Capdevilla. Lab. Fisicoquímica de Enfermedades Infecciosas. Fundación Instituto Leloir. Argentina.

Structure-function study of an atypical thioredoxin (EglsTRP) using Ancestral Sequence Reconstruction (ASR) and Resurrection. Luciana Rodríguez Sawicki. Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes-CONICET, Instituto de



Investigaciones Bioquímicas de La Plata (INIBIOLP). Facultad de Ciencias Médicas. Universidad Nacional de La Plata. Argentina.

A strategy for detection of COVID-19 virus by fluorescence without PCR. María Victoria Capellari. Centro de investigaciones en Bionanociencia (CIBION), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). Argentina.

17:00 – 19:00 h Minipresentations I-even IDs

Thursday, December 2

8:45 – 9:00 h Entry to virtual platform

9:00 – 10:00 h Plenary Lecture

Chair: Gerardo Fidelio

NMR Structure Determination of Antimicrobial Peptides from Model Membranes to Live Bacteria. Frances Separovic. School of Chemistry, University of Melbourne. Australia.

10:00 – 10:30 h Coffee break

10:30 – 12:30 h Symposium Computational Biophysics

Chair: César Avila

The SARS-CoV-2 spike protein is vulnerable to moderate electric fields. Martín García. Theoretical Physics and Center for Interdisciplinary Nanostructure Science and Technology, Universität Kassel. Alemania.



Ligand binding and migration in heme proteins: an intrinsically multiscale problem. Luciana Copece. INQUIMAE - CONICET. FCEN, Universidad de Buenos Aires. Argentina.

SARS-CoV2 ORF3a structural analysis: K channel or hydrophobic chloride pocket? F. Danilo Gonzalez-Nilo. Center for Bioinformatics and Integrative Biology, Universidad Andres Bello. Chile.

Prediction of stabilizing mutations using bioinformatic tools and its application on the design of a thermostable xylanase. Sol Canale. Departamento de Química Biológica- FCEN- UBA/ IQUIBICEN-CONICET. Argentina.

Target identification for repurposed drugs active against SARS-CoV-2 via inverse docking. Marcos Villarreal. INFQIQC-CONICET, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba. Argentina.

12:30 – 14:00 h Lunch

14:00 – 16:00h Symposium Membrane proteins and transporters. In memoriam Prof. Dr. Alcides Rega

Chairs. Mariela Ferreira Gomes & Irene Mangialavori

P-ATPasas con funciones inesperadas: del transporte de iones a la translocación de péptidos. Hugo Adamo. Facultad de Farmacia y Bioquímica-Universidad de Buenos Aires. IQUIFIB (UBA-CONICET). Argentina.

Exploring membrane proteins in UniProt Cecilia Arighi. Center of Bioinformatics and Computational Biology Department of Computer and Information Sciences. University of Delaware. USA.

Role of Fatty Hydrogen-bonds in activation of transmembrane kinases. Larisa Cybulski. Departamento de Microbiología – Facultad de



Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario. Argentina.

Polymer Nanodiscs as new platforms for Membrane Protein Research and Applications. Mariana Fiori. Texas Tech University. USA.

A Region of the SARS-CoV-2 Spike Protein functionally interacts with the Human $\alpha 7$ Nicotinic Receptor. Juan Facundo Chrestia. Instituto de Investigaciones Bioquímicas de Bahía Blanca (INIBIBB). UNS-CONICET CCT Bahía Blanca. Argentina.

16:00 – 18:30 h Minipresentations II-odd IDs

Friday, December 3

9:00 – 9:30 h Entry to virtual platform

9:30 – 11:30 h Symposium Young Initiative on Biophysics

Chair. Florencia González Lizarraga

Lipid droplets: biophysics of the first steps of biogenesis. Paula Muratori Brest. Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba. Argentina.

A compressive computational study on the post-transcriptional regulatory mechanism associated with the RSMZ-RSME system. Agustín Ormazábal. Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes. Argentina.

Improving inhibition potential of an ACE2-derived peptide against SARS-COV2 spike protein by rational design. Carolina Sarto. IQUIBICEN-CONICET. Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires. Argentina.



Featuring ACE2 binding SARS-COV-2 through a conserved evolutionary pattern of amino acid residues. Patricia Duzi Carvalho. Universidade de São Paulo. Brasil.

Exploring the role of the linker peptide in galectin-4 activity. Mora Massaro. Lab. de Glicómica Funcional y Molecular, IBYME-CONICET. Argentina.

Lysine 107 is critical for lipid-free apolipoprotein A-I tertiary conformation. Ivo Díaz Ludovico. INIBIOLP. Facultad de Medicina, Universidad Nacional de La Plata. Argentina.

11:30 – 12:00 h Coffee break

12:00 – 13:00 h 2017-2018 SAB Award Lecture

Chair: M. Soledad Celej

Protein nanoparticles and their application as antitumoral drug vectors. Macarena Siri. Max Planck Institute of Colloids and Interfaces. Alemania.

13:00 – 15:00 h Lunch

15:00 – 16:00 h Plenary Lecture

Chair: José María Delfino

The Amyloid states of proteins. David Eisenberg. Molecular Biology Institute. University of California, Los Angeles. USA.

16:00 – 16:30 h Closing Ceremony and Ponce Hornos Awards

Chairs: José María Delfino & M. Soledad Celej

Lectures



Division of Labor and Mechanism of Translocation in a Ring ATPase

Bustamante C^a

a - University of California, Berkeley, USA

Many transport processes in the cell are performed by a diverse but structurally and functionally related family of proteins. These proteins, which belong to the ASCE (Additional Strand, Conserved E) superfamily of ATPases, often form multimeric rings. Despite their importance, a number of fundamental questions remain as to the coordination of the various subunits in these rings. Bacteriophage phi29 packages its 6.6 mm long double-stranded DNA using a pentameric ring nano motor. 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. Interestingly, the packaging rate decreases as the prohead fills, indicating that an internal pressure builds up due to DNA compression attaining the value of ~3 MegaPascals at the end of packaging, a pressure that is used as part of the mechanism of DNA injection in the next infection cycle. We have used high-resolution optical tweezers to show that the motor packages the DNA in alternating phases of dwells and bursts. During the dwell the motor exchanges nucleotide, whereas during the burst, the motor packages 10 bps of DNA per cycle. We have also characterized the steps and intersubunit coordination of this ATPase. 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 during the motor's cycle. Surprisingly, a division of labor exists among the subunits: while only 4 of the subunits translocate DNA, all 5 bind and hydrolyze ATP, suggesting that the fifth subunit fulfills a regulatory function. Furthermore, 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 the symmetry-breaking mechanism in the motor. Finally, we use dsRNA, and RNA/DNA hybrids to establish what factor determines the size of the motor burst, which together with recent structural data, allows us to propose a novel mechanism of translocation for this motor.

Protein nanoparticles and their application as antitumoral drug vectors

Siri M^a

a - Max Planck Institute of Colloids and Interfaces, Alemania

My PhD thesis focus on the biophysical and biochemical characterization of a γ -irradiated bovine albumin serum-based nanoparticle (BSA-NP). It contributes to the design, manufacture and application of drug delivery systems using a biodegradable non-toxic albumin. The nanoparticle was achieved by the desolvation method using γ -irradiation as crosslinker.

During my presentation, I am going to highlight the biophysical studies carried out in which, by combining spectroscopic techniques (such as infrared and fluorescence spectroscopy) and microscopy, we correlated the NP structure with its function. I will also show the potential of the BSA-NP as a drug delivery system through the studies carried out on the binding of the nanoparticle with a hydrophobic drug.

The findings of this thesis also compromise cytotoxic assays, and in-vivo studies that, together with the biophysical studies, make an exhaustive study on the BSA-NP. As a result, not only does the BSA-NP became the basis for further understanding more complex formulations of this and other albumin-nanoparticles, but also explains the advantages of the γ -irradiation preparation method.

NMR structure determination of antimicrobial peptides from model membranes to live bacteria

Separovic F^a

a - School of Chemistry, Bio21 Institute, University of Melbourne, Australia

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

Contact: fs@unimelb.edu.au

The expanding amyloid family: structure, stability, function, and pathogenesis

Eisenberg DS^a, Sawaya MR^a, Seidler PM^a, Boyer D^a, Hughes MP^a, Rodriguez JA^a, Abskharon RA^a, Cao Q^a, Murray KA^a, Lu J^a, Jiang S^a, Cascio D^a, Hou K^a, Pan H^a, Bowler JT^a, Cheng X^a, Gregory R^a, Tayeb-Fligelman E^a

a - UCLA-DOE Institute, HHMI Institute, UCLA, Los Angeles, CA 90095 USA

The hidden world of amyloid biology has suddenly snapped into atomic level focus revealing over 80 amyloid protein fibrils, both pathogenic and functional. Many of the most prevalent degenerative diseases, including Alzheimer's, Parkinson's, ALS, and type 2 diabetes are associated with particular proteins in amyloid fibril form. Fibrils structures determined X-ray and electron crystallography, as well as particle averaging by cryoEM, and solid-state NMR have contributed to deepened understanding of the formation, stability, and pathology of structures have led to design of compounds that inhibit fibril formation as well as some compounds that disaggregated fibrils. A subclass of functional amyloid-like fibrils are formed by reversible interaction of low complexity domains, having underrepresented members of the 20 coded amino acids. When mutated or at high concentration reversible amyloid fibrils can transition to irreversible pathogenic form. Unlike globular proteins, amyloid proteins flatten and stack into unbranched fibrils. Also unlike globular proteins, a single protein sequence can adopt wildly different two-dimensional conformations, yielding distinct amyloid fibril polymorphs. Hence, an amyloid protein may define distinct diseases depending on its conformation.

I will describe the energetic basis for the great stability of pathogenic amyloid, the structural differences found in reversible amyloid, and chemical methods for inhibiting and disaggregating amyloid. Our database of amyloid structure and energy is available at <https://people.mbi.ucla.edu/sawaya/amyloidatlas/>

Reference

The Expanding Amyloid Family: Structure, Stability, Function, and Pathogenesis. Michael R. Sawaya, Michael P. Hughes, Jose A. Rodriguez, Roland Riek, David S. Eisenberg. *Cell*, **184**, 4857 (2021)

Symposia



Atomic force microscopy-based assessment of cardiovascular risk

Santos N^a

a - Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal

Erythrocyte aggregation is an indicator of cardiovascular risk, which is influenced by plasma fibrinogen concentration. Fibrinogen levels are elevated during cardiovascular diseases. Our main goals were to understand how fibrinogen-erythrocyte membrane binding influences erythrocyte aggregation and how it constitutes a cardiovascular risk factor in different diseases, namely, chronic heart failure (CHF), essential arterial hypertension (EAH), stroke and carotid artery disease (CAD). Fibrinogen-erythrocyte membrane and erythrocyte-erythrocyte adhesion measurements were conducted by atomic force microscopy (AFM)-based force spectroscopy. Upon increasing fibrinogen concentration, there was an increase in the work and force necessary for cell-cell detachment, both for healthy donors and EAH patients. Nevertheless, higher values were obtained for the EAH patients at each fibrinogen concentration. Fibrinogen-erythrocyte (un)binding forces were higher in EAH and CHF patients, when compared with the control group, despite a lower binding frequency. Ischemic CHF patients showed increased binding forces compared to non-ischemic patients. Importantly, a 12-month clinical follow-up shows that CHF patients with higher fibrinogen-erythrocyte binding forces, probed by AFM at the beginning of the assessment, had a significantly higher probability of being hospitalized due to cardiovascular complications on the subsequent year. Our results show that AFM can be a promising tool for clinical prognosis, pinpointing those patients with increased risk for cardiovascular complications, for which special personalized medicine strategies should be envisaged. More recently, this assessment strategy was extended to stroke and carotid artery disease patients, including the assessment of the biomechanical properties of patients' atherosclerotic plaques surgically removed by preventive carotid endarterectomy.

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- Guedes AF et al., *Nanoscale.* 2019;11:2757–66.
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- Carvalho FA et al . *Int J Nanomedicine.* 2018;13:1985–92.

Increasing Complexity in Membrane Computer Biological Simulations

Tieleman P^a

a - Centre for Molecular Simulation and Department of Biological Sciences, University of Calgary, Canada

Molecular dynamics simulations have become a widely used tool to study properties of biological membranes. In the late 1990s they modeled bilayers of 60-120 lipids of a single lipid type on a time scale of up to a nanosecond. Currently the state of the art involves complex models with tens of thousands of lipids, dozens of lipid types, proteins, and time scales of microseconds to hundreds of microseconds, using a combination of sampling methods and force fields. I'll discuss recent work from our group on several different problems, including general properties of lipid-protein interactions, specific interactions of ligands with ion channels, progress towards larger scales, and features of the recently published Martini 3 model.

Relevance of the protein macrodipole in the membrane binding and orientation of fatty acid binding proteins

Montich G^a, Galassi V^b, Villarreal MA^c

a - Departamento de Química Biológica Ranwel Caputto Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Centro de Investigaciones en Química Biológica de Córdoba (CIQUIBIC), CONICET

b - Facultad de Ciencias Exactas y Naturales (FCEN), UNCUYO, Mendoza, Argentina

c - Departamento de Química Teórica y Computacional, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba. Instituto de Investigaciones en Físico-Química de Córdoba (INFIQC), CONICET

Peripheral, transient interactions of soluble proteins with lipid membranes are dominated by electrostatic interactions. In spite that obvious positively charged patches on the protein are the candidates for a binding site with anionic lipid membranes, the intricacy of the electrostatic profiles and the non-linear dependence of electrostatic forces with the distance may produce protein binding in an unexpected way. Two members of the superfamily of the fatty acid binding proteins, chicken liver bile acid-binding protein (L-BABP), and the regulatory protein of the squid nerve sodium calcium exchanger (ReP1-NCXSQ), with identical structure, have a resultant molecular dipole pointing in opposite directions. The positive end points to the bottom of the barrel in L-BABP while it points to the α -helix domain in ReP1-NCXSQ. Molecular dynamics simulations shows that both proteins, whether positively or negatively charged, bind to anionic and cationic lipid membranes oriented in the lower energy configuration for the interactions of the molecular dipole with the membrane electric field. Filtration binding assays of anionic and cationic lipid membranes support the results of the simulations.

Acknowledgments

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A tale of two pandemics, the role of cryo-EM in the fight against enveloped viruses

Borgnia M^a

a - Molecular Microscopy Group, Genome Integrity and Structural Biology Laboratory, National Institute of Environmental Health Sciences, NIH. Research Triangle Park, North Carolina, USA.

Enveloped viruses including SARS-CoV-2 and HIV rely on surface glycoproteins to enter their target cells. These type I fusion proteins are metastable trimers of dimers that recognize specific receptors in the surface of their hosts. Binding to the receptor triggers a conformational change that results in the fusion of the viral envelope with the plasma membrane. Because of their viral origin these envelope glycoproteins are a prime immune target of considerable interest in the development of vaccines. Their metastability makes these integral membrane proteins notoriously difficult to isolate in the prefusion state. For over a decade, native viral spikes have eluded structure determination using X-ray diffraction and NMR. Here, I will discuss the important role that methods in cryo-EM continue to play in the understanding of the mechanism of fusion and in the development of therapies and vaccines against the many infectious diseases caused by enveloped viruses.

How immunotherapies catch oncdrivers to treat solid tumors

Gabelli S^a

a - Dept of Medicine, Oncology, Biophysics and Biophysical Chemistry, Johns Hopkins University School of Medicine

We designed two novel immunotherapies that selectively target the killing of cancer cells and not normal cells. Today, most therapies target receptors overexpressed on the surface of cancer cells. Since most driver mutations occur in genes encoding intracellular proteins, we designed antibodies that target the mutation-associated-neoantigens display by Major Histocompatibility Complex class I. These designed precision cancer immunotherapies have the advantage of being broadly applicable to the patients with a specific mutation-associated neoantigen (MANA) and an HLA-allele. We selected MANAs from oncogenes and onco-suppressors that give a selective advantage to cancer cells using a combination of next generation sequencing, and proteomics. We identified antibody fragments against MANAs presented by specific HLAs using phage display and grafted the candidates into two therapeutic platforms, chimeric-antigen-receptor-T-cells (CAR T cells) and bispecifics single-chain-diabodies. Binding kinetics, and thermal stability was measured to demonstrate the biochemical basis underlying the selectivity of binding for each platform.

For the antibody fragment, 2Q.1, developed into CAR T cell therapy platform, structural analysis of the complex structure established the basis of specificity and selectivity of the IDH2 R140Q peptide-HLA:B*07 2 . The structures of the IDH2 R140 and IDH2 R140Q peptide-HLA:B*07 complexes show the peptide bound with the mutant residue buried within the HLA-B*07 binding cleft and without major conformational differences. Differently to the buried epitope in IDH2 R140Q - HLA:B*07, the histidine residue in the p53 neoantigen-HLA:A*02 structure is exposed and available for the candidate antibody fragment binding cloned into a bispecific therapeutic, called H2 1 . A ‘cage like’ structure formed by aromatic residues gives structural basis of selectivity of the antibody against p53 R175H . Grafting of H2 anti-p53 R175H into a bispecific antibody with an anti CD3 fragment is effective in activating T cells to secrete cytokines and kill cancer cells. Moreover, treatment with the H2 bispecific resulted in regression of human tumor xenografts in mice.

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Role of conformational entropy in building allosteric connections in a family of transcriptional regulators

Capdevila DA^a

a - Lab. de Fisicoquímica de Enfermedades Infecciosas -Fundación Instituto Leloir, Ciudad de Buenos Aires, Argentina

In bacteria, transcription is tightly regulated by diverse mechanisms; for a bacterial pathogen colonizing the infected host, this allows swift adaptation to host insults by “sensing” changes in a specific molecule or metal ion inside the cell. We are particularly interested in “one-component” sensors that detect a wide range of inorganic stressors, from transition metals to reactive sulfur species. We propose that driving forces arising from dynamics can be harnessed by nature to evolve new allosteric ligand specificities. To test this hypothesis, we are currently investigating a wide range of sensors from a family of transcriptional regulators that share the same molecular scaffold but respond to a binding event in a distinct recognition site. Our structural studies on different allosteric states strongly suggest that this allostery may be inherently dynamic; which is further supported by our initial NMR characterization of fast internal side-chain dynamics.

Ligand Binding and migration in heme proteins: an intrinsically multiscale problem

Capece L^a

a - INQUIMAE - Conicet/Departamento de Química Inorgánica, Analítica y Química-Física, Facutad de Ciencias Exactas y Naturales, Universidad de Buenos Aires

Computer simulation studies of the molecular basis for ligand migration in proteins allow the description of key events such as the transition between docking sites, displacement of existing ligands and solvent molecules, and open/closure of specific "gates", among others. On the other hand, binding of external ligands is related to protein function, and usually can trigger conformational changes and allosteric transitions. In heme proteins, ligand migration from the solvent to the active site preludes the binding to the heme iron and is related to different functions. In particular, in human hemoglobin, binding of O₂ to the heme iron preludes the T to R allosteric transition. In this talk I will present different computational strategies and models that we use in our group to study these processes in heme proteins, which range from QM/MM calculations to coarse grain simulations, and Markov State Models.

SARS-CoV2 ORF3a structural analysis: K channel or hydrophobic chloride pocket

Gonzalez-Nilo FD^a

a - Center for Bioinformatics and Integrative Biology, Universidad Andres Bello, Avenida República 330, Santiago, Chile

SARS-CoV-2 ORF3a is believed to form ion channels, which may be involved in the modulation of virus release, and has been implicated in various cellular processes like the up-regulation of fibrinogen expression in lung epithelial cells, downregulation of type 1 interferon receptor, caspase-dependent apoptosis, and increasing IFNAR1 ubiquitination. ORF3a assemblies as homotetramers, which are stabilized by residue C133. A recent cryoEM structure of a homodimeric complex of ORF3a has been released. A lower-resolution cryoEM map of the tetramer suggests two dimers form it, arranged side by side. The dimer's cryoEM structure revealed that each protomer contains three transmembrane helices arranged in a clockwise configuration forming a six helices transmembrane domain. This domain's potential permeation pathway has six constrictions narrowing to about 1 Å in radius, suggesting the structure solved is in a closed or inactivated state. At the cytosol end, the permeation pathway encounters a large and polar cavity formed by multiple beta strands from both protomers, which opens to the cytosolic milieu. We modeled the tetramer following the arrangement suggested by the low-resolution tetramer cryoEM map. Molecular dynamics simulations of the tetramer embedded in a membrane and solvated with 0.5 M of KCl were performed. Our simulations show the cytosolic cavity is quickly populated by both K⁺ and Cl⁻, yet with different dynamics. K⁺ ions moved relatively free inside the cavity without forming proper coordination sites. In contrast, Cl⁻ ions enter the cavity, and three of them can become stably coordinated near the intracellular entrance of the potential permeation pathway by an inter-subunit network of positively charged amino acids. Consequently, the central cavity's electrostatic potential changed from being entirely positive at the beginning of the simulation to more electronegative at the end.

The SARS-CoV-2 spike protein is vulnerable to moderate electric fields

Garcia M^a

a - Theoretical Physics and Center for Interdisciplinary Nanostructure Science and Technology, FB10, Universität Kassel, Kassel, Germany.

Most of the ongoing projects aimed at the development of specific therapies and vaccines against COVID-19 use the SARS-CoV-2 spike (S) protein as the main target. The binding of the spike protein with the ACE2 receptor (ACE2) of the host cell constitutes the first and key step for virus entry. During this process, the receptor binding domain (RBD) of the S protein plays an essential role, since it contains the receptor binding motif (RBM), responsible for the docking to the receptor. So far, mostly biochemical methods are being tested in order to prevent binding of the virus to ACE2. Here we show, with the help of atomistic simulations, that external electric fields of easily achievable and moderate strengths can dramatically destabilise the S protein, inducing long-lasting structural damage. One striking field-induced conformational change occurs at the level of the recognition loop L3 of the RBD where two parallel beta sheets, believed to be responsible for a high affinity to ACE2, undergo a change into an unstructured coil, which exhibits almost no binding possibilities to the ACE2 receptor.

We also show that these severe structural changes upon electric-field application also occur in the mutant RBDs corresponding to the variants of concern (VOC) B.1.1.7 (UK), B.1.351 (South Africa) and P.1 (Brazil). Remarkably, while the structural flexibility of S allows the virus to improve its probability of entering the cell, it is also the origin of the surprising vulnerability of S upon application of electric fields of strengths at least two orders of magnitude smaller than those required for damaging most proteins. Our findings suggest the existence of a clean physical method to weaken the SARS-CoV-2 virus without further biochemical processing.

Moreover, the effect could be used for infection prevention purposes and also to develop technologies for in-vitro structural manipulation of S. Since the method is largely unspecific, it can be suitable for application to other mutations in S, to other proteins of SARS-CoV-2 and in general to membrane proteins of other virus types.

Exploring the role of the linker peptide in Galectin-4 lectin activity

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Galectin-4 (Gal-4) is a tandem-repeat carbohydrate-binding protein mainly expressed in the gastrointestinal tract. It presents two carbohydrate recognition domains (CRD) at the N- and C-terminal ends connected by a linker peptide. Even though both domains display lectin activity themselves, the nature and structural significance of the linker regarding the biological activity of the protein is not well understood. While the structure of each domain has already been solved by experimental techniques, no structural data has yet been reported for the full-length Gal-4 protein nor the linker region. In this work, we employed an interdisciplinary approach to gain insight into the relation between structure and function of linking both domains. For this purpose, we recombinantly expressed human Gal-4 and each of its isolated CRDs, and their glycosidic preferences were evaluated. In doing so, we identified different affinities for both full-length Gal-4 and each isolated CRD for specific glycan moieties. To assess key determinants of these differences, we employed different computational approaches to construct three models of the intact Gal-4 using Modeller, Rosetta and AlphaFold2 programs. After careful inspection, molecular dynamic simulations were performed using Amber19 package programs. Simulations were visualized with VMD software, and characterized by structural parameters such as atomic fluctuations, distances and dihedral angles in order to evaluate the orientation and direction of each domain with respect to the linker. Together, these analyses allowed the characterization of the whole protein, mainly its CRD positions and linker peptide conformation. A discussion on the importance and function of the linker region in the protein biological function will be presented.

Featuring ACE2 binding SARS-CoV and SARS-CoV-2 through a conserved evolutionary pattern of amino acid residues

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Abstract

Spike (S) glycoproteins mediate the coronavirus entry into the host cell. The S1 subunit of S-proteins contains the receptor-binding domain (RBD) that is able to recognize different host receptors, highlighting its remarkable capacity to adapt to their hosts along the viral evolution. While RBD in spike proteins is determinant for the virus-receptor interaction, the active residues lie at the receptor-binding motif (RBM), a region located in RBD that plays a fundamental role binding the outer surface of their receptors. Here, we address the hypothesis that SARS-CoV and SARS-CoV-2 strains able to use angiotensin-converting enzyme 2 (ACE2) proteins have adapted their RBM along the viral evolution to explore specific conformational topology driven by the residues YGF to infect host cells. We also speculate that this YGF-based mechanism can act as a protein signature located at the RBM to distinguish coronaviruses able to use ACE2 as a cell entry receptor.

Acknowledgments

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Improving inhibition potential of an ACE2-derived peptide against SARS-CoV-2 spike protein by rational design

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In the recent SARS-CoV-2 pandemic, the importance of designing neutralizing strategies for the virus is evident. During viral infection, the spike protein is responsible for the attachment to the host cell surface via binding to angiotensin converting enzyme 2 (ACE2) and for the fusion of viral and cell membranes releasing the viral genome into the cytoplasm. In this sense, some peptides are capable of competitively inhibiting the interaction between SARS-CoV-2 and ACE2. Earlier studies of the interaction between the spike receptor binding domain (RBD) of the SARS-CoV-1 and ACE2 identified characteristic regions of the receptor, from which a derived peptide, called p6, was found to have potent antiviral activity. In this work, we propose five new peptides derived from ACE2. Using atomistic MD simulations, we started analyzing p6, and then we rationally designed five new peptides using two different strategies: On the one hand, the hydrophobic and solvent-exposed residues were mutated expecting a stabilization of the secondary structure and on the other hand, we tried to improve the interaction based on per-residue energy calculations and published mutational analysis. We compare the analysis of the computer simulations to experimentally obtained Kd values, and highlight key factors that contribute to the tight binding of the p6 peptide to the SARS-CoV-2 RBD.

Lipid droplets: biophysics of the first steps of biogenesis

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"Lipid Droplets" (LDs) are intracellular structures formed by a neutral lipid nucleus surrounded by a single layer of phospholipids (PLs) and proteins. They are recognized as key organelles for intracellular physiology since they are involved in multiple roles within cell biology. Furthermore, they play a role in several diseases (such as dengue, coronavirus and non-viral infections), cancer and obesity. The dynamics of LDs among them vary greatly, but these differences can be accounted for by their processes of assembly and disassembly. For this reason, it is now considered of key importance to study their biogenesis, which occurs in the endoplasmic reticulum (ER) membrane. This work aims to contribute to this objective from a biophysical approach, focusing on the molecular mechanisms that induce the first stages of formation of LDs, particularly, those which trigger the accumulation and budding of triglycerides (TGs) within the ER membrane bilayer. For this purpose, we use Langmuir films and Brewster Angle Microscopy to study, on model monolayers, the influence of certain PLs (with different spontaneous curvatures: POPE (-), POPC (0) and LPA (+)) on the stability and topology of the 3D structures formed ("lenses"), being able to study each lens individually. Thus, we aim to describe the energetic properties (interfacial tension and rheology) that will predict the accumulation and permanence of the TGs in the interface or their budding as an independent structure, as well as characterize the molecular conditions that predict the accumulation of "blisters" of TGs inside bilayers.

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A comprehensive computational study on the post-transcriptional regulatory mechanism associated with the RsmZ-RsmE system

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The CsrA family of binding proteins is widely distributed in bacteria. Its function is associated with the post-transcriptional mechanisms that regulate the expression of genes implicated in the metabolic adaptation of bacteria. The role of CsrA is allowed by its capability to recognize specific target mRNAs, thus modulating its expression. In *Pseudomonas* protegens, implicated in the plant protection against phytopathogens, the CsrA homologous protein is named RsmE. Its role is regulated by small RNAs (sRNAs) that cooperatively capture RsmE, thus releasing its target mRNA. RsmZ is one of those regulator sRNAs. Its structure attached to three RsmE homodimers was recently solved. Furthermore, it has been described that the attachment of RsmE to the RsmZ binding motifs follows a specific sequential order. In previous works, we have presented a structural model that may explain the feature so mentioned. That work corresponds to the first simulation reported for the available structures of the RsmZ-RsmE complex. More recently, we have simulated the attachment process itself. Here, we present the methods performed for both the simulations and the analysis of the results so obtained. Thus, Molecular Dynamics and Umbrella Sampling simulations were employed to study the dynamic behavior of the complex and the binding process, respectively. Furthermore, we performed Principal Component Analysis (PCA) and clustering methods on the results obtained. Consequently, the Free Energy profile of the binding process was calculated, and the main structural features of the molecules involved were described. As a whole, our results suggest that the structures adopted by the two molecules during the attachment pre-exist in equilibrium in their free forms. That observation, added to the structural pattern proposed by our group that explains the order followed by RsmE when joining RsmZ, implies a comprehensive understanding of the system's post-transcriptional regulation mechanism.

Lysine 107 is critical for lipid-free apolipoprotein A-I tertiary conformation

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Apolipoprotein A1 (APOA1) is the primarily protein component of high-density lipoproteins (HDLs) which play important roles in metabolic disorders such as obesity, diabetes, and cardiovascular disease (CVD). Situated at the water-lipid interface, APOA1 adopts a variety of structural conformations on the lipid surface to accommodate docking with various enzymes, lipid transfer proteins, and cell surface receptors that remodel HDL during its lifespan. In this way, APOA1 acts as a master regulator of both HDL composition and function. The deletion mutant Lys107del (Δ K107) is a natural variant of APOA1 that occurs in humans which is associated with hypertriglyceridemia and accelerated atherosclerosis and CVD. The purpose of these studies was to determine if structural differences exists in the Δ K107 APOA1 molecule that could explain the observed alteration in lipid metabolism in carriers of Δ K107 variant. Using a combination of high-resolution size exclusion chromatography, chemical cross-linking, and liquid chromatography-mass spectrometry we performed deep structural analysis on WT APOA1 and Δ K107 APOA1. We found the absence of Lys 107 severely impeded the ability of APOA1 to self-associate into higher order oligomers. Differences in the cross-linking pattern between APOA1 and Δ K107 APOA1 indicate that deletion of Lys 107 severely disrupted intermolecular interactions between APOA1 molecules in the N-terminal region of the molecule. Given that APOA1 self-association is thought to be a critical step in HDL biogenesis, our findings suggest the absence of Lys 107 results in a structural change that could alter HDL formation and/or function which may underlie the dyslipidemia observed in Δ K107 carriers.

Acknowledgments

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From ion transport to peptide translocation. P-ATPases with unexpected functions.

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More than 60 years ago the Danish biochemist Jens Skou first associated the Na^+/K^+ -dependent ATPase activity with the transport of Na^+ and K^+ by the sodium/potassium pump. Since then, the family of proteins known as P-ATPases has been regarded as active ion transporters. The frenetic cloning activity of the '90s led to the identification of hundreds of P-ATPases spread through all kingdoms of life and exhibiting a variety of ion specificities. However, among them, a group of eukaryotic proteins that was called group P5 had an enigmatic transport activity. The P5 group developed more interest when it was shown that in humans, the dysfunction of P5-ATPases caused severe neurological diseases. The mysterious transport specificity of P5-ATPases has recently been solved. A paper published in *Science* has reported that, instead of small ions, the P5-ATPases of the so-called subgroup P5A, translocate membrane-inserted peptides of proteins that are misincorporated in the ER membrane. This remarkable finding indicates that the P5 are the first P-ATPases involved in protein quality control. I will present our results on the structure and ATPase activity of the yeast Spf1p P5A-ATPase in connection with its unique transport function.

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Polymer nanodiscs as new platforms for membrane protein research and applications.

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Membrane proteins (MPs) are essential to many organisms' major functions. They are notorious for being difficult to isolate and study, and mimicking native conditions for studies *in vitro* has proved to be a challenge. Lipid nanodiscs (LNDs) are discoidal nanostructures that consist of a lipid bilayer membrane patch encased within membrane scaffold proteins (MSPs). LNDs are playing increasingly important roles in studies of the structure and function of MPs but their use is undermined by the fluidic and labile nature of lipid bilayers. These limitations have led to the testing of copolymers in new types of nanodisc platforms. The use of styrene-maleic acid (SMA) copolymers for solubilization and reconstitution of MPs into polymer-encased nanodiscs (SMALPs, SMA lipid particles) is promising for studies of MPs in a near-physiologic environment without the use of detergents. Here, we introduce new approaches to address some of the drawbacks of SMALPs and LNDs by using a set of new block copolymers to replace the MSPs and another set of block copolymers to replace the lipid bilayer. Polymer-encased nanodiscs and polymeric nanodiscs support functional MPs and address some of the limitations present in other MP reconstitution platforms.

Exploring membrane proteins in UniProt

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UniProt mission is to provide the scientific community with a comprehensive, high-quality and freely accessible resource for protein sequence and function information. It contains a number of databases catering different user needs. The UniProt Knowledgebase (UniProtKB) is the core database covering the tree of life and over 220 million protein entries. In this talk, I will give a brief overview of UniProt, followed by details on the annotation of membrane proteins, predictions of transmembrane domains, and available structure predictions by AlphaFold. I will also cover the ongoing crowdsourcing curation activity that involves domain experts in UniProt biocuration. Finally, I will highlight new aspects of the upcoming new UniProt website

Interhelical hydrogen bonds in the membrane core drive activity of transmembrane kinases.

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Hydrogen bonds (HB) are a primary driving force for de novo protein folding and represent one of the main intermolecular forces responsible for protein interactions. Here we study inter-helical HB in the low permittivity environment of the lipid membrane core. We show how re-localization of HB residues in transmembrane segments of membrane receptors modulates their kinase activity to the extent of inverting the output response to the stimulus.

We also explore the role of a particular type of HB of the peptide backbone which are not totally protected from water attack. This kind of HB, called dehydrons, play an important role in the activation of catalytic residues because they can function as activators of nucleophilic groups. By exploring this concept, we forced a canonical bacterial histidine kinase to phosphorylate alternative residues.

Oral Communications



Arginine based cationic surfactants interaction with stratum corneum model membranes

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Skin delivery's efficiency is seriously restricted due to the barrier function of the stratum corneum (SC). Surfactants represent one of the most employed chemical permeation enhancers (CPE) used to overcome this issue.

N^α-benzoyl-L-arginine decyl- and dodecylamide (C_{10} and C_{12}) are cationic surfactants we have obtained through means of a biocatalytic strategy. The present work reports their surface activity and their interaction with a quaternary lipid membrane model (SCM), which resembles the SC structure and barrier capacity. Lipid monolayers were used to mimic the simplest unit layer of the SC. We further explored the in-plane structure and elasticity of the SCM-enhancer systems at macro-, micro- and nanoscopic scales. Oleic (OA) and stearic acid (SA) were used as positive and negative CPE controls, respectively.

Experiments revealed that C_{10} and C_{12} were able to modify the lipid film's elasticity, dropping the Cs^{-1} value of SCM up to 30 and 15-20% for C_{10} and C_{12} respectively, and showing a dose-dependence effect, stronger than that of OA. They were able to insert themselves into highly packed preformed monolayers, up to an initial surface pressure (p) of 43–45 mN/m. Cut off values for OA and SA were significantly lower.

Direct observation of the films by BAM showed that the addition of 35 mol% of C_{10} or C_{12} stabilized liquid-liquid phase separation at lower p , probably due to the surfactants' immiscibility with the most hydrophobic components of the SCM. Notably, this was not the case for SCM films containing OA, which showed an even surface at low p .

We finally analyzed the lateral structure of the films at nanoscale by means of AFM. The incorporation of the amphiphiles to the SCM changed the domain shape, as rounded-border structures appeared. The presence of C_{12} and OA did not alter the phase equilibrium, whereas C_{10} and SA increased the thick phase area coverage by 32 and 69%, respectively.

Evidence collected in these assays suggests both C_{10} and C_{12} could be employed as CPE.

Transtegumental electrical potentials of Protoscoleces of the cestode *Echinococcus granulosus* from bovine origin.

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Cystic echinococcosis encompasses a group of important zoonotic diseases caused by the metacestode (larval stage) of tapeworms belonging to the genus *Echinococcus*. It is mainly transmitted in livestock raising areas and one the most important species is *E. granulosus*. Metacestodes constitute fluid-filled cysts with an inner thin germinal layer where large numbers of protoscoleces (PSCs) are formed by asexual multiplication. PSCs remain invaginated (invaginated PSC) within the mucopolysaccharide-coated basal region, for protection until evagination occurs in the definitive host (evaginated PSC). Little information is available as to the electrical activity of the tegument considering that this complex cellular syncytium is where all metabolic interchange in the PSC takes place. To gain insight into the electrophysiology of the parasite at this developmental stage, here we conducted microelectrode impalements of *E. granulosus* PSCs from bovine origin in normal saline solution. We observed two statistically different ($p < 0.001$) intraparasitic potentials, a transient potential of approximately -65 ± 1.4 mV ($n=49$) and a stable potential of about -23 ± 0.3 mV ($n=49$), most likely representing tegumental and intra-parasitic extracellular space electrical potential differences, respectively. These values changed upon evagination and also had different values depending on the anatomical region of the PSC and ionic concentration in the bathing solution. Changes in electrical potential differences of the parasite provide an accessible and useful parameter for the study of transport mechanisms and potential targets for the development of novel antiparasitic therapeutics.

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Structure-function study of an atypical thioredoxin (EgIsTRP) using Ancestral Sequence Reconstruction (ASR) and Resurrection.

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Thioredoxins(Trxs) is a family of proteins with two Cys in the active site responsible of the oxidoreductase activity in a thiol-dependent form. EgIsTRP is an atypical Trx from *E. granulosus* (Bisio et al., 2016). It exhibits a Trx fold and binds Fe-S clusters but it lacks oxidoreductase activity. Our aim is to determine the structural bases of the differential biological behavior of EgIsTRP as well as its evolutionary origin using ASR techniques and protein resurrection.

Evolutionary analysis revealed that EgIsTRP is part of a cluster composed by members of the Taenidae family close to the cluster of Hymenolepididae family. Both clusters are embedded in a major cluster composed by canonical Trxs, mostly from mammals. Using NMR structures of EgIsTRP (kindly provided by Dr. Bellanda) and a human Trx (TrxH) we observed that the conformational diversity of EgIsTRP is greater than the observed in TrxH (maximum RMSD 1.65 Å and 0.35 Å respectively). Same trend is observed with other canonical Trxs examined using CoDNAs database. Interestingly, EgIsTRP enhanced flexibility modifies the amino terminal Cys pKa increasing its basicity making it unlikely to act as an oxidoreductase since Cys of canonical Trxs exhibits an acidic pKa. To extend our findings, we estimated the degree of flexibility in the phylogenetic clusters using Dynamine (Cilia et al., 2014). Taenidae cluster had the members with higher flexibility. Experimental data from another member of this cluster also showed to be negative for oxidoreductase activity. Low flexibility values were found in Hymenolepididae and the mammalian proteins. Unpublished experimental data of a member of Hymenolepididae cluster showed it is positive to oxidoreductase activity but negative to Fe-S cluster formation. This evidence supports the hypothesis that EgIsTRP and close homologous proteins have evolved under a selective pressure to increase their conformational diversity and flexibility, gaining the capacity to bind Fe-S clusters while losing the oxidoreductase activity.

We have already predicted the ancestral sequence of the protein node dividing Hymenolepididae and Taenidae families. We are now expressing and characterizing this protein and some of its variants in order to understand the structural determinants that lead the change of function in this atypical thioredoxin.

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A strategy for detection of COVID-19 virus by fluorescence without PCR

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The pandemic caused by SARS-CoV-2 virus (COVID-19) highlighted the need for rapid and accurate virus detection. PCR is the reference gold standard for its low limit of detection and specificity. These features are attained by the polymerase reaction and by the appropriate design of the polynucleotide sequence, respectively. It is not adequate for massive and fast tests, as might be the case in airports or railway stations.

In this work, we develop a test strategy that uses RNA virus extracts, as current tests, but is based on a direct fluorescence detection of the target virus without PCR. The strategy involves the selection of the complementary virus sequence, its immobilization on a platform, and the design and builds up of a portable fluorescence detector with a very low limit of detection.

We use a fluorescent beacon, comprising a fluorophore and a quencher, kept close by a DNA hairpin sequence. The DNA sequence is designed for specific detection of SARS-CoV-2, without interference of human, or of common disease ARN (like common cold o flu). When the viral ARN binds to the beacon, the hairpin opens, and the luminescent signal develops.

First, we tested the performance of the beacon in solution by fluorescence spectroscopy in the presence of the complementary sequence used as a positive control. Afterwards, amino functionalized beacon was covalently attached to epoxy functionalized microscopy glass coverslips. Saturating concentration of an amino functionalized 10 bp polyT was used to react with remaining epoxy groups in the glass and served as isolating layer on glass. Coverslips with sensor were measured on a microscope under STORM conditions as prepared and after addition of negative control, positive control or sample. These results served as a reference for the performance of the compact fluorescence excitation-detection instrument we designed, based on a 520 nm laser and a single photon counting PMT. This prototype has limit of detection similar to STORM.

Finally, a set of RNA extracts from healthy and sick patients was measured by STORM microscopy. The results showed that the light signal doubles in the presence of the virus with sufficient load to consider the individual as infectious. These preliminary results give a 10% false positive or negative rate, more than adequate for the stated objectives.

Acknowledgments

CONICET

Prediction of stabilizing mutations using bioinformatic tools and its application on the design of a thermostable xylanase

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The design of active enzymes with increased stability is of high interest both for basic and applied research. There are a number of methods developed to predict changes in stability upon mutation using structure based energy potentials or evolutionary information derived from homologous sequences. In this work we evaluated many of these programs: Foldx, PopMusic, EV-Mutation and a MSA-based predictor coded in our group. We compared their individual and combined performance for predicting experimental data of a large set of single residue mutants extracted from the Fireprot database. Predictions made using different structural models and/or different Multiple Sequence Alignments inputs were compared. The performance varied between different methods and the protein studied. In general, a good prediction was observed on average for all methods. The percentage of true destabilizing mutations predicted (60-90%) was higher than the true stabilizing ones (30-70%). We then used these methods for predicting stabilizing mutations on a relevant biotechnological enzyme with xylanase activity. Xylanases are widely studied for their potential applications in the bioconversion of xylans for biofuels, prebiotics and feed industries. CsXyn10A from *Cellulomonas sp. B6* is a bi-modular (GH10 and CBM2) endo-xylanase that hydrolyzes the (1→4)-beta-D-xyloside linkages in xylan, it does not degrade cellulose and its activity is optimal at 50°C, in a pH range of 5 to 7.5. However, its activity rapidly decays at this temperature, limiting its use in bioprocesses. Here, we used the best individual and combined methods to design thermostabilizing mutations of the catalytic GH10 module of this enzyme to improve its performance for biotechnological applications. The selected mutations were based on a ranking of predictions that were filtered to avoid changes in residues near the catalytic site or highly conserved residues. The experimental analysis of these predictions will help in the validation of the method used for the design and to increase the optimal temperature and/or half-life of CsXyn10A for its use in different applications.

Target identification for repurposed drugs active against SARS-CoV-2 via inverse docking

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Screening already approved drugs for activity against a novel pathogen can be an important part of global rapid-response strategies in pandemics. Such high-throughput repurposing screens have already identified several existing drugs with potential to combat SARS-CoV-2. However, moving these hits forward

for possible development into drugs specifically against this pathogen requires unambiguous identification of their corresponding targets, something the high-throughput screens are not typically designed to reveal. We present here a new computational inverse-docking protocol that uses all-atom protein structures and a combination of docking methods to rank-order targets for each of several existing drugs for which a plurality of recent high-throughput screens detected anti-SARS-CoV-2 activity. We demonstrate validation of this method with known drug-target pairs, including both non-antiviral and antiviral compounds. We subjected 152 distinct drugs potentially suitable for repurposing to the inverse docking procedure. The most common preferential targets were the human enzymes TMPRSS2 and PIKfyve, followed by the viral enzymes Helicase and PLpro.

All compounds that selected TMPRSS2 are known serine protease inhibitors, and those that selected PIKfyve are known tyrosine kinase inhibitors. Detailed structural analysis of the docking poses revealed important insights into why these selections arose, and could potentially lead to more rational design of new drugs against these targets.

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A region of the SARS-CoV-2 spike protein functionally interacts with the human $\alpha 7$ nicotinic receptor

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Coronavirus disease 2019 (COVID-19) is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The binding of the viral spike protein (S) to angiotensin-converting enzyme 2 in host cells is crucial for infection. The S protein has been suggested to interact with nicotinic acetylcholine receptors (nAChRs), and their contribution to the COVID-19 inflammatory pathophysiology has been proposed. $\alpha 7$ is an interesting candidate target because it is present in neuronal and non-neuronal cells, and it has neuroprotective and anti-inflammatory actions. By whole-cell and single-channel recordings we revealed that the Y674-R685 region of the S protein shows a direct functional interaction with human $\alpha 7$ nAChR. The S fragment exerts a dual effect, acting as a low-efficacy agonist and a non-competitive antagonist. In agreement with molecular dynamics simulations showing stable binding of this region to the ACh binding pocket, the S fragment activates $\alpha 7$, but only in the presence of a potentiator, supporting its action as a very low-efficacy agonist. In addition, it allosterically inhibits $\alpha 7$ responses elicited by ACh, which may result in the predominant effect. This study provides unequivocal evidence supporting a functional $\alpha 7$ -S protein interaction, which may play a role in infectivity and/or disease progression and may be explored for new therapeutic opportunities.

Minipresentations



Study of the biochemical changes in the cytoplasm of *in vitro* matured bovine oocytes in media enriched with Vascular Endothelial Growth Factor-D by Raman microscopy

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In recent years, numerous studies have focused on the optimization of *in vitro* embryo production (IVP) of animals of productive interest, including bovines. *In vitro* maturation (IVM) of oocytes is the first stage of the IVP and can determine the performance of the process and the quality of the future embryo. Therefore, the optimization of the IVM media would make it possible to increase the number of competent oocytes. The aim of this work is to evaluate the impact of vascular endothelial growth factor-D (VEGF-D) on the cytoplasm of bovine oocytes during the IVM, by Raman microscopy.

The spectra were taken from the cytoplasm of oocytes that were subjected to the IVM process for 18 and 22 h in the absence (control: 18H and 22H) and in the presence of the factor (18H-F and 22H-F). For a more precise analysis of cytoplasmic changes during IVM, the zona pellucida was removed from the oocytes by enzymatic digestion. The average spectrum of the 18H-F group showed a marked increase in the intensities of the characteristic protein bands with respect to the 18H spectrum. When the spectra that completed the maturation time (22h) were related to each other, only subtle changes in intensity were observed in some bands assigned to amino acids. A principal component analysis (PCA), multivariate statistical calculation, was implemented for the signals that showed the greatest spectral difference. The scatter graph obtained by the PCA revealed that the oocytes of groups 18H-F and 22H-F are grouped in the sector of the graph representing the presence of intense characteristic protein bands. We previously demonstrated that, throughout IVM, there is a gradual accumulation of proteins in the cytoplasm of bovine oocytes, being greater at the end of the process (22h). All these data confirm that the supplementation of the maturation media with VEGF-D increases the protein content in the cytoplasm of bovine oocytes and, therefore, could have a favourable impact on the performance of IVP.

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The microtubule oscillator as a universal phenomenon

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Microtubules (MTs), formed of cylindrical arrays of $\alpha\beta$ tubulins, are essential cytoskeleton elements of eukaryotic cells. Previous studies from our laboratory (Priel 2006, 2008) demonstrated the ability of MTs to act as biopolymer transistors that may underlie the production of electrical oscillations by different assemblies from the mammalian brain (Cantero 2016, 2018, 2019, 2020). Although its electrical behavior may have important implications in neuronal cell function, no information is available as to whether MTs from non-excitatory cells may also share similar properties. Here, we explored and compared the ability of LLC-PK1 renal epithelial cells MTs to produce electrical oscillations. Further, we compared them with those of FtsZ, a prokaryote protein and evolutionary ancestor of tubulin.

Tubulin from LLC-PK1 cells was isolated by polymerization and serial steps with ultracentrifugation. FtsZ from *E. coli* was purified by $(\text{NH}_4)_2\text{SO}_4$ precipitation. Samples were assembled into 2D-sheets by incubation with a polymerization buffer and then subjected to voltage-clamp under gigaseal conditions. Electrical recordings from output currents were obtained and filtered using Clampfit 10.7, showing a complex oscillatory behavior. Power spectra and EMD analysis were performed using MatLab (2019a).

Both LLC-PK1 MT and FtsZ had a similar oscillatory regimen with frequency peaks at 39 Hz and 91-93 Hz, which correlates with brain MT frequency of 39 Hz. EMD analysis of the signals showed 6-7 intrinsic modes (IMF) for all 2D sheets (brain n=15, LLC-PK1 n=4, FtsZ n=3), disclosing more fundamental frequency peaks. In most cases (n=19), the first IMF had a 91-93 Hz frequency and the second was at 38-39 Hz, revealing the predominance of these frequencies in all samples.

Data support the hypothesis of MTs electrical oscillatory behavior as a highly conserved evolutive phenomenon, preserving similarities between brain and renal epithelial cells, and even a tubulin-related bacterial protein.

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Regulation of plasma membrane calcium ATPase (PMCA) by actin cytoskeleton

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The Plasma Membrane Calcium ATPase (PMCA) is a calmodulin-modulated P-type ATPase responsible for the maintenance of low intracellular concentrations of Ca^{2+} in most eukaryotic cells. Our group have previously shown that purified actin can exert a dual modulation on the activity of Ca^{2+} -ATPase 4b isoform (hPMCA4b): F-actin inhibits it while short actin oligomers may contribute to its activation. These studies had to be performed with purified proteins given the nature of the biophysical and biochemical approaches used.

On the other hand, in HEK293 human cells that overexpressed PMCA2w/b isoform, the actin depolymerization upon Cytochalasin D (CytD) treatment significantly increased PMCA2-mediated Ca^{2+} extrusion and when F-actin was stabilized using jasplakinolide, PMCA2w/b activity was completely abolished.

In order to assess whether the functional interaction between the hPMCA4 isoform and the actin cytoskeleton may be of physiological relevance, we decided to further characterize it in the context of a living cell by monitoring in real-time the changes in the actin polymerization and cytosolic Ca^{2+} concentration ($[\text{Ca}^{2+}]_{\text{cyt}}$). For this, hPMCA4 isoform was transiently expressed in HEK293T cells. The dynamics of $[\text{Ca}^{2+}]_{\text{cyt}}$ was performed using the fluorescent probe Fluo-4 and studying the alterations in $[\text{Ca}^{2+}]_{\text{cyt}}$ generated by Ca^{2+} release from the endoplasmic reticulum, and by extracellular Ca^{2+} entry through store-operated Ca^{2+} channels. The dynamics of actin polymerization was performed transiently expressing LifeAct-Ruby.

Results show that the alteration of actin polymerization by CytD treatment significantly increased hPMCA4 activity (102%). On the other hand, in absent of CytD, actin polymerization dynamics did not change after TG stimulus, while after Ca^{2+} stimulus, an actin reorganization was observed. This reorganization takes place at the same times that the hPMCA4 increases its activity, suggesting that hPMCA4 may be activated by actin depolymerization in the cells.

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Biophysical Characterization of lipid diacetylene mixtures during UV exposure

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Abstract

Interest in polymer lipids arose as an option to combine both liposomes and polymer characteristics in one system, extensively showing its biocompatibility.

The design of nano-photopolymerizable liposomes relies on two advantages: lipid self-assembly properties and photoactivatable bonds in diacetylene lipids moiety along their acyl chain. Closely packed and adequately ordered, diacetylene lipids undergo polymerization when they are irradiated with UV photons. Under these illumination conditions, liposomes show 1,4-addition to form alternating ene-iyne polymer chains.

Thus, this study aimed to evaluate the potential use of the binary and ternary mixtures of polymerizable diacetylene lipid 1,2-bis (10,12-tricosadiynoyl) -sn-glycero-3-phosphocholine (DC_{8,9}PC) with DMPC, DOTAP, DSPE PEG₂₀₀₀ and DSPE PEG₂₀₀₀ amine, which will be used in the design of a photosensitive nanocarrier for drug delivery. UV-Vis, FTIR spectroscopy, Bright Field microscopy, DLS, Z potential, and rheology were studied in the liposomes.

An overview of UV diacetylene nano-size, its structural and functional characterization is provided. The degree of polymerization and polymer length was estimated between 5-11 monomers units. We performed a deconvolution analysis of ATR-FTIR. The deconvolution revealed at least three prominent peaks in the monomer state and broad background peaks that appear upon UV exposure. Peaks of the DC_{8,9}PC over the range 2300-1900 cm⁻¹ show an expected significant change: reducing the triple carbon bond to double carbon-carbon bond (C = C). Region 1750-1650 cm⁻¹ shows the vibrational modes of hydrogen bonding of the headgroup. The region 1500-1450 cm⁻¹ corresponds to CH₂ in-plane bending and CH₂ symmetric stretching, hence packing alkyl chains.

The formulations developed show good stability (high z potential), small size enough (15 nm average, measured by DLS), and good cellular uptake and biocompatibility probability.

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Development of artificial models for the study of amphiphilic derivatives of L-ascorbic acid that interact with mitochondrial membranes

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Amphiphilic derivatives of ascorbic acid (ASCr), such as ascorbyl palmitate, ascorbyl myristate, and ascorbyl laurate, are compounds that have shown promising anticancer activity. Given that the mechanism of action of ASCn is poorly elucidated, one of the preliminary hypotheses is that they act by uncoupling the mitochondrial pH gradient. This is why we set out to study the mechanism of action of ASCn using liposomes as mitochondria models.

For this, we have generated giant unilamellar vesicles (GUVs), by means of electroformation. The GUVS contained POPC or the mixture that resembles the composition of the inner membrane of mitochondria (PC/PE/PI/Cardiolipin/Cholesterol, 46:30:7:7:10), to which a fluorescent membrane was added and a biotinylated lipid to anchor them to the bottom of the well. These vesicles encapsulate pyranine at pH 8, which is a fluorescent probe that presents different absorption maxima as a function of pH.

Once the GUVs were obtained, they were diluted in citrate buffer pH 6, in order to mimic the pH gradient present on both sides of the inner mitochondrial membrane. Finally, the GUVs were exposed to the different ASCn and carbonylcyanide-p-trifluoromethoxyphenylhydrazone (FCCP), a compound known to uncouple the mitochondrial pH gradient.

The intravesicular pH change caused by these compounds was monitored by means of confocal fluorescence microscopy, and subsequently, determining the ratio of the relative intensity of fluorescence absorption at 450 nm and 400 nm of pyranine. These, correspond to the absorption maxima at pH 8 and pH 6 respectively of pyranine, setting the emission at 510 nm. With the development of this technique, we hope to differentiate the mechanism of action of ASCn on the inner mitochondrial membrane, discriminating a pH dissipation action from general permeabilization or lysis of the membrane.

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Effect of ionic strength on Ibuprofenate adsorption on a lipid bilayer of dipalmitoylphosphatidylcholine from molecular dynamics simulations

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In this work, the free energy change in the process of transferring ibuprofenate from the bulk solution to the center of a model of the dipalmitoylphosphatidylcholine bilayer at different NaCl concentrations was calculated. Two minima were found in the free energy profile. A local minimum -located in the vicinity of the membrane- and the global free energy minimum is found in the tail region. The downward shift of free energy minima, with increasing NaCl concentration, is consistent with the results of previous works. Conversely, the upward shift of the free energy maximum with increasing ionic strength is due to the competition of sodium ions and lipids molecules to coordinate with ibuprofenate and neutralize its charge. In addition, normal molecular dynamics simulations were performed to study the effects of the ibuprofenate on lipid bilayer and in presence of a high ibuprofenate concentration. The effect of ionic strength on the properties of the lipid bilayer and on lipid-drug interactions was analyzed. The area per lipid shrinking with increasing ionic strength, volume of lipids and thickness of the bilayer is consistent with the experimental results. At a very high ibuprofenate concentration, the lipid bilayer dehydrates, and it consequently transforms into the gel phase.

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Evaluation of the effect of Miltefosine on membrane curvature

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Miltefosine is an amphiphilic drug originally conceived as an antitumor agent. Nowadays, its use is focused on the treatment of some forms of leishmaniasis and breast cancer metastasis. Although its mechanism of action is not completely known, several studies have demonstrated its influence on cholesterol homeostasis, and the physical properties of biological membranes such as the relaxation of highly curved structures.

The aim of our work is to study the effect of miltefosine on highly curved membrane structures. For this, we propose the analysis of two different artificial membrane systems.

The first system consisted of using confocal microscopy to observe giant unilamellar vesicles (GUVs) of POPC:GM1 (90:10) that, under specific conditions of electroformation and a ten-fold dilution in HEPES solution, spontaneously exhibit highly curved structures in their interior produced by an area excess in the GUVs' inner leaflet. This allows us to evaluate the effect of miltefosine when added from the outside of the vesicles, showing that the highly curved structures retract due to the incorporation of the drug, causing the recovery of symmetry in the bilayer.

The second system is focused on the evaluation of the phase transitions of DOPC:glycerol monooleate (GMO) lipid dispersions, which, in the presence of miltefosine, undergoes a transition from a cubic phase (highly curved structure) to a hexagonal phase, a more relaxed structure. For this purpose, we used the spectral phasors approach to analyze the fluorescence emission spectra of the LAURDAN probe of several DOPC:GMO mixtures and in the presence of the drug. This probe is highly sensitive to its environment and gives information about the lipid's phase state.

Overall, our results highlight the occurrence of a scarcely-studied modulation of the membrane physical properties by the incorporation of an amphiphilic drug, opening a new perspective on the manipulation of membrane-modulated cellular processes.

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Gabaergic insecticides exploration: interaction with biological membranes. An experimental and *in silico* approach.

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The study of many insecticidal compounds includes the knowledge of their interaction with a specific receptor protein under the ligand-receptor model. However, considering membrane receptors and mainly to lipophilic ligands, this interaction can be non-specifically modulated by changes in the physical properties of the membrane, due to the activity of these same products on the surrounding lipid molecules modulating the supramolecular organization of the receptor environment. The objective of this work is to describe how the lipophilic gabaergic insecticide Fluralaner affects the biophysical properties of DPPC Langmuir monolayers used as model membranes. We analyzed these effects by experimental and *in silico* complementary approaches. Experimental penetration isotherms indicated that the Fluralaner can be absorbed into the monolayer. Langmuir compression isotherms showed that Fluralaner molecules produce an expansion in the DPPC isotherm, by acting as spacers between the phospholipids. Also, the partition of Fluralaner molecules into the lipidic phase affects the characteristic DPPC phase transition, making it occur at higher surface pressure values. To provide a molecular insight into the behavior of the system, we performed ALL ATOM detail Molecular Dynamics simulations of DPPC monolayers in the presence of Fluralaner at different molecular packing states of the phospholipid. We were able to determine the probable distribution of Fluralaner at different regions of the DPPC monolayer and to observe an expansion of the simulated DPPC compression isotherm in the presence of Fluralaner, as observed experimentally. We are currently performing a series of umbrella sampling simulations to compute the potential of mean force (PMF). This will allow us to gain a thermodynamic insight on the partition of the insecticide into the lipidic phase.

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Interaction of polymyxin loaded polypeptide with bacterial membrane models

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It is now known that the interaction of peptides with biological membranes is a key process in the study of the different cellular mechanisms and metabolic pathways necessary for cells to carry out their function, since this interaction can affect the properties of the lipid bilayer such as selective permeability and modulate curvature. The biological role of peptides in membrane interactions is determined by the charge present in the sequence of amino acids that form these biomolecules. Certain cationic polypeptides act as antimicrobial agents, altering the cell membrane of bacteria, due to the binding with the outer membrane in Gram-negative bacteria, finally causing their death, as is the case of the cyclic polypeptide, Polymyxin or also called Colistin B. In this work we seek to gain knowledge about the mechanism of action of this antimicrobial peptide at the molecular level, as well as its effects on biological membranes, by studying different models of mimetic membranes using Langmuir- Blodgett phospholipid monolayers. The effect of pH and lipid composition on the interaction with polymyxin was studied by performing fixed-area Gibbs isotherms and Langmuir isotherms, measuring surface pressure and surface electric potential simultaneously. The results of this work indicate that pH modulates the surface activity of the peptide at clean interfaces and when interacting with negatively charged lipid monolayers. The insertion would be through a mechanism not yet elucidated, which is being studied.

Analysis of the structure and surfactant activity of formulations containing exogenous pulmonary surfactant and antibiotics

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Inhaled antibiotics (Atb) allow the delivery of high drug concentrations into the lungs and reduce systemic toxicity. Nowadays, local administration of Atb in the lung is only possible by nebulization of intravenous formulations, with undesirable side effects. The incorporation of Atb in the membranes of an exogenous pulmonary surfactant (EPS) could be a novel alternative to avoid the side effects and improve drug delivery. EPS would act as a carrier with therapeutic effect, so it is essential that Atb s incorporated into the EPS do not affect its biophysical properties.

Objectives: To study the effects of Gentamycin (Genta), Amphotericin (Amph), Itraconazole (Itra), and Levofloxacin (Levo) on the structure and biophysical properties of EPS.

Methods: EPS alone or added with each Atb were labeled with nitroxide spin labels to study the fluidity (order parameter S) and the lateral structure (S/W ratio) of the EPS membrane by Electron Spin Resonance. The EPS macrostructure (heavy and light subtypes), the amount of Atb incorporated in the EPS membranes and the tensioactive properties (ST) were also analyzed.

Results: Amph and Itra were incorporated into EPS bellow 1 and 5 %m/m respectively and Levo was incorporated until 20%m/m. Genta is an hydrosoluble Atb, so did not incorporate to EPS membranes. Only Genta altered EPS macrostructure increasing the % of heavy subtype.

Genta 10% and 20%m/m and Amph 3%m/m increased membrane fluidity ($p < 0.01$) while Itra and Levo did not significantly change this property. Amph 3%m/m was the only Atb that modified the lateral structure. The presence of none of the Atb tested, at any concentration, significantly changed the tensioactive properties of the surfactant.

Conclusion: Incorporation or encapsulation of these Atb to the EPS did not affect its physiological properties since all the values obtained were within the acceptable limits of surfactant activity, so the EPS can be considered a good alternative as carrier of these drugs.

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Brain cortex lipid extracts from hypertensive rats with omega-3 fatty acids-supplemented diets analyzed by Langmuir monolayers and Brewster Angle Microscopy

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Consumption of polyunsaturated fatty acids (PUFA's) of the omega-3 series - eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)- produces beneficial effects in patients with neurodegenerative diseases. The incorporation of omega-3 in membranes may affect their biophysical and biochemical properties and, ultimately, cellular pathophysiology.

In this work, we evaluated the effect of early supplementing in diet with EPA and DHA over membrane composition and properties. We analyzed Langmuir monolayers composed of total lipids of rat brain cortex isolated from male wistar normotensive (W) and spontaneously hypertensive (SHR) rats. W and SHR animals were randomly separated into four groups: W control (W-C), SHR control (SHR-C), W treated (W-T) and SHR treated (SHR-T). All groups were fed with standard rodent chow diet; the treated groups received omega-3 PUFA orally every day (EPA 20:5 and DHA 22:6 doses 200 mg/kg body mass/day) during 16 weeks. On sacrifice day, cortex samples were isolated, and the total lipids were extracted.

Lipid extracts were spread at the air/buffer interface and the phase behavior and rheological properties of the lipid monolayers were studied by recording the isothermal compression of the films and micrographs acquisition by Brewster Angle Microscopy (BAM) for the four conditions.

All lipid extracts showed coexistence of liquid phases (liquid-expanded or Le and liquid-ordered or Lo) at low lateral pressures. W-T cortex lipids showed slightly slower miscibility surface pressures than W-C samples (~6.7 mN.m-1 vs. ~8.5 mN.m-1). Interestingly, in SHR-T samples the miscibility pressure was higher compared to SHR-C (~14.5 mN.m-1 vs. 7.8 mN.m-1). Accordingly, SHR-T samples showed higher differences in the mean gray values between the Le and Lo phases with a decrease in the gray levels compared to SHR-C. These findings evidence an alteration in the lipid composition of cortex membranes related to both the pathological and treated condition.

Determination of the refractive index and thickness of phospholipid Langmuir monolayers from reflectivity

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Using the reflectivity and refractive index obtained on lipid monolayers (DMPC & DPPC) and a mathematical model (Drude's Approximation), the thicknesses of the monomolecular films were calculated. The thicknesses were then compared with reference thickness values measured directly by Grazing Incidence X-Ray Off Specular Scattering (GIXOS) at the beamline XRD-2, Laboratorio Nacional de Luz Sincrotron (LNLS, Brazil) also in Langmuir monolayers. The reflectivity was obtained in a Brewster's Angle Microscopy (BAM) and the refractive index was obtained in the same setup via Contrast Matching by varying the refractive index subphase. The results showed an acceptable approximation of thickness for one of the lipids (DMPC) with an overestimation of around 5.5% and an error margin 1 order of magnitude smaller than the reference technique (GIXOS). If GIXOS are used with a correction proposed in our group, then 0.17 nm should be added to the raw measurement, the agreement is also acceptable. Nevertheless, the other lipid (DPPC) showed a big overestimation and the same error margin as before. If correction of 0.17 applies, then the thickness (2.14 nm) is closer to the 2.6 nm by BAM. This side-by-side comparison allows us to better asses the limitations of each technique when measuring monolayer thickness and provides insight into plausible sources of error, such as unaccounted anisotropy in the liquid condensed phase of DPPC, due to axial tilt of lipid molecules, this deviations from the ideal Fresnel Interface on which the model is based and thus help to improve future experiments.

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Liposomes in skin: penetration enhancers, nanoparticles or active drugs?

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Due to their amphiphilic nature, phospholipids self-organize into liposomes when dissolved in water. We tend to think of them as forming particles. In particular when preparing liposomes to be used for drug delivery, nanosized particles are the configuration of choice. In this process, the individual nature of the lipid being used is lost: any phospholipid, or indeed any amphiphilic molecule, will do.

In the case of liposomes for topical use however, the effects observed can (and should) be described in other terms. Lipids can be considered either as a penetration enhancer (a solvent, a continuum) or as an active drug (individual molecules with a specific chemistry). We will present evidence for this argument with some examples from our lab.

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Liquid-liquid phase separation modulates membrane structure and hydration

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Cells can condensate some of their intracellular components into compartments known as membraneless-organelles (also called liquid droplets or protein condensates). Among them are nucleoli, Cajal bodies, P-bodies, and stress granules, all existing as liquid protein-rich droplets within the cell and arising from the condensation of cellular material in a process termed liquid-liquid phase separation. Although termed “membraneless”, these droplets can come into contact, wet, and reshape membranous compartments. Whilst there are some reports exploring membrane remodelling by liquid polymer-rich droplets, this is still an underexplored field, and the molecular origin of membrane reshaping remains unresolved. Here, we use giant unilamellar vesicles (GUVs) either encapsulating aqueous two-phase systems (ATPS) or in contact with soy glycinin condensates to explore the molecular interactions with the membrane when liquid-liquid phase separation takes place near the bilayer. We use a microfluidic setup to have a better control of individual vesicles and the external milieu, together with two-photon excitation fluorescence microscopy to exploit the spectroscopic properties of LAURDAN, an environmentally sensitive fluorescent probe. Hyperspectral imaging and fluorescence lifetime imaging (FLIM) combined with a multidimensional phasor analysis, provides us with spatial and time resolved information on these systems. The phasor plot analysis allows us to evaluate the changes that occur when the polymers/protein condensates interact and wet the membrane, enabling us to identify regions of different hydration properties within the membranes. Altogether, this approach provides new information on the relationship between membrane wetting, hydration, and spontaneous curvature generation driven by protein/polymer liquid-liquid phase separation.

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Model lipidic membrane as a Losartan bioindicator

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Liposomes formed by phospholipids can be used as model membranes to study the behavior of biological membranes because they show similarities such as their structure, composition, and selective permeability [1]. Therefore, lipidic membranes can also be used to predict the effects of exposure of living organisms to contaminants of emerging concern (CECs) [2-4]. CECs are bioaccumulative compounds of different chemical nature that can be potentially toxic to the environment, and they have gone unnoticed due to lack of information or adequate techniques for their identification. Among them are products such as: drugs, pesticides, cosmetics, cleaning supplies and personal hygiene among others.

The liposome becomes a bioindicator that will provide information on the toxicity and interaction of CECs with cell membranes, detecting the action of emerging pollutants on living organisms. In this way, the impact of toxic substances in terms of bioaccumulation and bioavailability in the body can be anticipated, using a membrane model that mimics the cell membrane, minimizing the use of biological membranes and living beings for experimentation.

In this work, the interaction of an emerging contaminant present in water was explored: Losartan, an antihypertensive agent, with a mixed model membrane composed of phosphatidylcholines: DPPC-POPC (0.75: 0.25) through studies of zeta potential, particle size, conductivity, density, and speed of sound. It was observed that the presence of Losartan in the water affects the surface and mechanical properties of the model membrane.

The results are expected to provide insight into the toxicity, bioavailability, and bioaccumulation of emerging pollutants in living beings.

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Natural membranes: lipidomic, membrane fluidity and phospholipid order.

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Biological membranes are complex assemblies constituted by a wide variety of lipids and proteins. It is generally accepted that biological membranes have lateral heterogeneity derived from non-homogeneous distribution of their components and are usually assumed as coexisting liquid disordered- liquid-ordered phases.

In this study, the lipid composition of *Bos taurus* (Bt) synaptosomal membranes and *Triatoma infestans* (Ti) ganglionic membranes was determined by HPLC-MS/MS. Membrane order and fluidity of whole natural membranes (NMs) and extracted lipid membranes (LMs) was evaluated by Laurdan generalized polarization (GP) and anisotropy (A) determinations in steady state fluorescence spectroscopy experiments. There are no reports neither about the lipid composition of NMTi nor biophysical studies of them. Also, we analyzed a 4-lipid component model membrane (BMs) as a potential biomimetic membrane of the lipid fraction of the natural membranes. LMs were obtained by a modified Blight & Dyer partition, the content of PC, PE, PS, SM, PG, PI, and Sterols was determined.

We observed that in LMBt, PC polar group was the most abundant, with 18C fatty acids and 2 unsaturations being the most frequent. On the other hand, LMTi had PE as the main polar group, with 18C fatty acids and 3 unsaturations being the most common. A 14/28% of plasmalogen was detected and 17/11% of sterols respectively.

It was observed that in all cases Laurdan GP values and anisotropy diminish with temperature increase and that the values of NMTi are lower than those of NMBt. This result is in accord with a previous report with DPH probe and with the sterol % present in each membrane.

Laurdan fluorescence spectra were compared at physiologically relevant temperatures (37°C for Bt and 28°C for Ti) and was observed that both the NMs and LMs presented similar λ_{max} values (434nm for Bt and 436nm for Ti) with a 490nm shoulder in both cases, suggesting phase coexistence. BMBt were capable of simulate the order of NMBt and LMBt since their anisotropy values were similar. However, BMTi showed higher anisotropy values meaning a more ordered membrane.

Interestingly, GP and anisotropy values obtained at relevant temperatures were similar for NMs and LMs pointing to a conserved compensation balance between cholesterol and PE content that might be needed for membrane fluidity control in the NMs studied as was proposed in bibliography.

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Photopolymerizable phospholipids and lipids from lung surfactants in association with mucolytics

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In the present work, we obtained polymeric diacetylene liposomes¹ that can associate N-Acetyl-L-Cysteine (NAC), a broad spectrum mucolytic. The reason for studying these formulations is that they could be applied in the future as NAC delivery systems, with a possible dose reduction but maintaining its effect. Liposomes used herein are obtained by a photopolymerization reaction, thus gaining stability and rigidity. Lipids belonging to lung surfactant² were added in different ratios to the formulations in order to maximize its possible interaction with the lung tissue.

This formulation could efficiently transport NAC to exert its mucolytic.

Formulations so obtained presented high levels of polymerization. Also, they present small hollow fibers structures with a high number of polymeric units. These types of arrangements could present advantages in the field of drug delivery, giving the possibility of a controlled release³. Lipopolymers with lipids from lung surfactant associated with NAC are promising complexes in order to treat not only respiratory illnesses. The stability of the formulation would allow its inoculation through other routes such as the oral one, helping the reposition of NAC as an antioxidant drug.

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Studies of the physicochemical properties of membranes from Barley roots exposed to cold stress and recovery periods

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Barley tissues can respond to cold stress by modifying the fatty acid composition of the membrane. What remains unknown is whether the modifications in membrane composition are maintained or rapidly adapted during stress relief. The present study attempted to compare the lipid profile and physicochemical properties of membrane from barley roots both under chilling stress treatment and in the subsequent recovery to stress. Lipids were obtained through a single-extraction method with a polar solvent mixture, directly from seedlings which had been exposed at 4°C for 36h (stress condition) and 25°C for 2h or 24h (recovery conditions). Mass spectrometry analysis indicate that lipid metabolism was significantly affected by chilling. Most of the glycerolipids analyzed returned to control values during short- and long-term recovery, whereas several representative phosphatidic acid (PA) molecular species were edited during long-term recovery. The natural extracts were studied using monolayers at the air/liquid interface and large unilamellar vesicles (LUVs). Surface pressure and surface potential compression isotherms, as well as monolayer observation with Brewster-angle microscopy (BAM) were performed at 25°C and 4°C. All isotherms showed a similar shape, with a kink at about 25 mN/m indicating a structure rearrangement of the monolayer, also detected by the compressional modulus. At 4°C the collapse pressure decreases from 45 (at 25°C) to 35 mN/m. BAM images of monolayers during compression showed the apparition of light gray dots (thick regions) upon compression, with no significant differences between conditions. LUVs were studied with zeta potential determinations as well as Laurdan fluorescence data. Regarding to zeta potential, significant differences were found between each condition and also between measurements at 25°C and 4°C during control, stress and short recovery, but in long recovery the values at both temperatures were similar. The generalized polarization (GP) value determinate by Laurdan fluorescence intensity decreased during stress condition and restored to control values during long recovery. All these results highlight the impact of the phospholipid remodeling on the physicochemical properties of membranes that may be responsible for plant membrane adaptation and tolerance.

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TM4 peptide, as a representative model of the nAChR, conditions its lipid microenvironment

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Nicotinic acetylcholine receptors (nAChRs) are integral membrane pentameric proteins that belong to the Cys-loop superfamily of ligand-gated ion channels. Given that the nAChR is a transmembrane protein, the properties of the membrane where it is embedded are essential for its correct functioning, and since even small changes in nAChRs activity can cause great effects on human biology, the interaction between lipids and the nAChR is of great relevance. The transmembrane domain of each subunit of the nAChR is composed of four segments (TM1-TM4) in which TM2 segments form the ion channel pore and TM1, TM3, and TM4 are located more externally. TM4 segment is the most exposed and it is in intimate contact with both the surrounding membrane lipids and the rest of the transmembrane segments, these being two facts that make it a key participant in lipid-nAChR interaction. Due to the abundance of Chol in neural membranes and its importance and implication in different human diseases, in this work we studied the relationship between domains either rich in cholesterol (Liquid order domains, Lo) or poor in cholesterol (Liquid disorder domains, Ld) and the nAChR. To this end, we worked with GUVs, giant unilamellar vesicles that can be observed under the microscope, formed by two different lipid compositions (with nanoscopic or microscopic Lo and Ld domains) containing or not a synthetic peptide corresponding to the TM4 segment of the nAChR. Confocal Fluorescence Microscopy, Fluorescence Recovery After Photobleaching (FRAP) measurements and experiments of Miscibility Transition Temperature showed that TM4 peptide concentrates in Ld domains. Furthermore, we observed that its presence alters the intrinsic properties of the domains as well as the whole microscopic membrane organization. It is well known that lipids condition nAChR functioning, here we demonstrate that just this peptide, as a minimalist but still representative model of the nAChR, can perturb its lipid microenvironment as well.

Differential functional properties between homoeric and heteromeric 5-HT3 receptors

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The serotonin type 3 receptor (5-HT3) is a ligand-gated ion channel that converts the binding of serotonin (5-HT) into a transient cation current and mediates fast excitatory responses in peripheral and central nervous systems. Five human subunits (A-E) have been identified to date, of which only the A subunit can form homomeric receptors (5-HT3A). We performed single-channel and macroscopic current recordings from cells expressing different subunit combinations to determine how the accessory subunits (B-E) contribute to the receptor functional properties. The incorporation of the B subunit increased about 5-fold the EC50 value of 5-HT responses with respect to 5-HT3A receptors. At the single-channel level, 5HT3A receptors cannot be studied due to their reduced conductance. Thus, we also used a high-conductance A subunit (AHC) that forms channels of about 4.5 pA, with openings grouped in long activation episodes of 287 ± 123 ms (-70 mV). The heteromeric 5-HT3AB channel showed reduced amplitude (about 2.0 pA) and briefer activation episodes with respect to the homomeric receptor (47.1 ± 4.4 ms). The pattern of channel activation did not show a clear 5-HT concentration dependence for 5HT3A and 5HT3AB receptors. Also, both receptors were activated and potentiated by the allosteric agonist carvacrol. Expression of AHC with C, D or E subunits showed opening events of different amplitudes, indicating that A can assemble with one of these accessory subunits. However, the frequency of opening was very low, suggesting that more complex subunit arrangements may occur. Molecular docking studies provided insights into how the different accessory subunits may contribute to the binding site. This study provides information required for identifying functional heteromeric receptors in native cells and for understanding their distinct roles and opens doors for the development of specific ligands.

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GABA_A receptor modulation by acetone

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Ketogenesis is a metabolic process that generates ketone bodies (acetone, acetoacetate and β-hydroxybutyrate) from the β-oxidation of fatty acids. Ketogenic pathway occurs sporadically under normal conditions, but is positively regulated when carbohydrate stores decrease significantly or by increases in the concentration of fatty acids, for example during fasting, prolonged exercise, excessive alcohol consumption, low insulin levels or ketogenic diets. Ketogenic diets are commonly indicated as a therapy for treating central nervous system disorders, including Parkinson's and Alzheimer's disease, amyotrophic lateral sclerosis, refractory epilepsy and schizophrenia. However, the actions of ketone bodies on neurotransmission have been poorly explored and the mechanisms responsible for the therapeutic benefits of ketogenic diets are under study.

Recently, Pflanz et al. (2019) reported the effects of ketone bodies on a ionotropic GABA receptor, describing acetone as a positive modulator, and β-hydroxybutyrate as a negative modulator, of the GABA_Aα1β2γ2 receptor function. In order to analyze the effects of ketone bodies on GABAergic neurotransmission, and since subunit composition confers specific pharmacological properties to the GABA_A receptor subtypes, we evaluated the sensitivity of different phasic and tonic GABA_A receptors to acetone. Human GABA_A receptors were expressed in *Xenopus laevis* oocytes and chloride currents recorded by two-electrode voltage-clamp. Bath applications of acetone were performed at the plateau of GABA-evoked responses. Acetone inhibited GABA_Aρ1 and potentiated GABA_Aα1β2, GABA_Aα5β3 and GABA_Aα4β3δ receptor responses. Effects depended on both acetone and GABA concentration and were completely reversed after washout. Docking experiments with acetone are being carried out in order to identify the interaction sites.

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Inhibition of the gastric H,K-ATPase by potassium competitive acid blockers

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The gastric H,K-ATPase is a membrane protein found in the parietal cells of the stomach, where it couples H⁺ extrusion to the uptake of K⁺, leading to the acidification of gastric juice (1). Acid-related diseases are an important public health issue where the mainstay of treatment has been the suppression of H,K-ATPase activity. As K⁺ plays a vital role in this catalytic cycle, for the dephosphorylation of the H,K-ATPase and the subsequent conformational changes, acid secretion can be inhibited by agents that are competitive with respect to K⁺ binding. This argument led in the past decades to the development of a new class of acid suppressants, known as potassium competitive acid blockers (P-CABs). Since a systematic investigation of enzyme-inhibition mechanisms has become a fruitful way to

design and test new drugs, the effects of P-CABs-type inhibitors have been extensively studied analyzing how the apparent Michaelis and Menten parameters are affected (2). Working with the non-compartmentalized enzyme preparation, we analyzed the interactions between K⁺, the H,K-ATPase, and two different inhibitors under steady state conditions. Our results from ATPase activity as a function of K⁺ concentration was described by a rational function where the maximal exponent on [K⁺] is 2. Data show that K⁺, as a product, can inhibit the reaction steps that involve its release, which implies that ATPase activity would not obey the Michaelis-Menten equation. This can lead to mistakes when analysing the results according to variations in V_{max} and K_M. Here we propose a minimal model to describe

the binding of K⁺ to different enzyme conformations and the inhibition by P-CABs compounds allowing a more realistic evaluation of their effects.

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Montelukast activation of BK (Slo1) channels: Role of the β accessory subunits.

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The large conductance voltage- and Ca^{2+} - dependent K^+ channel (BK channel) has been proposed as a possible pharmacological target in smooth muscle and central nervous system pathologies. Recently, the channel modulation through its β accessory subunits emerged as the key to obtaining compounds showing a tissue selective activity. It has been shown that leukotriene B4 activates the BK channel only in presence of $\beta 1$. So, we hypothesize that Montelukast (MTK), a leukotriene receptor antagonist, can share this property. Here, using the patch-clamp technique in the inside-out configuration, we studied the effect of MTK on the BK channel expressed in HEK cells with or without different β subunits ($\beta 1$, $\beta 2$, and $\beta 4$). 1 μM MTK slightly activated BK channel expressed without any β subunits: MTK changed the voltage dependence of channel activation (left shift of the G-V curve, $\Delta V_{1/2} = -10.8 \pm 3.7$ mV; $n=3$; $p<0.05$). This effect was increased in presence of $\beta 1$ ($\Delta V_{1/2} = -66.9 \pm 19.4$ mV; $n=8$; $p<0.05$) or $\beta 4$ ($\Delta V_{1/2} = -55.2$ mV ± 16.5 ; $n=4$; $p<0.05$). Moreover, when the channel was expressed with $\beta 2$, the resultant inactivating BK current was not affected by MTK (peak current ratio: $I_{\text{MTK}}/I_{\text{control}} = 0.87 \pm 0.07$, NS, $p>0.05$). Then, we studied in more detail the effect of MTK on BK channel expressed with $\beta 1$ (BK $\alpha/\beta 1$). MTK activated the BK $\alpha/\beta 1$ channel in a concentration-dependent manner inducing channel activation from submicromolar concentrations as 30 nM, MTK $\Delta V_{1/2} = -11.8 \pm 1.0$ mV; $n=5$; $p<0.05$). Moreover, MTK effect was characterized by a significant delay in the time constant of the BK $\alpha/\beta 1$ deactivation process (for 1 μM MTK, Deactivation: $\tau_{\text{control}} = 0.8 \pm 0.1$ ms; $\tau_{\text{MTK}} = 10.0 \pm 0.8$ ms, $n=9$, $p<0.05$) without affecting the activation kinetic. Our results indicate that MTK at very low concentrations directly activates the BK channel probably stabilizing the open conformation and, this modulation is increased in the presence of $\beta 1$ and $\beta 4$, while is abolished when the channel is expressed with $\beta 2$.

A novel receptor target for old anthelmintic drugs evaluated in the nematode *Caenorhabditis elegans*

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Ivermectin (IVM) and piperazine (PZE), which are agonists of Glutamate-gated chloride channels and GABA_A receptors, respectively, are marketed drugs used in anthelmintic therapy. Here we discovered a novel target of these drugs by evaluating their effects on the free-living nematode *C. elegans*. Nematodes contain a homomeric 5HT-gated chloride channel, MOD-1, that modulates locomotor behavior. Due to its absence in vertebrates, MOD-1 emerges as an attractive anthelmintic drug target. By electrophysiological recordings from cells expressing MOD-1, we deciphered its pharmacological properties and searched for novel ligands. Macroscopic currents activated by 5-HT showed that MOD-1 desensitizes slowly and recovers from desensitization in about 1 s. Dose-response curves revealed an EC₅₀ for 5-HT of ~1 µM, similar to that of 5-HT₃A receptors. The partial agonists tryptamine and 2-Me-5HT showed very different efficacies between MOD-1 and 5-HT₃A receptors. IVM and PZE did not activate MOD-1 but acted as non-competitive antagonists. IVM produced a slight and irreversible inhibition whereas PZE led to a profound and reversible inhibition, indicating that MOD-1 may be involved in their anthelmintic effects. Also, the specific GABA_A receptor agonists, muscimol and isoguvacine, inhibited MOD-1 currents. We performed locomotor activity assays of wild-type (WT) and mutant strains to establish MOD-1 as a novel anthelmintic target. We found that 5-HT produced a rapid paralysis of WT worms while the MOD-1 mutant strain was resistant, thus confirming MOD-1 as the functional target of 5-HT. The exposure of worms to 5-HT combined with IVM or PZE at concentrations at which they do not act at their canonical receptors reduced the 5-HT paralyzing effect, thus supporting the negative modulation of MOD-1 detected in electrophysiological recordings. This study contributes to our understanding of the action of drugs to treat parasitic diseases and to guide future drug discovery efforts.

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DESK could phosphorylate residues different from the conserved H188

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Thermosensor DesK is a transmembrane histidine-kinase that allows the bacterium *Bacillus subtilis* to adjust the levels of unsaturated fatty acids required to optimize membrane lipid fluidity. The cytoplasmic catalytic domain of DesK behaves as a kinase at low temperature and as a phosphatase at high temperature, regulating in this way the phosphorylation state of the response regulator DesR.

Induced conformational changes create a vulnerable metastable state due to enrichment of packing defects, the so-called dehydrons, which are solvent-exposed backbone hydrogen bonds. These transient intramolecular hydrogen bonds promote their own dehydration and therefore drag the binding partner into their proximity, thus, illustrating that are not only determinants of protein structure, but also promoters of protein association and functional modulation.

We propose that the presence of dehydrons in the ATP binding domain (ABD) of DesK will determine its reactivity, so we employed an extension of Pymol software to identify dehydrons near the gamma phosphate in the ABD, and that could participate in phosphorylation reactions. Besides the conserved histidine H188 phosphorylatable residue located in the dimerization-histidine-phosphorylation domain (DHP), we identified H335 as an alternative phosphorylatable residue which is located at 3.7 Å from the gamma phosphate and would be activated by dehydrons Ser332 (dehydron A: His335-Ser332) and Gly339 (dehydron B: His335-Gly339), both located in the solvation sphere. These aminoacids are part of the C-terminal of the ATP lid which has been previously reported to interact with helix α 1 of the DHp catalytic domain. Therefore, to test the role of His 335 in DesK activity, we constructed a series of mutants replacing H335 and its dehydrons. We obtained mutants that gained activity compared to wild-type DesKC and, while others loss kinase activity. Our results are consistent with the hypothesis proposed above and reflect that DesK phosphorylate residues different from the conserved H188.

Effect of the lipid environment on plasma membrane Ca^{2+} ATPase in the absence of its auxiliary subunit neuroplastin/basigin

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The plasma membrane Ca^{2+} -ATPase (PMCA) is a highly regulated transporter responsible for the extrusion of Ca^{2+} from all eukaryotic cells. PMCA isoforms are expressed in different tissues and perform their function through the adjustment Ca^{2+} concentration in membrane microdomains that are related in Ca^{2+} signaling events. In humans, the variants PMCA1 and 4 are ubiquitously expressed whereas PMCA2 and 3 are expressed in specialized tissues. ATPase activity is modulated by several modulators being calmodulin one of the most effective by binding to the C-terminal domain of PMCA.

It has recently been discovered that neuroplastin and basigin, two glycoproteins, would act as a mandatory subunit of PMCA¹. PMCA forms a heterodimer with neuroplastin or basigin depending on the neuroplastin/basigin ratio in each tissue². The interaction of PMCA with neuroplastin/basigin is essential for its correct targeting towards the plasma membrane. It has also been proposed that basigin or neuroplastin would be necessary for PMCA to be active⁴, but these studies remain controversial.

The aim of this work was to study the effect of the mandatory subunit of PMCA on its Ca^{2+} -ATPase. To this end, we used the PMCA of human erythrocytes as a model, whose majority variant is PMCA4. Our results showed that: (1) Basigin, but not neuroplastin, was present after purification of PMCA, (2) PMCA reconstituted in detergent micelles, was inactive in the absence of basigin; (3) PMCA reconstituted in mixed micelles of detergent-phospholipids, was active in the absence of basigin, (4) The apparent affinity for Ca^{2+} and Mg^{2+} of PMCA in erythrocytes plasma membranes was similar to that PMCA reconstituted in mixed micelles. This suggests that PMCA's activity and Ca^{2+} apparent affinity depend on the biophysical characteristic of the lipid environment in the absence of its auxiliary subunit. Moreover, we study the activation of PMCA by calmodulin and the accessibility of the C-terminal auto-inhibitory domain in these different lipid environment.

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Inhibition of the plasma membrane calcium pump by aurintricarboxylic acid

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The plasma membrane calcium pump (PMCA), is a high-affinity calcium pump that ejects calcium from the cell into the extracellular environment, using the hydrolysis of ATP. There are four isoforms of PMCA, encoded by four different genes. PMCA1 and PMCA4 are expressed in many tissues, while PMCA2 and PMCA3 are more tissue or cell-specific. PMCA4 is the most common isoform expressed in red blood cells. It has been found to negatively regulate angiogenesis, which makes this PMCA blockade of interest for research¹. In living cells, aurintricarboxylic acid (ATA) has been shown to be a specific inhibitor of PMCA at low concentrations², although its inhibitory mechanism remains unknown.

This study aims to characterize the PMCA inhibition of ATA. For this purpose, PMCA was purified from human erythrocytes, containing 80% PMCA4 and 20% PMCA1, and the inhibition of PMCA by ATA was studied under different conditions.

Our current results show that: (1) ATA inhibits PMCA Ca^{2+} -ATPase activity with an apparent affinity (K_i) of 0.25 μM ; (2) The inhibition and activity of PMCA Ca^{2+} -ATPase were completely restored after dilution of ATA in the medium (3) The apparent affinity for ATA increased with Mg^{2+} concentration, but did not modify with ATP or Ca^{2+} ; (4) In the absence of Ca^{2+} and ATP, ATA also inhibited PMCA phosphatase activity with a K_i of 0.1 μM ; (5) When increased concentrations of ATA were added to the purified PMCA, the fluorescence of ATA increased suggesting that ATA binds to the pump.

Our results suggest that ATA does not bind to the ATP binding site of PMCA and that Mg^{2+} is required for complete inhibition of activity. Moreover, the variation in fluorescence of the binding can make it possible to obtain information on the interaction between this molecule and different structural states of PMCA.

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New functional caffeine analogs as possible multitarget potentiators of the cholinergic system

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Since cholinergic deficit is characteristic of Alzheimer's disease, two possible molecular targets for its treatment are the acetylcholinesterase (AChE) and the nicotinic acetylcholine receptors (nAChRs). In previous studies of our group, we found that caffeine behaves as an agonist of the nAChRs and confirmed that it inhibits the AChE. We subsequently synthesized hybrid caffeine analogs by connecting a theophylline group with a pyrrole group via a carbon linker of different lengths (3 to 7 carbon atoms). All the compounds inhibited the AChE and activated the nAChR with higher potency than caffeine. Some of them conduct the receptor to a desensitized and agonist-refracting state, while others make the receptor quickly return to a resting, agonist-responding state. Based on these results, in this work we synthesized three new hybrid analogs of the synthetic compound with a linker of 5 carbon atoms, which belongs to the desensitizing group, maintaining the theophylline structure but changing the pyrrole group by piperidine, 1-methylpiperazine or dimethylamine. All analogs inhibited the AChE with higher potency than the precursor. Using Crystal violet (CrV) fluorescence probe, an open channel blocker with higher affinity for the desensitized than for the resting state of the nAChR, we observed that the compounds with piperidine and 1-methylpiperazine caused nAChR conformational changes that could be related to a conformational transition corresponding to receptor activation followed by stabilization in the desensitized state. In contrast, the compound with the dimethylamine group did not conduct the nAChR to a desensitized state. Our results provide new insights into structure-activity relationship for this group of functional multi-target drugs giving valuable tools to transform the search of new drugs from random screening into a detailed rational drug design of new interventional therapies in neurological diseases.

Structural and functional characterization of the sensor/transducer MecR1 protein of *Staphylococcus aureus*

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Methicillin resistant *Staphylococcus aureus* (MRSA) is a pathogen that poses a worldwide threat. Resistance to β-lactams in MRSA is regulated by the membrane proteins BlaR1 and MecR1. We are interested in unveiling how β-lactams activate these metalloproteases, resulting in manifestation of resistance. Mechanistic and structural information of these sensor proteins is needed to rationally design compounds capable of blocking signal transduction that could be used in combination therapies with currently available β-lactam antibiotics. We have informed a model for full-length MecR1 combining modeling, experimental mapping of transmembrane topology, the X-Ray structure of the sensor domain and molecular simulations. However, no high-resolution models of full-length MecR1 or BlaR1 are available. In this work we intended to structurally and functionally characterize full-length MecR1. We have been able to over-express full-length MecR1 (E205A mutant) as a fusion to Mistic in *E. coli* BL21 StarTM (DE3) membranes. Furthermore, we have purified it by affinity and size-exclusion chromatography. To determine the homogeneity and oligomerization state of Mistic-MecR1 we have performed SEC-MALS and AUC experiments, which showed two oligomerization states in detergent micelles: a monomer (major species) and a dimer. Both species presented an active sensor domain that was irreversibly acylated by the fluorescent penicillin Bocillin-FL. We also performed Circular Dichroism spectra in different conditions. The protein was thermostable and could only be denatured in the presence of SDS and urea. We carried out crystallization assays, but we only obtained poly-crystals that could not be optimized. We concluded that Mistic-MecR1 is not a homogeneous sample, as it is in equilibrium between monomer and dimer, which might be interfering with crystallization. We will hence explore the possibility of characterizing Mistic-MecR1 using Cryogenic electron microscopy (cryo-EM).

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Unravelling the physiological and molecular function of the betaine-sensitive receptor in *Caenorhabditis elegans*: a new target for anthelmintic drugs.

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The free-living nematode *Caenorhabditis elegans* is a model of parasitic nematodes. This worm has the most extensive family of Cys-loop receptors, which are pentameric ligand-gated ion channels that include nicotinic receptors (nAChRs). *C. elegans* contains an extended family of nAChRs but the functional properties and roles of many of these nAChRs remain unknown. ACR-23 is a nAChR present in neuronal and muscle cells of nematodes and is not conserved in vertebrates. It is a cation-selective channel activated by betaine (BE) and sensitive to monepantel (MNP), a new anthelmintic drug. Given the limited information about its functional role in nematodes, we explored ACR-23 from a pharmacological, physiological, and molecular perspective. Locomotion assays of adult worm showed that BE significantly increased motility. This effect was not observed in *acr-23* mutants, indicating that BE acts through ACR-23. MNP decreased worm motility in the adult stage in a concentration-dependent manner with an EC₅₀ of about 30 µM. The *acr-23* mutant showed different MNP sensitivity compared to the wild-type strain, indicating that, in addition to ACR-23, other receptors may be targeted by MNP. By using a primary culture of *C. elegans* muscle cells, we described for the first time the properties of BE-elicited single-channel currents. Opening events showed a mean duration of 0.3 ms and amplitude of about 2.4 pA at a holding potential of 100 mV. The identification and functional characterization of receptors for BE and MNP provides insights into the molecular basis of anthelmintic action, which pave the way for anthelmintic drug design.

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Bioinspired Rhamnolipids: new bioactive amphiphiles with nanobiotechnological potential

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Surfactants find applications in almost all chemical industries, but their use can have a negative impact on the surrounding and health. Growing environmental concerns have led to the development of innovative and environmentally friendly surfactants. Thanks to their emulsifying, antimicrobial and activating properties of the innate defense of plants, the rhamnolipids (RL) show a high potential for their use in areas such as biomedicine and agriculture^[1]. However, natural RLs (produced by *Pseudomonas* and *Burkholderia* bacterias) are a difficult mixture to isolate and purify, and they have deleterious effects on eukaryotic cells thus limiting their widespread use.

In this work, we study the physicochemical properties of four new bio-inspired mono-rhamnolipids with same acyl length but different sugar-lipid bonds synthesized by our collaborator, Dr. Patrick Martin. We propose that small chemical variations to the natural rhamnolipids can result in new compounds that show enhanced bioactive properties. These molecules were firstly characterized through ¹H and ¹³C NMR, and then their physicochemical and surface activity properties were evaluated in comparison with the natural mixture of RLs produced by *Pseudomona aeruginosa*. The experimental methodologies used such as Langmuir films and Dynamic Light Scattering allowed us to obtain parameters as the critical micelle concentration (CMC), mean molecular area (MMA), surface tension and their interfacial stability. We found that CMC values were between 0.04 mM to 0.5 mM and MMA values were between 50 to 90 Å²/molecule, being both parameters highly dependent on the sugar's chemical function. On the other hand, all the compounds studied showed a low stability at the air/water interface, since we observed a decrease in the area occupied by monolayers of RLs spreaded onto a saline solution at pH 7 registered at a constant surface pressure of 20 mN/m as a function of time.

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Biophysical characterization of Dendrimer-Vismodegib complexes and skin penetration studies

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Vismodegib (VDG) is an antineoplastic drug for basal cell carcinoma (BCC) treatment. Although it is effective it has severe side effects. The complexation of VDG with polyamidoamine dendrimers (PAMAM-D) could contribute to the reduction of its side effects by means of site-specific topical delivery and using lower amounts of drug. PAMAM-D are three-dimensional macromolecules with hydrophilic chemical groups on the surface and hydrophobic ones inner. The aim of this work is to design and optimize PAMAM-D complexes with VDG for the topical delivery of the active principle.

We have used generation 4.0 and 4.5 PAMAM-D, with amino-ended (D.NH₂), hydroxyl-ended (D.OH), and carboxyl-ended (D.COOH) groups. VDG was purified from commercial tablets. The complexes were obtained by incubation of VDG and PAMAM-D in methanol, and after drying under vacuum pellets were reconstituted in PBS. These complexes were characterized by spectroscopic techniques such as UV-Vis, fluorescence, among others, which provided a possible D-drug interaction mechanism.

The VDG solubility in PBS reached after complexation was 67.3 μM with D.NH₂, 54.0 μM with D.OH, and 63.0 μM with D.COOH. In the three complexes obtained it was corroborated that VDG interact with the inner hydrophobic pockets of PAMAM-D. Particularly, the electron clouds of VDG could interact electrostatically with the terminal amino groups of PAMAM-D. In contrast, VDG would not interact with the hydroxyl or carboxylic groups. On the other hand, the fluorescence characterization allowed us to select VDG complexes with D.NH₂ and D.COOH to monitor skin penetration. Additionally, VDG accumulated in the skin was quantified by HPLC. The D.NH₂:VDG penetrated deeper into human skin explants than D.COOH-VDG. The concentration of VDG recovered from the skin was 4.6 and 13.0 μM for D.NH₂ and D.COOH, respectively.

These results show a promising first approach to therapeutic potential use of these formulations in the treatment of BCC.

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Self-assembly of Trypanosoma cruzi aldo-keto reductase protein: Insights from simulations

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Chagas disease, caused by the protozoan *Trypanosoma cruzi*, affects a total of 6 million people in the countries of the Americas. Particularly, in Argentine, about 1.5 million people are infected and it is estimated that 30% of them will eventually develop progressive heart disease. Recent research suggests that the *T. cruzi* aldo-keto reductase (TcAKR) enzyme may be involved in the metabolism of action of trypanocidal drugs such as Benznidazole (one of the drugs used to treat *T. cruzi* infection)¹ and β-lapachone². On the other hand, experimental kinetic studies demonstrated that TcAKR exhibits aldo-keto reductase activity with Michaelian (hyperbolic) kinetics and quinone oxide reductase activity with sigmoidal kinetics. In agreement with the sigmoidal kinetics, different experimental techniques indicate that TcAKR is found as a monomer, dimer and tetramer³. In this work we used computer simulations to elucidate the possible aggregation of the TcAKR protein. In particular, through extensive Molecular Dynamics simulations of octamers, dimers and monomers, we found that the protein aggregates in supramolecular, such as dimers and tetramers. We were able to identify affinity regions (specific interactions) between monomers as well as amino acid residues at the monomer interfaces that stabilize the formation of supramolecular arrays of TcAKR. Furthermore, structural stability was evaluated looking at the RMSD. We identified regions with higher mobility, mainly the last 30 residues at the C-terminus. The findings of self-aggregation capacity of TcAKR could explain the differential kinetic behavior of the enzyme. Aggregation affects both the structure and dynamics of the system, suggesting that it would also affect the binding of potential drugs.

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Protein auto association, concentration and temperature: Insulin as an example.

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Insulin is one of the most medically relevant biomolecules and, at least in part, its fame is due to its close link with diabetes, a major public health problem worldwide. Like most recombinant proteins that are used as biotherapeutics, conformational changes suffered by insulin might lead to reduced clinical efficacy and, ultimately, to immunological reactions that preclude its use. Despite its low molecular weight (<6 kDa), insulin presents complex features typical of much larger proteins. In aqueous solution, this molecule is in equilibrium between, fundamentally, two species: monomers and dimers. Its small and well-known structure allows us to show how modifications in its concentration and/or in the working temperature affect the equilibrium position. In this sense, circular dichroism (CD) shows variations in molar ellipticity as a function of concentration in the range 0.06-0.73 mg/mL, as well as changes in spectral shape after a slight increase in temperature (<45°). Needing minimal sample handling (just serial dilutions) and aided by CD, this system presents a robust and versatile model that allows multiple levels of analysis. Although often underestimated, information provided by these experiments allows comparison with other techniques, such as SEC-FPLC and SLS/DLS, and shed light on relevant aspects such as conformational changes, monomer-dimer equilibrium, among others. As such, insulin becomes a simple and interesting case study to be implemented for the teaching of biophysical techniques, relevant for the structural study of biomolecules with therapeutic application.

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Gangliosides smelt nanostructured amyloid A β (1–40) fibrils in a membrane lipid environment

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Gangliosides induced a smelting process in nanostructured amyloid fibril-like films throughout the surface properties contributed by glycosphingolipids when mixed with 1-palmitoyl-2-oleyl-phosphatidylcholine (POPC)/A β (1–40) amyloid peptide. We observed a dynamical smelting process when pre-formed amyloid/phospholipid mixture is laterally mixed with gangliosides. This particular environment, gangliosides/phospholipid/A β (1–40) peptide mixed interfaces, shown complex miscibility behavior depending on gangliosides content. At 0 % of ganglioside covered surface respect to POPC, A β (1–40) peptide forms fibril-like structure. In between 5–15 % of gangliosides, the fibrils dissolve into irregular domains and they disappear when the proportion of gangliosides reach the 20%. The amyloid interfacial dissolving effect of gangliosides is taken place at lateral pressure equivalent to the organization of biological membranes. The domains from the monolayers are clearly evidenced by using Brewster Angle Microscopy and Atomic Force Microscopy when the films are transferred onto a mica support. The domains are Thioflavin T (ThT) positive when observed by fluorescence microscopy. We postulated that the smelting process of amyloids fibrils-like structure at the membrane surface provoked by gangliosides is a direct result of the interfacial properties and a new interfacial environment imposed by the complex glycosphingolipids. We add experimental evidence, for the first time, how a change in the lipid environment (increase in ganglioside proportion) induces a rapid loss of the asymmetric structure of amyloid fibrils by a simple modification of the membrane condition (a more physiological situation).

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Role of extracellular matrix components in apolipoprotein A-I amyloidosis: Differential interactions and organ-specificity

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Protein misfolding is involved in chronic diseases known as amyloidosis. Among other, human apolipoprotein A-I (apoA-I) natural mutants are retained specifically in different tissues, inducing protein aggregates that cause organ damage. The reason of such selectivity is far to be known. The variant Arg173Pro, is associated with cardiac amyloidosis. With the hypothesis that the extracellular matrix (ECM) plays a role in the retention of this variant, we have previously shown that Arg173Pro (but not the Wt) interacts with heparin (HEP), a model of glycosaminoglycans (GAGs) present in proteoglycans. We here expand those results and used different approaches (electrophoresis, fluorescence chromatography), to test and characterize the interaction with more biological GAGs such as dermatan sulphate (DS). Our results indicate that this variant binds with higher affinity than the Wt to both GAGs (HEP and DS). Elution profiles through a column with HEP covalently linked indicated that the binding to DS displaces the interaction with HEP at physiological pH. The protein-GAGs complexes are detected by the fluorescent dye toluidine blue and are characterized by native electrophoresis. The GAG: protein complexes were resolved by polyacrylamide gel electrophoresis, and developed by silver nitrate staining. The Arg173Pro variant is mostly retained by interacting with DS and HEP.

This finding is interesting, since both GAGs are associated with amyloid deposits

The results obtained here partially support the particular tropism that this variant may have for the tissues it affects, since GAGs such as HEP and heparan sulfate are concentrated in myocardium. In addition, the study of the protein-GAGs interaction is clue for the synthesis of sulphated compounds, which could act as inhibitors of this interaction. Previous laboratory results confirm in synthetic models that apoA-I Arg173Pro interacts more with matrices with a higher sulphated content.

We conclude that a delicate specificity may determine the interaction of apoA-I variants with the components of the ECM. Particularly, with the charge provided by dermatan sulphate and heparan sulphate, these results suggest that the retention of Arg173Pro could be mediated by this interaction.

Quantification of molecular association from single molecule localization microscopy data

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Single molecule localization microscopy (SMLM) provides super resolution images that contain information of spatial molecular localization as well as a map of the underlying cellular structure responsible for the molecular distribution. The reliable computation of quantitative parameters for association of identical or different molecules must take into account the labeling-detection efficiency, multi blinking effects, the volume in which molecules are located, its shape, and the density of each association partner. Detection efficiency and multi blinking have been extensively treated in the literature. In this work, we will concentrate the analysis on the determination of the effect of the size and shape of the volume containing the molecules, as well as on the influence of molecular density. To this aim, we will use the association parameter:

$$Q_{as} = [N_{AL} / (N_A \cdot N_L)] \cdot V$$

where N_i represents the number of localizations of each partner in the volume V . Simulations guided us to optimize a procedure and criteria to characterize this volume. For this we used and compared methods based on density kernel and Voronoi and Delaunay tessellations. We could obtain density parameters from the distribution that could recover the input volume with >90% coincidence by either method. Finally, we analyzed literature data [1] of SMLM of 5 receptors and evaluated Q_{as} for the 15 possible auto and crossed associations, taking into account the size and shape of the containing volume.

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CONICET

Aggregation properties of the synthetic peptidic amphiphile OOCWW-palmitic acid studied by molecular dynamics simulations

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The synthetic peptide based amphiphile (PA), built by binding the aminoacid sequence OOCWW with a palmitic acid molecule, has been studied using molecular dynamics simulations (MD). This molecule has an important biotechnological relevance as a carrier that favours the DNA transfection in host cells. These PA molecules can form dimeric complexes in aqueous solutions at appropriate pH through the oxidation of their C residues, generating a gemini like amphiphile (PGA). Atomistic MD of these PA and PGA molecules, isolated and in lipid aggregates in aqueous solution, have been performed to provide a basis for interpreting de fluorescence spectra at different pH. The most populated structural conformations of the isolated PA and PGA in solution have been determined together with the interactions that favors such behavior and the solvent distribution in the environment of the indole W moiety. The MD have shown that PA or PGA molecules, aggregate spontaneously in aqueous solution, building H-bonds among their charged groups and lowering their solvent accesible surface. The simulations have also shown that miceles of 30 PA or of 10 PGA molecules respectively, with their hidrocarbonated tails toward their centre and the peptidic moiety exposed to the solvent, are stable through the reached simulation times. The gyration radius of these miceles, the stabilizing interactions and the solvent distribution in the environment of the W residues have been determined and analyzed. A semi-classical calculation of the changes in the dipolar transition energy, due to the interaction of the W residues with the charged groups of the micellar and solvent environment, has been performed for all the studied cases along the simulated time. The environment of the W residues dynamics along the time, give rise to a dipolar transition energy distribution that can be used to correlate the fluorescent spectra features with the aggregation state of the molecules.

Assessment of insect RDL receptor homology models for virtual screening: impact of the template conformational state in pLGICs

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Pentameric ligand-gated ion channels (pLGICs) constitute a large family of transmembrane receptors. This family includes the γ -aminobutyric acid (GABA) receptors. The RDL homopentamer is the main GABA receptor in the insect nervous system. It presents structural differences with vertebrate GABA_A receptors that result in a particular pharmacological profile. Therefore, the RDL receptor (RDL-R) is one of the most relevant targets for insecticides. Due to the difficulties related to pLGICs crystallization, many studies have used homology modeling to obtain the structure of these proteins and to perform computational studies about their ligands binding. However, the impact that the template conformational state could have on the model virtual screening (VS) performance has not been studied in detail. The aim of this work is to obtain RDL-R homology models in different conformational states and to evaluate their performance in a retrospective VS of channel-blocker insecticides. Fifteen RDL-R models were obtained, based on different pLGICs templates, whose structures represent three conformational states: closed, open and desensitized. With these models, molecular docking assays were performed with a set of active ligands and decoys. To evaluate the VS performance, the area under the ROC curve and the BEDROC score were calculated for each of the models. In addition, molecular dynamics simulations (MDS) were performed for the best models among each of the conformational states. The initial structures were obtained from the docking poses of the insecticide fipronil. VS performance parameters showed variations according to the conformational state of the templates. The correlations of these parameters with different variables were evaluated to analyze which were the determinant factors for a correct identification of active ligands. Structural properties of the channel pore, such as the solvent-accessible area and volume and the pore diameter at some specific residues could explain the differences in VS performance. The best results were obtained for a model based on a closed template. MDS confirmed that the expected interactions between the binding site residues and fipronil were present only in the closed model. These results show that different templates should be explored to obtain accurate RDL homology models, particularly focusing on the template conformational state. The model that presented the best performance parameters could be used in a prospective VS.

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Computer simulation of chemical reactivity without choosing a reaction coordinate

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Modeling realistically chemical reactive processes requires the use of QM schemes for the reactive portion of the system and extensive sampling to obtain the free energy profiles that yield the necessary kinetic and thermodynamic information. Moreover, free energy simulation has to overcome two main difficulties: 1) The long simulation time required for the sampling of activated processes (i.e. those which involve crossing potential energy barriers); 2) The necessity of choosing a reaction coordinate *a priori* to describe the process of interest.

The first obstacle is usually addressed by performing biased sampling, a method which modifies the potential energy surface in order to artificially favour the sampling of originally high energy configurations. Regarding the simulation of free energy profiles, methods like umbrella sampling and steered molecular dynamics belong to this family. These approaches, however, require the user to select a coordinate to describe the processes. For simple processes, this coordinate could be a combination of geometric parameters like bond distances, angles and torsions. For complex processes, choosing an adequate reaction coordinate is not trivial. Unfortunately, what is the meaning of simple and complex in this context is not an easy question, given that even chemical reactions involving less than a few atoms can exhibit complex mechanisms. In the context of potential energy profiles simulations, there are a few options to avoid the problem of the reaction coordinate selection for the simulation. However, the options available for free energy profiles obtention are limited and, in most cases, have not been applied to biological relevant processes.

In this work, we present our implementation of the Nudged Elastic Band method for finding minimum free energy paths on the free energy surface, which works by analysing AMBER trajectory files. We applied this method to study conformational changes on the alanine dipeptide and other model systems, and to study the S-Sulphydratation of methanesulfonic acid in aqueous solution. Our results suggest that reliable estimations of free energy barriers and free reaction energies can be obtained by affordable simulations at the QM-MM level.

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Gabaergic insecticides exploration: characterization of one GABA_A receptor binding site.

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The insect GABA_A receptor (RDL) is an important protein target for natural and artificial insecticides that act by blocking the channel and inhibiting the ions flux through it. We aim to characterize the non-competitive antagonists II (NCA-II) binding site, to develop tools that allow us to obtain new insecticidal compounds that share this same blocking site. This site is targeted by isoxazolines, being Fluralaner a canonical representative of this chemical class. We pretend to evaluate whether these compounds could interact with the membrane where the receptor is embedded and module it. Different 3D models of the *Aedes aegypti* homopentamer RDL were developed in our group by homology modelling, due to the fact that there is no available crystallographic structure of the receptor. Distinct templates corresponding to different conformational states of the channel were used. We have evaluated the performance of the models by molecular docking assays of Fluralaner, in order to determine which of them is capable of replicating the Fluralaner binding pose. The model obtained from the 3RHW template (PDB ID 3RHW), which has an open channel conformation, was the one that best replicated both the interactions reported by biochemical assays, as well as the spatial orientation of the Fluralaner molecule at the receptor blocking site. These molecular docking assays of the Fluralaner at the NCA-II site allowed us to select a model of the *Aedes aegypti* GABA_A receptor that reproduces that reported by previous works. We performed ALL ATOM detail Molecular Dynamics simulations to characterize the membrane-receptor-ligand interactions, which allowed us to validate the selected model since the interactions between the Fluranaler and the rest of the system components were as expected.

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Immunoinformatics approach to epitope-based peptide vaccine design and receptor binding prediction for EIAV ENV polyprotein

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Equine infectious anemia virus (EIAV) is a lentivirus causing a persistent infection in horses characterized by recurrent febrile episodes and high levels of viremia associated with a novel antigenic strain of the virus. Retroviral envelope glycoproteins are synthesized as polyprotein precursors that are cleaved, during transport to the surface of infected cells, into the surface (SU-gp90) and transmembrane (TM-gp45) subunits, being the most antigenic viral proteins.

In this report, we identified some novel T-cell (ELA-I, ELA-II) and B-cell epitopes within of the most antigenic EIAV ENV polyprotein with the potential to induce humoral and cell-mediated immunity, using a comprehensive set of immunoinformatics tools. In particular, we worked with American strains to propose an epitope-based peptide vaccine that involves our geographical zone as the main target.

Two regions were found in SU-gp90 and 5 regions in TM-gp45 with great potential to be used as CD8+ peptide epitopes and 3 regions of SU-gp90 and 6 in TM-gp45 to be used as CD4 + epitopes. Eighteen B-cell regions had epitopes localized within both proteins, Taken together we selected those regions that contained B-cell and T-cell epitopes, localized between the following aminoacid residues 130-190; 430-530; 590-670 and 700-790.

The prediction of the transmembrane zones in TM-gp45 allowed to establish the probable location of the regions previously found as well as the fusion peptide.

From the consensus sequences of each of the proteins, their 3D structure was modeled using the new machine learning-based algorithm, AlphaFold 2. Only a central region within SU-gp90 could be reliably modeled and was used for the prediction of conformational B epitopes. Since this region also contained the likely receptor binding region for EIAV, docking of this region with the Equine Lentivirus Receptor was performed to try to further delineate the shape of the binding site. In order to select the best docking pose, the predicted glycosylation sites were taken into account. The coiled-coil region of GP45 was also modeled but with a lower confidence level.

Our results were compared with experimental data from our own laboratory and also with those of other American researchers, taken from de Immuno Epitope Data Base (IEDB). Some of the epitopes found in this work are consistent with those previously reported, while others are new and will be used in the design of a new epitope-based vaccine.

Machine learning multi scale QM-MM schemes: validation of possible ways to evaluate the subsystem coupling energy

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Machine Learning is gradually changing the paradigm of theoretical chemistry. The development of faster computational algorithms and hardware is producing a transformative impact on chemical sciences, amplifying insights available from computational chemistry methods.

In this context, we are working on the application of a machine learning methodology called ANI -developed at the University of Florida by our collaborator Adrián Roitberg- in a QM-MM multiscale scheme called QM(ANI)-MM.

In this scheme the QM region is described with the ANI method, while the MM region, much less demanding in numerical terms, is described by classical force fields. Specifically, the ANI algorithm yields, in addition to the energy, a set of atomic charges which will make possible to determine the coupling energy between the quantum and classical subsystems by a simple partial charges approach called mechanical embedding.

In this work, we propose to validate the mechanical embedding approximation of the value of the QM-MM energies calculated with different partial charges partition schemes. We will compare it with the reference values provided by a QM-MM approach which uses density functional theory for the QM description. We will employ a set of 18 amino acid-like species composed in our benchmark set. The latter is reported in the database ANI-1x, for which three different schemes of partial charges are reported. The species are solvated with 4000 water molecules, and a 100 ps long classical MD is performed to relax the solvent, and generate representative solvated structures.

Furthermore, we implement a correction term considering the polarizability of the atoms present in the QM region, that has the potential of improving the quality of the scheme obtained.

Membrane surface charge and their effect on hIAPP structural stability

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Human Langerhans islets amyloid polypeptide, or hIAPP, is the main component of amyloid deposits found in the pancreas of patients with type-2 diabetes (T2D). In aqueous solutions, hIAPP monomers mainly adopt unstructured conformations, which give intrinsically disordered protein (IDP) characteristics. In presence of lipid membranes, hIAPP binds to the surface of the membrane acquiring α -helical conformations. Experimental studies have shown that hIAPP aggregation is accelerated in lipid bilayers rich in anionic phospholipids. This same phenomenon has been observed in membrane phospholipids that contain oxidative modifications in one of their aliphatic chains. Thus, as the ratio of peptides bound to the membrane surface increases, hIAPP monomers are cooperatively transformed from α -helical intermediates to amyloid aggregates in β -sheets. With this in mind, we have carried out an atomic description using Molecular Dynamics (MD) simulations, analyzing the interaction of the hIAPP monomer with zwitterionic, anionic and, hydroperoxidated lipid bilayers. Our results suggest that in zwitterionic bilayers the peptide-membrane interaction is not stable. However, in anionic bilayers despite the stability of the peptide-membrane interaction, the high charge on the surface reduced the conformational stability of hIAPP decreasing its helical content, in contrast to the hydroperoxidated bilayer where the charge balance inside the bilayer promotes the peptide-membrane interaction allowing an increase in helical content of hIAPP.

Molecular dynamics simulations reveal that the immunogenic 33-mer peptides can adopt both folded and unfolded conformations

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The 33-mer peptide is an immunodominant fragment of α 2-gliadin protein. It is found in gluten, and is proposed as the responsible of the immune response in celiac disease and other gluten-related disorders. This peptide is deamidated in the small intestine which allows its recognition by the immune system, initiating the inflammation process. Both peptides are intrinsically disordered peptides (IDPs); this means that they do not have a uniquely defined structure, which hampers a comprehensive description of their spatial configuration. Their structures have not yet been well characterized, so the aim of this work is to study the conformational landscape of wild-type and mutated peptides through computational methods.

Using GROMACS2020, 1.5 μ s atomistic molecular dynamics simulations were performed to evaluate the conformational space of peptide 33-mer and its deamidated bioactive form employing two force fields suitable for intrinsically disordered peptides, AMBER03ws and AMBER99-disp. In the present work the structures obtained from two different force fields were assessed, evaluating on one hand, the possibility of using these force fields with these IDPs and, on the other hand, the structural differences due to the deamidation. In general, both peptides show a preference for extended conformations. However, the 33-mer peptide adopts more folded structures than the deamidated one. The trajectories with both force fields describe similar regions of the conformational landscape, but histograms of Ramachandran plots show more content of polyproline II secondary structure when using AMBER99-disp force field. The structures obtained for the peptides from both force fields are suitable for examining their aggregation.

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Rational design of peptides with antimicrobial activity against the pathogenic bacterium *Pseudomonas aeruginosa*

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The Gram-negative bacterium *Pseudomonas aeruginosa* (PA) is an opportunistic pathogen capable of producing different infections. It has a marked tendency to acquire resistance to antibiotics for clinical use. It is among the main bacteria with priority for the development of new antimicrobials, according to the WHO.

We propose as a new strategy the *in silico* development of peptides whose mechanism of action consists of interrupting protein-protein interactions (PPI) vital for PA. With this purpose, we have focused on the node proteins that participate in fundamental processes for the survival of the bacteria.

The periplasmic protein TolB was chosen as a target, since it participates in the division and transport of other proteins through the PA membrane. Its essential role has been demonstrated in the literature and there are several crystalline structures of its homologue in *E.coli* alone (1C5K and 1CRZ) or interacting with one of its interaction pairs, such as PAL protein (2HQ5 and 2W8B) and colicin fragments (2IVZ, 3IAX and 4JML).

We generated a TolB model of PA using MODELLER and a multi-template homology modeling approach. This model was used to build four different TOLB-partners complexes using PAL, and three colicines as partners, resulting in 4 different protein-protein structures. These complexes were used as starting points for 30 ns molecular dynamics simulations (DM) in order to evaluate if the complex remains stable. Also, we determined the energy contribution of the residues of the ligands in the interaction with TolB using GROMACS. We found that the complexes showed low RMSD values and high affinity energies demonstrating that PAL and the colicins fragments can interact with TolB of PA.

Finally, we designed various 10 amino acid length peptides that mimics the interaction of PAL and colicins using the residues that showed the stronger affinity with TolB of PA and we performed a virtual screening of them by docking using the Autodock CrankPep program.

Structure-based analysis from cestodes' FABPs towards virtual screening campaigns

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Echinococcosis and Cysticercosis are listed among WHO's list of Neglected tropical diseases (NTD's), affecting people in tropical and subtropical areas. *Echinococcus granulosus* and *Echinococcus multilocularis* are the causative agents of cystic and alveolar echinococcosis, respectively, while *Taenia solium* is the parasitic agent involved in cysticercosis.

In general, cestodes present an incomplete lipid metabolism lacking many enzymes involved in the synthesis, so they must obtain these molecules from their hosts. In this sense, Fatty Acid Binding Proteins (FABPs) have been proposed as essential for cestodes' life cycle, because they are small intracellular proteins that bind fatty acids and other hydrophobic ligands, being important in lipid traffic and delivery of such compounds.

In the present study we propose the tertiary structure of FABPs from *E.granulosus*, *E.multilocularis* and *Taenia solium*, obtained from *in silico* methodologies such as Homology Modelling (HM) and Molecular Dynamics (MD). Each model generated by HM was validated using the knowledge-based potential QMEAN4 and Ramachandran Plot analysis.

On the other hand, MD simulations allow us to analyze the dynamic evolution of an atomic system and its interactions in a certain time period. From each validated-model, we performed 200 ns Molecular Dynamics Simulations with explicit water molecules. Full MD trajectories were then submitted to a Principal Component Analysis (PCA) and k-means clustering algorithm, in which we were able to obtain the main binding site conformers for each FABP model.

We developed 3D FABPs models for three clinical-relevant cestodes. This study will allow us to perform Molecular Docking protocols to carry out virtual screening campaigns using different drug databases in order to discover new potential compounds with biological activity against these etiological agents.

Keywords: Homology Modelling. Molecular Dynamics. Neglected tropical Diseases. FABP. *Echinococcus spp.* *Taenia solium*.

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Study of gene regulatory networks with interacting mRNAs and microRNAs, using a theoretical-experimental approach

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Encoding and regulating protein expression are equally important functions of RNA molecules, and their dysfunction might cause diseases. Understanding how these processes occur is critical from both a basic and applied point of view.

MicroRNAs are small RNA molecules that do not code for proteins and regulate their target, messenger RNA (mRNA), through a post-transcriptional mechanism. It is generally accepted that miRNAs repress gene expression by promoting the degradation of target mRNA and/or inhibiting its translation.

From a theoretical point of view, it has been proposed that the way miRNAs interact with their targets is through a sequestration mechanism. Additionally, different species of mRNA can compete to bind to the same miRNA. Together, these mechanisms, sequestration and competition, are believed to create an indirect interaction between the target mRNAs of the same miRNA, commonly called cross-talk, through which the expression levels of the targets could influence each other. These ideas have been called hypotheses on the effect of competition between endogenous RNAs (ceRNAs).

This project aims to characterize gene regulation networks with mRNAs and interacting microRNAs, to establish the principles that govern this interaction and to identify emerging behaviors, with potential applicability to other networks similarly organized.

Among the many aspects that could be studied in these networks, we propose to pay special attention to the regulation originated in the competition for limited and shared resources and the ability to generate responses optimal according to the temporal dynamics of the inputs received and the characteristics of the network.

A simple model of the biological interactions in the infection of the airways with Sars Cov 2

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The biology and pathogenesis of Covid 19 are not yet completely understood. Biochemical and anatomopathological observations in culture systems and human disease were mainly focused on the viral particle-ACE2 receptor interaction and also pointed to the strong involvement of innate cellular immunity in the acute form of the disease. Recently Baral et al, 2021 (<https://doi.org/10.1101/2021.07.17.452576>), presented a Molecular Dynamics model for the interaction between S protein RBD and its specific receptor ACE2, which includes the effects of mutations present in Delta strain on the affinity of the interaction and the possibility of immune evasion. Mathematical modeling of the disease covered mainly epidemiological as well as spreading control issues, mainly vaccination. Here we present our advances on modeling the biology and pathogenesis of Covid 19. The models are based on Lotka-Volterra equations and take into account clinical immunology biochemical determinations. One of the authors previously remarked upon the analogies between virus-immune system interactions and parasite-immune system interactions (*Comm. Theor. Biol.* 8, 1-21, 2003). Our description of the interaction leads to understanding the coexistence between species (mild cases) or excluding one of them (healing or death) during airways infection. Since S protein- RBD affinity, cellular activation, and extracellular viral particles were considered, the model could be extended to describe specific humoral and cellular responses during post-acute events.

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In silico approach for the study of interaction between the Mycobacterial phosphatase PtpA and the eukaryotic hTFP α

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Mycobacterium tuberculosis PtpA is a key virulence factor required for mycobacterial survival within host macrophages and it is a secreted bacterial factor that dephosphorylates several host proteins. One of those putative physiological substrates of PtpA is the human trifunctional enzyme subunit alpha (*hTFP* α) which catalyzes two of the four reactions of the mitochondrial beta-oxidation pathway. This eukaryotic protein is synthesized in the macrophage cytosol and then translocate to the mitochondria. Curiously, *hTFP* α was no longer detected in the mitochondria of macrophages during infection with the *M. tuberculosis* H37Rv. Therefore, our hypothesis is that the mycobacterial phosphatase PtpA acts on *hTFP* α in the cytosol of the macrophage, altering its subcellular location and probably contributing with the decrease of mitochondrial activity described during *M. tuberculosis* infection.

In this work, we perform a docking simulation approach between PtpA- *hTFP* α in order to identify the *hTFP* α phospho-tyrosine residue involved in the interaction. In addition, we performed long molecular dynamics simulations to analyze the stability and the structural effects of the interaction. The results allowed us to determine the P-Tyr271 of *hTFP* α as the potentially tyrosine targeted by PtpA. In the docking simulations this is the only tyrosine that, when phosphorylated, can fit surprisingly well within the catalytic site of PtpA. The interaction between both proteins stabilizes the catalytic D-loop of PtpA where the phospho-tyrosine of *hTFP* α is positioned. All the interacting groups from both proteins are located in an optimal position and the interactions appear to be stable over time in such a way that they allow the catalytic reaction to take place. Additionally, this tyrosine is located in a helix relevant for *hTFP* α membrane localization and activity, absent in bacterial TFP from the TB complex. Finally, these results are compatible with the SPR experiments which demonstrate that *hTFP* α interaction involves the active site of PtpA.

Lipid interchange in a hFABP. Questioning the portal opening model

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Fatty Acid Binding Proteins (FABP) are responsible for intracellular traffic of lipids and small hydrophobic molecules. Their structure is a beta barrel surrounding an inner cavity that accommodates fatty acids, enabling their transport in an aqueous medium inside the cell. The inspection of the crystallographic structure of the heart FABP (and, by extension, of the other proteins of the same family) induces to think in a model for lipid exchange that involves at least three stages: opening the so-called portal of the protein, ligand entry/release, and portal closure. Up to now, there is no experimental structural biology technique that could allow the direct observation of these stages. We performed Molecular Dynamics (MD) simulations of this protein in a crystal environment with an all-atom forcefield where the lipids, instead of being in the interior cavity, are in the crystal channels. In these simulations, we found more than ten events of lipid entry. From our MD simulations we can say that the dramatic conformational changes that are intuited from the observation of the crystallographic structure are not necessary for the entry of the lipid. Local fluctuations of the positions of the atoms of the portal region together with the elastic movement of the lipid, allow the entry. This entry does not occur by a unique path, so it should be defined better as a landscape rather than as a path even in our crystal system. The multiplicity of paths can be explained because the fatty acid interacts with the protein surface by hydrophobic effect in an nonspecific fashion proper to this interaction, until it stabilizes electrostatically by the interaction of the lipid head with residues 126 and 128. This counterintuitive result highlights the important role of MD simulations to show possible mechanisms at atomic level where we lack experimental evidence. As this behaviour was observed in a crystal confinement, it is expected even more variability in solution.

Spectroscopic (FTIR, Raman and UV-visible) and computational studies of Matrine alkaloid using DFT calculations

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Matrine is a bis-quinolizidine tetracyclic alkaloid that exhibit a wide range of pharmacological activity such as anti-cancer, anti-neuropathy, anti-viral and anti-parasitic [1]. The Matrine skeleton contains a quinolizidinic A/B ring system fused with a quinolizidinone C/D ring system and present four stereocenters, which generate eight pairs of enantiomers [2]. Despite this molecule is apparently rigid, the different conformations for each ring (chair, boat, half-chair, etc.) and the alternative trans-/cis-fusions for the ring junctions offer conformational variability, therefore a combined experimental and theoretical investigation into the structural and spectroscopic properties of this alkaloid was necessary. In this work, we present a complete spectroscopic and structural study of Matrine in solid phase and aqueous solution. The conformational preferences of this compound were investigated with the DFT/B3LYP hybrid functional with 6-31G(d,p) basis set. Infrared, Raman and UV-visible spectra were registered and the structural analysis was carried out using the Gaussian 16 program. Our results show that only one structure is most stable and it presents a good agreement with X-ray structure reported previously by Jin et al [3].

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Time correlations in a model of tissue growth

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Tissue growth is controlled by cell proliferation and apoptosis. The interplay between both cellular processes is crucial during physiological embryonic development as well as in pathophysiological conditions like cancer, among many other examples of biological and medical importance. In this study, we investigate the problem of tissue growth with a minimal Markovian mathematical model of the number of cells in a tissue subjected to both, cell proliferation and apoptosis. The model shows three dynamical phases, depending on the difference between the two relevant model parameters, the proliferation and the apoptosis rates: a growth phase and a decline phase in which proliferation is greater than apoptosis and vice versa, respectively, together with a homeostatic phase, where both rates are identical. The extinction probability is one in the decline phase and it is less than one (but not zero) in the growth phase. In the homeostatic phase, the extinction probability is one while the average number of cells is constant. To quantitatively characterize tissue growth dynamics, we computed the time correlation function for our model in these three regimes. We found that the time correlation is clearly different in the three dynamical phases and we verified this result numerically. Noteworthy, we found that that time correlations follow a power-law behavior in the homeostatic phase, suggesting that this phase is critical. We discuss the implications of this minimal model in the context of biophysics of tissue growth.

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Development and characterization of silver nanoparticles obtained by green synthesis from leaves of Aguaribay (*Schinus areira*).

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The increased emergence of antibiotic-resistant bacteria is a serious health problem worldwide. This situation has motivated the research of new antimicrobial agents. In this sense, nanoparticles have received increasing attention for their antimicrobial activity. The objective of this work was to obtain silver nanoparticles (AgNPs), from AgNO₃, by green synthesis using an aqueous extract of leaves of *Schinus areira* as a reducer and stabilizer agent, and characterization of its antimicrobial action against gram-positive and gram-negative bacteria. The AgNPs obtained were evaluated by UV-vis spectroscopy, Zeta Potential and DLS, confirming the presence of AgNPs with a maximum absorbance at 420 nm and the absorption intensity of the resulting spectra increased as a function of the amount of extract used (2 %, 4% and 8%). The AgNPs obtained with the low concentration of extract show the highest size and polydispersity (66,3±35,8 nm) while there were no significant differences between the other two remaining conditions with an average size around 40 nm. Later, the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (CBM) of the AgNPs obtained using 4% and 8% of leaves extract were determined in *E. coli* and *S. aureus*. Both nanoformulations showed high antibacterial activity with MIC values of around 2 pM and CBM of 4 to 8 pM. To a better knowledge of the antibacterial action, death curves were carried out. In agreement with the CBM data, both syntheses at 2 x MIC managed to reduce 90% of the bacterial population after 1 hour of incubation. Finally, to characterize the mechanism of action, determination of the oxygen reactive species (ROS) and membrane damage were evaluated by spectrofluorometry. Both AgNPs tested were able to increase ROS levels in *E. coli* and *S. aureus*, as well as induce some damage in the bacterial membrane, but with some differences among each synthesis. Overall, it can be concluded that these biosynthesized produce AgNPs that have colloidal stability and antibacterial activity toward gram-positive and gram-negative bacteria. Regarding the mechanism of action, our findings suggest that the intracellular ROS induction by AgNPs could induce oxidative stress followed by damage to bacterial membrane leading to cell death.

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

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The alpha-hemolysin of *Escherichia coli* (HlyA), induces the translocation of phosphatidylserine (PS) from the internal to the external layer of the plasma membrane of erythrocytes. On the other hand, during the malaria disease, *Plasmodium falciparum* grows inside erythrocytes and synthesizes proteins, like PfEMP1, that translocate to the erythrocyte membrane at specific membrane knobs. *P.falciparum* proteins and PS in the erythrocytes plasma membrane bind to specific proteins expressed by the endothelium, thereby inducing erythrocytes adhesion, a process that can lead to vascular obstruction and thrombotic events *in vivo*.

However, many aspects of the adhesion of erythrocytes are not yet understood. To investigate the adhesion mechanism in controlled conditions, we designed a biomimetic device that emulates the capillary architecture and the expression of adhesive molecules by the activated ECs.

Microfluidic chips were prepared in PDMS using molds fabricated by photolithography. The microfluidic chip was adhered to a glass surface by O₂ plasma treatment. The inner surface of the microfluidic channels was coated with the copolymer PLL-g-PEG-NTA.

We followed the surface functionalization by quartz microbalance and fluorescence microscopy using a GFP-His Tag. Then, we studied the adhesion of erythrocytes incubated with HlyA to a device functionalized with Annexin V-His Tag, at different controlled flows. We also studied the adhesion of *P.falciparum*-infected erythrocytes to a surface coated with CD36. Only the *P.falciparum*-infected erythrocytes displayed a higher adhesion to surfaces in comparison to non-treated erythrocytes. The infected erythrocytes remain adhered to the surface even at shear stresses up to 8 dyn/cm². Our preliminary results show that we can replicate the pathological adhesion of malaria-infected erythrocytes observed in tissues with this device. Future experiments will test the infected erythrocytes adhesion to surfaces where CD36 distribution on the surface is controlled at the nanometer scale using nanostructured surfaces.

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Obtaining protein hydrolyzates with antimicrobial activity from goat milk's whey

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The present work aims to obtain antimicrobial hydrolyzates from goat's whey obtained from a local cheese factory by means of enzymatic hydrolysis of the proteins present in it. For this, the goat's milk whey was pasteurized at 70 ° C, a process that caused the precipitate to obtain a soluble and insoluble fraction. Both of them were then lyophilized and stored at -20°C. The soluble fracción was analyzed by SDS-PAGE observing, as expected, that major proteins present are α -lactalbumin, β -lactoglobulin, and Sericalbumin. To obtain antimicrobial peptides from this fraction, enzymatic digestion with pepsin and rennet complex was performed. The results of SDS-PAGE demonstrated that both enzymes achieved the hydrolysis of proteins into smaller peptides. Likewise, the Bradford method was applied to determine the protein content in each of the samples. Assays were conducted to test the antimicrobial activity of the soluble fraction hydrolysates against *Escherichia coli* ATCC 25922 and *Staphylococcus aureus*. ATCC 25923 The results show that the hydrolyzates were able to inhibit the microbial growth of both strains on agar plates at 3 and 4 hours of incubation. The whey soluble fraction hydrolyzed with pepsin proved to be more efficient especially against *E. coli* strain, reducing the microbial load after 4 h of incubation by 2.5 times, with respect to 1 times reduction of the fraction with rennet. On the contrary, in the case of *S. aureus*, it was the fraction hydrolyzed with rennet that exhibited the highest antimicrobial activity with a reduction of 3 times with respect to less than one time of pepsin hydrolyzate. In addition, the duplication time of each bacteria in those conditions was also obtained, observing the higher effects in *E. coli* treated with the pepsin hydrolyzate with a duplication of this time. Afterward, by using ultrafiltration columns the peptides with less than 3000 KDa from both hydrolyzates (with Pepsine and Rennet complex) were purified, observing for both ultrafiltered hydrolyzates a significantly higher inhibitory activity confirming the presence of active peptides with low molecular mass.

Besides further experiments should be made to a better characterization, all these data confirm that whey goat milk is a good source for the obtention of antibacterial hydrolyzate peptides that could be applied in food, as coming from a safe source, with the advantage of its low cost as it is already considered waste from cheese manufacturing.

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BaCopA, a Cu(I) ATPase from the Antarctic bacterium *Bizionia argentinensis*

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Copper ions are cofactor for several enzymes and participate in some cellular redox reactions. Intracellular excess of copper ions generates reactive radicals that cause damage to DNA, proteins and lipids. For this reason, intracellular levels must be regulated to avoid toxic concentrations. A subfamily of P-ATPases (denoted as PIB-1) are present in prokaryotic and eukaryotic organisms, and constitute one of the main transporters responsible for the elimination of excess copper ions from the cytosol [1].

In this work we characterize a putative PIB-type ATPase belonging to *Bizionia argentinensis* (BaCopA), a gram-negative bacterium isolated from the superficial seawater of Potter Cove, Antarctica. BaCopA was cloned and expressed in *Saccharomyces cerevisiae* as a GFP-fusion His-tagged protein for its subsequent purification and detection. Activity assays indicate that purified BaCopA is able to catalyze ATP hydrolysis at 5°C. ATPase activity of BaCopA increases when Cu (I) and ATP are added to the reaction medium. However, an inhibitory effect of ATPase activity occurs with the addition of vanadate, a specific inhibitor of P-ATPase-type enzymes [2].

A structural model was built by homology modeling using the resolved structure of *L. pneumophila* CopA as template (PDB: 4BBJ). The structural alignment shows a high degree of similarity, with the typical topological pattern of PIB-1 ATPases. Comparison with its mesophilic and hyperthermophilic counterparts led to the identification of key residues conserved in functional domains and differences in non-covalent interactions and surface charges. The detailed analysis of this interaction network suggests greater structural flexibility in BaCopA and, therefore, a better adaptation to low temperatures.

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Biochemical and structural characterization of superoxide dismutases from the polyextremophilic *Acinetobacter* sp. Ver3 strain

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High-altitude Andean lakes (HAALs) along the central Andes area in South America undergo extreme environmental conditions such as high concentrations of salts and metalloids, wide daily temperature variations and high exposure to UV radiation. *Acinetobacter* sp. Ver3 is a polyextremophilic strain found in HAALs that is characterized by a high tolerance to UV-B radiation and pro-oxidants. Catalases and superoxide dismutases (SODs) are the most important enzymes involved in the protection against the oxidative stress. The Ver3 genome comprises the *sodB* and *sodC* genes encoding an iron-containing superoxide dismutase (^{AV3}SodB) and a copper/zinc superoxide dismutase (^{AV3}SodC), respectively; however, the role(s) of these genes in the extremophilic phenotype has remained elusive.

In this work, we studied the performance of *Acinetobacter* sp. Ver 3 SODs at different temperatures and pH conditions and tested their residual activity after incubated in presence of different chemical agents. ^{AV3}SodB showed a higher thermal stability and activity in a broader pH range, whereas ^{AV3}SodC^P activity was more susceptible to heat inactivation and extreme pHs. In order to explore the structural properties of ^{AV3}SodB, we solved the crystal structure of the enzyme to 1.34 Å resolution, one of the highest resolutions achieved for an enzyme of this type. ^{AV3}SodB bears ca. 90 % amino acid identity with the equivalent enzyme in *A. baumannii*, an important target for drug design. The high-resolution structural data presented here provides instrumental information for the design of SodB-specific inhibitors to control nosocomial infections caused by opportunistic bacteria. Additionally, the biochemical information reported for the SOD enzymes studied may be relevant for future applications in food, agriculture or industrial processes.

Contribution of water entropy to metal binding in *Staphylococcus aureus* transcription factor CzrA

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CzrA is a transcription factor that regulates the response of *S. Aureus* to high Zn(II) concentrations, and binds the metal with pM⁻¹ affinity. Enthalpic (ΔH) and entropic (ΔS) binding contributions were characterized by calorimetry, and ΔS was dissected into ΔS_{conf} -by means of sidechain dynamics NMR experiments- and ΔS_{solv} -based on Cp changes of the system. Our aim is to validate the determination of ΔS_{solv} for CzrA by Isothermal titration calorimetry (ITC) in D₂O and interpret at the atomistic level using computer simulations.

We carried out ITC experiments at different temperatures in H₂O buffers to obtain ΔC_p and use the “cratic model” to estimate ΔS_{solv} . In order to test the predictions from this determination, we propose an alternative method to obtain this contribution independent from the assumptions of the cratic model. We propose to perform ITC experiments in D₂O buffers, a strategy that has been used to determine enthalpic contributions of H₂O molecules in biomolecular association processes. Since those experiments also allow us to obtain changes in binding constants, we expect to estimate ΔS_{solv} in a more direct manner. We expect a significant difference in ΔH contributions as the metal process occurs coupled to proton release.

Computational strategies allow us to complement the macroscopic estimation of ΔS_{solv} , since they enable us to estimate that same quantity in a site specific manner. We focus on two strategies to calculate entropy of H₂O molecules from molecular dynamics simulations. In the first place we will calculate ΔS_{solv} from protein-water radial distribution functions. This will allow us to characterize H₂O molecules that visit the surface of the protein. In a second stage, we use a mutual information expansion strategy, considering first, second and third order contributions to ΔS_{solv} . This estimation allows us to map H₂O molecules entropy in the whole simulation box. In both schemes we will compare S_{solv} in Zn-bound and unbound states of CzrA.

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Cryo-EM structure and biochemical characterization of the hemocyanin of the invasive freshwater snail *Pomacea canaliculata*: understanding its role in the innate immune system

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Hemocyanins (HC) are respiratory pigments found in mollusks and arthropods also associated with innate immunity functions, including phenol oxidase (PO) activity and cleavage into antimicrobial peptides (AMPs). The aim of this work was to expand the structural knowledge and the derived physiological implications of gastropod HC within an evolutionary context. We focused on the molecular structure of the HC from *P. canaliculata* (PcH) by Cryo-EM and kinetically characterized its intrinsic and proteolytically induced PO activity. Additionally, we evaluated *in silico* the production of AMPs.

Purified PcH was analyzed by single-particle Cryo-EM from which we obtained a 5 Å resolution density map. We constructed an *ab initio* 3D model by homology modelling using its deduced aminoacid sequence, and the resulting structure was docked and real-space refined in the Cryo-EM map, retrieving a cylindrical rearrangement assembled in di-decamers related by D5 symmetry. Each decameric subunit is composed of five antiparallel dimers, each divided into sixteen parologue functional units showing conserved structural features among snail HC.

We also characterized PcH intrinsic PO activity, using catechol and dopamine as substrates. Like other gastropod HC, only catechol was catalyzed, showing a KM = 50.5 mM and a Kcat= 0.29·min⁻¹. Furthermore, PO activity was increased by limited proteolysis using trypsin, chymotrypsin and subtilisin. Finally, a bioinformatic analysis predicted the presence of several AMPs within the PcH sequence, likely to be generated under proteolytic treatment during a microbial infection.

As a whole, these results reinforce the idea that molluscan HC may be involved in innate immune system. The PcH structural model allows a deeper comprehension of these processes, triggered by still not well-known mechanisms that include structural changes by endogenous and/or exogenous proteases.

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Effects of ssRNA length and ATP presence on Dengue NS3 helicase (NS3h) dynamics

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Dengue Virus Non-structural protein 3 (NS3) is a multifunctional protein critical in the virus life cycle, in part by coupling helicase and ATPase activities. Though the NS3h has been crystallized bound to a ssRNA 7mer in several stages of the ATP hydrolysis cycle, recent in vitro experiments have shown that the minimal binding site of NS3 on ssRNA is actually 10 nucleotides. To reconcile these observations, we use MD simulations to compare NS3 interactions with bound ssRNA fragments of 7, 11, and 16 nucleotides in different stages of the ATP hydrolysis cycle.

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Exploring the dimerization domain loop in bacterial Histidine Kinases

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Histidine kinases (HKs) are sensor proteins that autophosphorylate in response to environmental signals. HKs usually act as dimers and can phosphorylate in cis (intramolecular) or in trans (intermolecular). What determines whether a HK acts in trans or in cis has been the subject of much research. It's been shown that replacing the loop connecting the two helices in the DhP domain from a cis HK to a trans one switches the mechanism, but why this happens hasn't been shown.

To explore this, we collected the sequences of the loop and surrounding residues of multiple HKs of known structure and ran them through a protein property prediction server. This showed a higher tendency to form beta-turns in cis HKs, usually around a glycine present at the beginning of the loop. To understand these results, we ran MD simulations of the loop region for all these HKs. The simulations showed the formation a beta-turn-like structure in cis HKs, where the glycine at the beginning of the loop forms a phi angle of around 90°. This structure might restrict the relative orientations of the two DHp helices, forcing the protein to fold in the cis-acting structure. Interestingly the HK DesK, which belongs to the poorly studied HisKA_3 subfamily, was significantly less mobile than all the other (HisKA) HKs. Since there are no other HisKA_3 structures, we used a model published by the AlphaFold database of the DosS HK. Similar to DesK, DosS showed reduced mobility with respect to HisKA HKs. In a previous work we showed that DosS acts in trans and DesK is also thought to act in trans. The reduced mobility of the loop among these HisKA_3 HKs might imply that all of them phosphorylate in trans.

We intend to expand our simulations to models of other HKs to further understand the difference between cis and trans HKs as well as the differences between HisKA and HisKA_3 families. We also intend to simulate point-mutations to key residues in the loop to analyze the role of each residue

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Flavonoid-Mg²⁺ complexes: stability, characterization, and effects on the Plasma Membrane Calcium ATPase

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The action of natural flavonoids as modulators of the catalytic activity of enzymes has been attributed to various related chemical species, such as the formation of complexes with metals or even oxidized forms thereof. We recently demonstrated that the flavonoids quercetin and gossypin inhibit the Ca²⁺-ATPase activity of plasma membrane calcium ATPase (PMCA) as a function of Mg²⁺ concentration, suggesting that the true inhibitor is quercetin-Mg²⁺ or gossypin-Mg²⁺. The aim of this work was to investigate the formation and stability of the quercetin-Mg²⁺ and gossypin-Mg²⁺ complexes and their effects on the inhibition of the activity of PMCA. We investigated the formation and stability of flavonoid-Mg²⁺ complexes using UV-Vis spectroscopy. The results show that flavonoids and their Mg²⁺ complexes in MOPS buffers (pH 7.4) were unstable and oxidized species were produced. These results are in agreement with the studies carried out by mass spectrometry.

On the other hand, the formation and stability of the flavonoid-Mg²⁺ were examined in the same medium in which the PMCA activity was carried out. This medium contained the reagents necessary for the determination of activity, including mixed micelles of C₁₂E₁₀ and DMPC. The results show that quercetin, gossypin, and their Mg²⁺ complexes remained stable without the appearance of oxidized species, suggesting that the presence of lipids increases the stability of flavonoids.

Our results show that quercetin-Mg²⁺ and gossypin-Mg²⁺ complexes were formed with a stoichiometry of 1:1 and a Kd of 13.5 ± 2.7 mM and 19.6 ± 1.4 mM, respectively. On this basis, the Mg²⁺-dependent inhibition of PMCA activity was expressed as a function of the concentrations of quercetin-Mg²⁺ and gossypin-Mg²⁺ showing that PMCA inhibition depends on the concentration of the flavonoid-Mg²⁺ complex, suggesting that these are the true PMCA inhibitors.

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$\gamma 2$ subunit-containing GABA_A receptors display resistance to ROS modulation

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Endogenous reactive oxygen species (ROS) are involved in neuronal signalling and plasticity in normal physiology, aging and neurodegenerative disorders. Besides, GABAergic neurotransmission is sensitive to several redox agents including ROS. For example, we previously shown that tonic responses mediated by GABA_{A $\alpha 1$} receptors can be modulated by hydrogen peroxide (H₂O₂), ascorbic acid, glutathione and nitric oxide, through thiol modification of cysteines. We have also identified endogenous redox agents that modulate GABA_A receptors involved in phasic inhibitory neurotransmission in the retina and hippocampus, but the molecular mechanisms of action remain elusive.

Our studies have been now extended in order to analyze the effects of H₂O₂ on tonic and phasic responses mediated by diverse GABA_A receptors subtypes. Heterologous expression of the GABA-gated Cl⁻ channels was performed in *Xenopus laevis* oocytes followed by two-electrode voltage-clamp recordings of the GABA-evoked ionic currents. H₂O₂ (1mM) potentiated GABA_{A $\alpha 1\beta 2$} and GABA_{A $\alpha 5\beta 3$} receptor responses elicited by submaximal concentrations of GABA (EC₅₋₁₀). In contrast, equivalent responses mediated by GABA_{A $\alpha 1\beta 2\gamma 2$} and GABA_{A $\alpha 5\beta 3\gamma 2$} receptors were not altered in the presence of H₂O₂, suggesting that $\gamma 2$ subunit conferred significant insensitivity to ROS modulation. H₂O₂ effects on GABA_{A $\alpha 1\beta 2$} responses were reversible, dose and use-dependent, voltage-insensitive and partially prevented by irreversible alkylation of sulphydryl groups with NEM. The degree of potentiation exerted by H₂O₂ was reduced by increases in agonist concentration. Concentration-response curves in the presence of H₂O₂ showed a leftward shift, compared to control values, and an increase in the maximal response. Potentiation induced by H₂O₂ on GABA_{A $\alpha 5\beta 3$} receptor responses showed a similar profile. Additional experiments are being performed to elucidate the mechanisms of action underlying the effects of H₂O₂ on GABA_A receptors.

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His-tag presence modulates enzymatic activity both in solution and at lipid interfaces.

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β -Galactosidase (β -Gal) is an important biotechnological enzyme used in the dairy industry, pharmacology and in molecular biology. This enzyme has a commercial application for lactose hydrolysis in dairy products. Milk processing with β -Gal before milk is commercialized is important to solve nutritional (lactose intolerance) and technological (crystallization of dairy products) problems. In this context, it is important that the activity of β -Gal be evaluated in heterogeneous media. β -Galactosidase (β -Gal) is an important biotechnological enzyme used in the dairy industry, pharmacology and in molecular biology. This enzyme has a commercial application for lactose hydrolysis in dairy products. Milk processing with β -Gal before milk is commercialized is important to solve nutritional (lactose intolerance) and technological (crystallization of dairy products) problems. In this context, it is important that the activity of β -Gal be evaluated in heterogeneous media.

In our laboratory we have overexpressed a recombinant β -galactosidase in *Escherichia coli* (*E. coli*). This enzyme differs from its native version (β -Gal_{WT}) in that 6 histidine residues have been added to the carboxyl terminus in the primary sequence (β -Gal_{His}), which allows its purification by immobilized metal affinity chromatography (IMAC). In this work we compared the functionality of both proteins and evaluated their catalytic behavior on the kinetics of lactose hydrolysis. We observed a significant reduction in the enzymatic activity of β -Gal_{His} with respect to β -Gal_{WT}.

Our studies also focus in studying the activity of both β -Gals in the presence of multilamellar vesicles (MLVs) of different composition. We conclude that the additional positive charges β -Gal_{His} (belonging from histidine residues) promotes the interaction of the protein with negatively charged interfaces favoring the effect shown against neutral interfaces.

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Influence of ATP and spermidine on the liquid-liquid phase behavior of the Parkinson's related protein a-synuclein

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Liquid-liquid phase separation (LLPS) of macromolecules is one of the most extensively studied phenomena in cell biology and biophysics in the last decade. Multiple biomolecules, including intrinsically disordered proteins (IDPs), can either phase transition or contribute to the liquid-liquid phase transition of others. a-synuclein (AS) is a widely studied IDP because of its role in Parkinson's disease (PD) and other related movement neurodegenerative disorders which are characterized by the accumulation of amyloid fibrils of AS in Lewy bodies inside neuronal cells. LLPS has been considered as an intermediate step in the process of protein fibrillation, due to the increase in the effective concentration of proteins that can act as nucleation centers. Biomolecules such as ATP or polyamines, which are dysregulated in pathological conditions, can modulate protein condensates and therefore amyloid formation. In this work we studied the influence of ATP and spermidine on AS liquid droplets. The formation of LLPS was monitored by differential interference contrast (DIC) and fluorescence microscopy. AS showed a low propensity for phase separation in a wide range of pH, ionic strength and crowding agent concentration. Strikingly, whereas ATP did not modify the phase behavior of the protein, spermidine effectively promoted the formation of AS liquid droplets. Results will be discussed in the context of the role of LLPS in health and disease pathogenesis.

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Lysine 107 is critical for lipid-free apolipoprotein A-I tertiary conformation

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Apolipoprotein A1 (APOA1) is the primarily protein component of high-density lipoproteins (HDLs) which play important roles in metabolic disorders such as obesity, diabetes, and cardiovascular disease (CVD). Situated at the water-lipid interface, APOA1 adopts a variety of structural conformations on the lipid surface to accommodate docking with various enzymes, lipid transfer proteins, and cell surface receptors that remodel HDL during its lifespan. In this way, APOA1 acts as a master regulator of both HDL composition and function. The deletion mutant Lys107del (Δ K107) is a natural variant of APOA1 that occurs in humans which is associated with hypertriglyceridemia and accelerated atherosclerosis and CVD. The purpose of these studies was to determine if structural differences exists in the Δ K107 APOA1 molecule that could explain the observed alteration in lipid metabolism in carriers of Δ K107 variant. Using a combination of high-resolution size exclusion chromatography, chemical cross-linking, and liquid chromatography-mass spectrometry we performed deep structural analysis on WT APOA1 and Δ K107 APOA1. We found the absence of Lys 107 severely impeded the ability of APOA1 to self-associate into higher order oligomers. Differences in the cross-linking pattern between APOA1 and Δ K107 APOA1 indicate that deletion of Lys 107 severely disrupted intermolecular interactions between APOA1 molecules in the N-terminal region of the molecule. Given that APOA1 self-association is thought to be a critical step in HDL biogenesis, our findings suggest the absence of Lys 107 results in a structural change that could alter HDL formation and/or function which may underlie the dyslipidemia observed in Δ K107 carriers.

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Membrane Protein Freezing. Characterization of changes in the plasma membrane calcium pump structure and function.

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Keeping intact the structure of a protein for a long period of time is a very desirable process. Freezing has been shown to be effective, but protein integrity is altered during this procedure. Knowing the characteristics of the structural and functional changes during the freezing process is a central issue but they are little known. In this study the inactivation of the plasma membrane calcium pump (PMCA) by freezing was examined by a combined kinetic and spectroscopic approach. Two cooling procedures were used to freeze the samples, with cooling rates at the freezing point of 0.05 K s^{-1} (slow cooling) and 4 K s^{-1} (fast cooling). After freezing, samples were thawed at a heating rate of 0.2 K s^{-1} . The Ca^{2+} -ATPase activity significantly diminished after freezing following the slow cooling procedure, being the decrease an exponential function of the storage time at 253K with $t_{1/2} = 3.9 \pm 0.6$ hs. On the contrary no significant changes on the enzyme activity were detected when the fast-cooling procedure was followed. Regardless of the cooling procedure used for freezing, successive freeze-thaw cycles produced an exponential decrease of the Ca^{2+} -ATPase activity, being the number of cycles at which the activity was reduced to half 9.2 ± 0.3 (fast cooling) and 3.7 ± 0.2 (slow cooling). Kinetic analysis shows that freeze-thaw inactivation significantly decreased V_{max} values whereas no significant differences were found in the apparent affinity for ATP, Ca^{2+} and calmodulin. Besides, the addition of these enzyme ligands to the freezing medium improves the stability against freeze-thawing. Moreover, the inactivation process was found to be associated with the irreversible partial unfolding of the polypeptide chain, as assessed by Trp and ANS fluorescence, and far UV circular dichroism. These results suggest that freezing inactivation of PMCA is the result of the irreversible partial unfolding of this protein and only full active and inactive species are involved in this process.

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Mesoscopic clusters of ICA512-RESP18HD

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ICA512 is a protein tyrosine phosphatase enriched in secretory granules (SGs) of the pancreatic β-cells and other neuroendocrine cells. In this work, we focused on the N-terminal extracellular domain of ICA512 called RESP18HD (glucocorticoid-responsive regulated endocrine-specific protein 18 homology domain) and on its condense properties. In previous studies, we discovered that RESP18HD displays an in vitro protein condensing activity at close to neutral pH, i.e. in the pH range found in the early secretory pathway in cells. Remarkably, this condensing activity is exerted towards insulin, which in the presence of isolated RESP18HD aggregates at pH 6.8. Now we could demonstrate that behaviour extends to proinsulin, the insulin precursor, which is present in early stages of the SG secretion. Also we found that the (pro)insulin condensing activity of RESP18HD, evidenced by turbidimetry assay, increases dramatically in reducing conditions, suggesting a potential thioredoxin-like activity of RESP18HD. In this regard, a FITC-labeled insulin reagent shows a change in the fluorescence signal in presence of RESP18HD and DTT according to the turbidimetry assays. Our main hypothesis is that ICA512-RESP18HD aggregation properties are related to a liquid phase separation known as mesoscopic clustering - the formation of nanometer-sized soluble conglomerates - leading to the formation of RESP18HD larger amorphous aggregates, which could contribute to the sorting of proteins from the endoplasmic reticulum to the Golgi in the secretory pathway.

Metal binding affinity and stability of *Bacillus cereus* phospholipase C variants.

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The phosphatidylcholine-preferring phospholipase C from *Bacillus cereus* is a monomeric exoenzyme of 28.5 KDa with three zinc ions in its active site. The enzyme is used in industry in oil degumming. Due to the harsh conditions of the reaction medium the enzymes used in this application are variants of the wild type that endure longer times while maintaining its activity. However, there is currently no available information on the relative stabilities of the whole enzyme or of the active site between the wild type and its variants.

Here we present information on metal off-rate constant of two variants as well as on structural destabilization by denaturants and temperature. We use the methyl and amide/indole fingerprint regions of the ^1H NMR spectrum of the protein to follow the processes. A group of signals between -0.25 and -0.32 ppm, among which presumably are the methyl groups of A54 and of I17, shift considerably being useful probes of active site integrity. The near UV CD spectra of the protein show significant changes between the apo and holo enzyme, as well as upon unfolding. The transition from holo to apo enzyme comes with a loss of activity that we measure with a turbidimetric assay.

We use the far UV CD spectrum to follow the thermal denaturation of the proteins. Since the unfolding is irreversible and we cannot use a melting temperature as a comparative parameter we focus on the kinetic parameters of the process. We obtained denaturation kinetics constants at different temperatures and the activation energy of the process.

Similar studies on new variants of the protein will help rationalize the effect of the remote mutations present in the industrial enzyme variants on their improvement relative to Bc PLC.

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Multienzymatic functionalization of an oligomeric scaffold for cellulose degradation enhancement

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The enzymatic degradation of lignocellulose generates sugars that upon fermentation produce bioethanol. The enzymes currently used in this process are expensive and have low efficiency. It is economically relevant to increase their activity and stability.

Cellulosomes are multienzymatic complexes that colocalize different cellulolytic enzymes and cellulose binding domains, increasing their degradation activity through enzymatic proximity and substrate targeting effects. The industrial production of natural cellulosomes is limited, due to the nature of the producing organisms and the properties of their scaffolding protein. Our goal is to use the structure of an oligomeric protein that is highly stable and highly expressed in bacteria as a scaffold for the colocalization of a consortium of cellulolytic enzymes. For the development of these artificial cellulosomes, we use a non-covalent coupling strategy through high affinity heterodimeric coiled coil peptides (leu1 and leu2) complementary fused to the oligomeric scaffold and the target enzymes.

For this purpose we cloned and expressed in *E. coli* the catalytic domain of a variety of enzymes fused to the coupling peptide: endoglucanases, exoglucanases, xylanases, beta glucosidases and cellulose binding domains from different organisms. The amount and solubility of these fusion proteins were compared to the isolated domains. At least one member of each functional category was solubly expressed in significant amounts and was purified by affinity chromatography and gel filtration. We present advances in their functional characterization.

We have successfully assembled several of the target proteins to the oligomeric scaffold, producing mono or multi-functional complexes. The interaction between the target modules and the subunits of the platform produce decameric complexes in agreement with the decameric structure of the scaffold.

Our results encourage further development of the artificial cellulosomes. It is expected that these complexes will help to increase the enzymatic degradation of lignocellulose, reducing the cost of bioethanol production.

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Mechanistic insights of hydrogen peroxide transport through PIP aquaporins pore

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Hydrogen peroxide (H_2O_2) is transported through membranes by aquaporins (AQP). In particular, some plant PIP aquaporins isoforms are efficient H_2O_2 channels. As water and H_2O_2 share physicochemical features, it was first supposed that all AQP that transport water could act as an H_2O_2 channel. However, experimental evidence showed that not all PIP that transport water can transport H_2O_2 . So, the mechanism of H_2O_2 transport is still an unsolved issue for AQP channels.

MtPIP2,3 is a plasma membrane AQP from the legume *Medicago truncatula* that permeates H_2O_2 . To understand the structural and chemical selectivity mechanisms leading to H_2O_2 permeability in PIPs, we characterized the particularities of H_2O_2 passing through *MtPIP2,3* pore by 1 μ s atomistic molecular dynamic simulations. As PIPs are tetrameric pH gated channels we constructed homology *MtPIP2,3* models in open and closed states, and with or without H_2O_2 . All models were conformationally stable along the simulation and H_2O_2 permeation events were found in the simulations in the presence of this molecule. We find that: i- H_2O_2 molecules can cross the pore in a single file, ii- dihedral angles adopted by H_2O_2 along the pore Z axis present a different distribution compared to the angles visited in the solution; in the selectivity-determining NPA region, H_2O_2 adopts the wider range of dihedral angles, iii- higher residence times are located around the selectivity filter zone in the open channel and moves to the cytoplasmic filter area in the closed channel; and iv- the constriction in the cytoplasmic filter area seems to be more stringent for H_2O_2 passage than for water.

Our results shed light onto the molecular mechanism of H_2O_2 passage through *MtPIP2,3* and represent the first steps to understand the structural determinants of AQP differential selectivity for these molecules and water.

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Proteins' Mutations Effects in Light of Evolution

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The primary aim of this work is to explore how proteins' point mutations impact their marginal stability and, hence, their evolvability. With this purpose, we show that the use of four classic notions, namely, those from Leibniz & Kant (1768), Maynard Smith (1970), Einstein & Infeld (1961), and Anfinsen (1973), is sufficient for a better understanding of the protein-evolution and, consequently, to determine the factors that could control it.

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Recombinant human proinsulin: oxidative refolding and biophysical characterization

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According to the World Health Organization, diabetes is a global health priority. Growing prevalence of diabetes worldwide will increase the demand for insulin in the near future, which is especially challenging in developing countries. For this reason, it is pertinent to reexamine the *in vitro* production of insulin. Moreover, our knowledge of insulin folding *in vivo* is insufficient and needs to be advanced.

Human insulin is synthesized *in vivo* as a single-chain precursor, proinsulin, from which a middle peptide, C-peptide, is post-translationally removed generating the biologically active insulin. To fold correctly, proinsulin must first form three disulphide bonds linking chains A and B of mature insulin. This critical step, both *in vivo* and *in vitro*, is incompletely understood. In this work we describe the optimization of the downstream processing of oxidative refolding of the recombinant human proinsulin expressed in *E. coli*. We successfully obtained native human proinsulin in large amounts, highly pure and properly folded. Furthermore, we report a preliminary biophysical characterization of human proinsulin.

Structural and functional characterization of Growth-Regulating Factors from *Arabidopsis thaliana*: a closer look at WRC DNA-binding domain

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Growth-regulating factors (GRFs) belong to a plant-specific family of transcription factors which display important roles in plant growth and development. GRF transcriptional activity is finely tuned by regulatory processes involving post-transcriptional silencing and protein-protein interactions exerted by miR396 and a family of co-transcriptional regulators known as GRF-interacting factors (GIFs), respectively. In this sense, expression of GRF target genes is modulated by a highly complex interplay between GRF/GIF isoform diversity and expression patterns along with miR396 and GIF gradients throughout plant tissues. At the protein level, GRFs are composed of two highly conserved domains known as QLQ and WRC and a less conserved C-terminal trans-activation domain. Whereas QLQ mediates GRF-GIF interaction, the WRC has been proposed as a putative zinc finger domain responsible for target DNA recognition. However, the structural aspects governing the domain function information, WRC-ligand interactions and GRF target recognition remain unknown. In this work, we study the Zn²⁺ and DNA binding properties of GRF7 WRC from *Arabidopsis thaliana* and explore the structural features of WRCs from GRF1, 3, 5 and 7 using Nuclear Magnetic Resonance.

Study of PDK1 kinase conformations and potential regulatory mechanism for substrate activation

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Phosphoinositide-dependent protein kinase 1 (PDK1) is a master kinase of the PI3-kinase signalling pathway that phosphorylates at least 23 other evolutionary related AGC kinases. It has an N-terminal kinase domain, a linker region and a C-terminal PH domain. Our laboratory has previously used a chemical and structural biology approach to study and characterize the bidirectional allosteric regulation between the *PIF-pocket*, a regulatory site located on the small lobe of the kinase domain, and the ATP-Binding site of PDK1. Phosphorylation by PDK1 is required for the activity of all substrates: they are phosphorylated either constitutively or with different timing upon PI3-kinase activation. Most substrates, like S6K, SGK, PKC, PRK/PKN, rely on a docking interaction where a C-terminal hydrophobic motif (HM) interacts with the *PIF-pocket* of PDK1. Interestingly, the interaction with the *PIF-pocket* of PDK1 is not a requirement for the phosphorylation of PKB/Akt after PI3-kinase activation, but both proteins have a PH domain that can bind PIP₃ at the cell membrane and colocalize. However, we believe that other mechanisms must regulate that interaction since there are reports of PKB/Akt activation by PDK1 in the absence of PIP₃. PDK1 has also been described to dimerize.

We describe the effect of inositol poliphosphorylated molecules and present results of a screening performed in order to find small compounds to regulate dimer formation. We conclude that PDK1 could exist as an equilibrium of dynamic conformations that may impact on the selective interactions with substrates. We suggest dimerization could also be part of the mechanism by which PDK1 phosphorylates some substrates like PKB/Akt. This potential new regulatory mechanism could be new approach to develop innovative drugs to target PDK1 and achieve, for example, PKB/Akt selective inhibition.

Study of the catalytic activity of β -Galactosidase encapsulated in silicate gels on the hydrolysis of lactose.

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Protein encapsulation in a solid matrix is of interest for biotechnological purposes and it also serves as a model of molecular crowding. The immobilization of enzymes is a process in which the protein is confined in a nanometric environment in which the encapsulated enzyme's behaviour does not necessarily resemble the dilute solution condition. We have successfully entrapped the enzyme β -Galactosidase (β -Gal) in silicate gels via a sol-gel reaction and, in previous studies, we determined that the encapsulated β -Galactosidase enzyme (E β -Gal) had the same or greater catalytic activity than the soluble enzyme for the artificial substrates ONPG and PNPG, and we proposed that the structuring of the water molecules in the nanopores of the gel would have significant importance in the differences observed for the hydrolysis of ONPG in fresh and aged gels at different time periods.

In the present work, we studied the catalytic activity of β -Gal and E β -Gal on the hydrolysis of lactose, due to the potential use in dairy industries for the production of free lactose food.

Preliminary results of the catalytic activity and efficiency of the hydrolysis of lactose catalysed by β -Gal and E β -Gal, are presented. First of all, we determined the amount of glucose formed after different times (10, 20, 30, 40 and 60 min), employing the soluble and encapsulated enzyme forms; it could be set that the reaction is in initial rate conditions up to 40 minutes for both β -Gal and E β -Gal. Then, the saturation curves for the hydrolysis of different initial concentrations of lactose were performed in fresh and aged gels, and the catalytic parameters V_{max} and K_m were obtained. The values of V_{max} did not vary significantly between the soluble and encapsulated enzyme. On the contrary, K_m values for E β -Gal double the values for the soluble enzyme. Finally, the efficiency of hydrolysis was analysed through tests done at 6°C and 37°C. It was observed that at 6°C the encapsulated enzyme is more efficient while at 37°C the soluble β -Gal is the more efficient one.

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Antibacterial activity of anionic peptides produced from tryptic hydrolysate of whey protein.

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Antimicrobial peptides (AMPs) show a broad spectrum of antimicrobial activity against a wide range of pathogens, and therefore play an important role in innate host defense. All AMPs are synthesized as proforms, which are subsequently processed into mature peptides of various lengths and structures. All the naturally occurring whey proteins have been reported to be the source of bioactive peptides when digested enzymatically. Tryptic whey protein hydrolyzate contains different anionic peptides. In the present work we analyze the antibacterial activity of one of these anionic peptides from β -lactoglobulin, β -Lg 125-135 (TPEVDDEALEK) which has net charge of -4 at pH 7. Antibacterial activity was expressed as $\log N_C/N_I$, where N_C is the 24 h increase in the number of colony-forming units without peptide, and N_I is the 24 h increase in the number of colony-forming units with peptide. The growth inhibition in the presence β -Lg₁₂₅₋₁₃₅ peptide of at a concentration of 2mM was 0.99 and 0.34 against *Staphylococcus aureus* ATCC 25923 *Escherichia coli* ATCC 25922 respectively. Usually, antibiotic peptides were described as cationic molecules that interact preferentially with anionic lipid membranes through electrostatic interactions. In this case, instead, the β -Lg₁₂₅₋₁₃₅ peptide was anionic and the interaction seems to occur through a cationic salt bridge between the peptide and the anionic lipid interface that mimics the microbial cell membrane.

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Bovine erythrocyte acetylcholinesterase (BEA) from natural membrane transferred to glass functionalized surface is modulated by essential oil compounds.

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Monomolecular layers at the air-water interface (Langmuir Films, LF) are useful tools for the study of biomembranes. This kind of systems provide a constant planar curvature and, different from other model membranes or even cells, allow the control of parameters such as packing degree and composition. In turn, LFs can be transferred, to functionalized substrates, maintaining their main properties, expanding the spectrum of tests to which they can be submitted and even enabling the construction of biosensors.

Acetylcholinesterase (AchE) is an enzyme with a crucial role in the nervous system. Currently, search for natural compounds with a modulatory effect on AchE activity is in high demand to be used as bioinsecticides and also in therapies for diseases such as Parkinson. The AchE (BEA) present (anchored) in Bovine Erythrocyte Membrane (BEM) serves as a model to study these issues.

In our laboratory FLs obtained by the spreading of BEA over the air-water interface could be transferred to alkylated glasses by applying different techniques in presence of monoterpenes (MTs). In these tests information was obtained on the modulating effect of the MTs Cineole, Camphor and Eugenol on BEA. For all MTs an inhibition of the enzymatic activity was observed, except for Eugenol which at low concentrations showed a slight increase in activity.

In the present work we applied a different transference method named Langmuir-Schaefer (LS) and we studied the effect of other MTs such as Thymol, Menthol and Geraniol on the activity of BEA in the MEB films packed at 35 mN/m. Menthol and Geraniol exhibited an inhibitory effect on BEA activity with an IC₅₀ of 6.73 mM and 2.27 mM, respectively. Thymol seemed to be inactive on BEA at low concentrations however, in a range from 2 mM to 15 mM it showed a marked activating effect. The modulatory mechanism can be through as a specific interaction with the substrate binding site on BEA or through a modification of the molecular environment on the enzyme in the LS film. Further experiments will be necessary to elucidate this matter. Moreover, in order to improve the quality of the data obtained with the transferred enzyme using the LS technique, activity and fluorescence tests were carried out to evaluate the homogeneity of the supported film. We found that slight modifications of some aspects of the technique used could notably improve the homogeneity of the transferred BEM.

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Copper-containing nitrite reductase from *Sinorhizobium meliloti* 2011: Site-directed mutagenesis of the aspartic acid residue from substrate sensing loop

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Cu-containing Nitrite reductases (NirK) catalyze the second step of the denitrification process catalyzing the reduction of NO_2^- to NO ($E^\circ' = 370\text{mV}$). *Sinorhizobium meliloti* NirK (*SmNirK*) presents a homotrimeric structure with two Cu atoms per protein monomer, one type 1 (T1, electron transfer center) and the other type 2 (T2, catalytic center). T1 and T2 are linked by two pathways: the Cys-His bridge and the substrate sensing loop (Fig. 1). It is thought that the substrate sensing loop works as a relay to trigger the T1 → T2 electron flow through the Cys-His bridge when the substrate is bound to the catalytic center (Cu-T2). An aspartic acid (D134) is present in the sensing loop and forms a H-bond with the T2 labile water molecule in the resting and with an O-atom from nitrite when the substrate binds the T2. Since this residue was proposed to be essential in the catalysis process, but NirK from *Thermus scotoductus* presents a serine residue in this position, the D134S was produced and studied by kinetic and spectroscopic techniques. The absorption spectrum of the as purified variant showed absorption maxima at 585nm and 459 nm with extinction coefficient values lower than the wild-type enzyme ($\epsilon_{585\text{nm}, \text{D134S}} = 2.2(2) \text{ mM}^{-1}\text{cm}^{-1}$ vs. $\epsilon_{585\text{nm}, \text{wt}} = 2.9 \text{ mM}^{-1}\text{cm}^{-1}$ and $\epsilon_{459 \text{ nm}, \text{D134S}} = 2.3(2) \text{ mM}^{-1}\text{cm}^{-1}$ vs. $\epsilon_{456 \text{ nm}, \text{ wt}} = 3.3 \text{ mM}^{-1}\text{cm}^{-1}$). Metal incorporation in the D134S structure was a more difficult process when compared with the wild type *SmNirK* and, in agreement with this, metal quantification showed 1.6(1) Cu/monomer. Although D134S is active using the physiological electron donor (pseudoazurin), the k_{cat} and K_m values decrease 240-fold and 10-fold, respectively, when compared with the wild-type *SmNirK*. D134S EPR signal of the as isolated enzyme showed two overlapped spectral components associated with T1 and T2. As expected, the g-values of the T2 copper show differences when compared with the wild-type enzyme. These spectroscopic and kinetic characteristics are presented.

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Effect of PEG-induced molecular crowding on β -Gal activity and thermal stability. Optimization of beta galactosidase function for GOS production using milk lactose as substrate.

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The yeast β -galactosidase or lactase [EC 3.2.1.23] (β -Gal) is a soluble enzyme capable of catalyzing lactose hydrolysis into its constitutive monosaccharides: glucose and galactose. In addition, and depending on the conditions of the environment, fundamentally high lactose concentration, β -Gal catalyzes the transglycosylation reaction whose products will be the Galacto-oligosaccharides (GOS). These molecules are considered prebiotics because they are not degraded in the digestive tract, reaching the intestine where they are a substrate for the growth of beneficial bacteria. GOS production is favored by: high lactose concentration, high reaction temperature and low water availability. These experimental conditions can be achieved if macromolecular crowded media (MCM) are used as the reaction medium. In this work we investigate the effect that molecular crowding induces on the activity and thermal stability of β -galactosidase from *Kluyveromices lactis*. PEG₆₀₀₀, a non-charged highly water-soluble polymer with well-known effects on water dynamics was used to produce the crowded environment.

The effect of PEG₆₀₀₀ on β -Gal kinetic parameters was studied using lactose as substrate. Results obtained showed that enzymatic activity is improved in MCM: the affinity increased while the V_{max} remained unchanged. Temperature-dependent β -Gal activity profile was studied both in the absence or in the presence of molecular crowded agent in a range from 37 to 50 °C. Results obtained showed that β -Gal thermal activity profile was enhanced in molecular crowded environment. The enzyme maintained its activity when it was incubated at temperatures 5 degrees higher in the presence than in the absence of molecular crowding agent. Thermal inactivation kinetic was also studied: in this type of experiments, the enzyme was pre-incubated at 37 and 50 °C during different periods of time and after that, the enzymatic activity was measured in optimal conditions. Results obtained show again that molecular crowding conditions protect the enzyme from heat denaturation. In this case, it was observed that the enzyme maintains its activity even when it is subjected for a considerable period of time at high temperature when it is in the presence of the molecular crowding agent.

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Interaction and association with lipids of Apolipoprotein A-I studied with Pyrene-labelled cysteine mutants

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

In water solution, apoA-I forms a bundle composed of several amphipathic alpha-helices. Depending on the concentration, apoA-I self-associates to form dimers and oligomers of higher orders with poorly characterized quaternary structures. It also interacts with phospholipids to form discoidal HDL (dHDL) in alternative anti-parallel helical arrangements. Previous studies from this laboratory have shown the participation of helices 5 and 10 in contact regions during certain oligomerization steps. However, these self-interactions during lipid interaction and lipid association are unknown.

The aim of the present study is to characterize the lipid interaction and lipid association to form dHDL with single labelled mutants of apoA-I, focusing on the proximity of helices known to be important to detect dHDL arrangements.

Selected cysteine mutants of apoA-I were purified and labelled with pyrenyl-maleimide in positions corresponding to helices 4 (K107C), 5 (K133C and L137C) and 10 (F225C and K226C). The monomer and excimer fluorescence of the labelled proteins were registered in apoA-I titrations with lipid vesicles and dHDL generated in vitro by different methods. Additionally, dHDL formation with K107C and K133C were evaluated in vivo by incubation with THP-1 macrophage cells.

The labelled mutants were stable in solution as indicated in a previous study and biologically active since they can form dHDL. Fluorescence emission spectra of pyrene showed lipid interaction by increased total fluorescence emission. It was also observed with P-value, showing little changes except with mutant L137C. In the same way, excimer formation indicated poor or absent oligomerization especially with mutants K133C, F225C and K226C, which self-associate in lipid-free environments. On the other hand, the pyrene emission in dHDL generated in vitro showed poor excimer formation except with K133C, which was minimal. It was also reported in vivo with mutants K107C and K133C, suggesting a 5/5 anti-parallel helical arrangement. In conclusion, we proposed that self-interactions in helix 5 could be critical to form dHDL since they are observed in lipid-free apoA-I and dHDL, except during the association with lipids where it can be different.

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Peptides derived from the α -core of a *Silybum marianum* defensin induced membrane permeabilization, oxidative stress and cell death in *Fusarium graminearum* conidia

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Plant defensins are antimicrobial peptides (AMPs); they are small basic, cysteine-rich peptides ubiquitously expressed in the plant kingdom and mostly involved in host defence. They present a highly variable sequence but a conserved structure. Two highly conserved regions have been identified in these proteins: the γ -core ($GXCX_{3-9}C$), a well-known determinant of the antimicrobial activity among disulphide-containing AMPs and the α -core ($GXCX_{3-5}C$), a less studied and not conserved motif among them. The importance of these motifs relies on the presence of positive residues which would allow the interaction with negative charges on the pathogen membrane and/or cell wall. In previous studies, we have demonstrated that synthetic peptides derived from these regions are active at micromolar concentrations against the conidia from the phytopathogenic fungus *Fusarium graminearum* and we have characterized their action. In order to gain insight into the understanding of the α -core, new peptides were designed by modifying the parent peptide SmAP_{a1-21} (Sequence: KLCEKPSKTWFGNCGNPRHCG; Minimum Inhibitory Concentration MIC: 32 μ M), which caused membrane permeabilization, induced morphological changes in the cell wall and the interaction with POPC/Erg monolayers (3:1) revealed its monolayer insertion. Three peptides were synthesized: SmAPH19R, where His19 was modified by Arg; SmAPH19A, in which His19 was modified by Ala and cSmAPC14S, a cyclic peptide synthesized promoting an intramolecular disulfide bond and Cys14 was modified by Ser. The new peptides were found to be active against *F. graminearum* (MIC SmAPH19R: 40 μ M, SmAPH19: 100 μ M and cSmAPC14S: 70 μ M). Both SmAPH19R and cSmAPC14S produced permeabilization of the conidia membrane, while SmAPH19A did not. All three peptides induced oxidative stress in the treated conidia through ROS production, as assessed by confocal microscopy and fluorometric measurements. SmAPH19R caused conidia death in 3 h, SmAPH19A in 6 h and cSmAPC14S in 30 min. Confocal microscopy of the peptides derivatized with fluorescein showed that SmAPH19R and SmAPH19A were localized in the conidia cell wall. The change of His by Ala reduced the activity compared with the parent peptide and would produce a change in the mechanism of action, since membrane permeabilization did not occur under the assay conditions. Although cSmAPC14S exhibited a higher MIC than the parent peptide, it was the most lethal on the time to kill assay.

Spectral analysis of SDS-induced reversible denaturation of AfCopA, an α -helical membrane protein

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Membrane proteins play a central role in life by helping cells to communicate with their surroundings. They represent approximately one third of the coding proteins in all genomes, and alterations in their folding could lead to a number of known pathologies [1]. However, when comparing with soluble proteins, few studies are focused on their folding and stability due to practical difficulties [2]. Furthermore, α -helical membrane proteins are typically resistant to classical denaturation agents like urea and GnHCl, but are susceptible to ionic detergents, like sodium dodecyl sulfate (SDS) [3]. In this work, we explore the SDS-induced denaturation of AfCopA, a thermophilic α -helical membrane protein from *Archaeoglobus fulgidus*, by analyzing the effect on the intrinsic fluorescence and the signal of a fluorescent probe, 1-anilinonaphthalene-8-sulfonic acid (ANS), bound to the protein. The intensity of both signals decrease by the addition of SDS to the protein, which is initially reconstituted in mixed micelles composed of phospholipids and a nonionic detergent. By changing the initial concentration of amphiphiles, we demonstrated that the effect on the signal are dependent on the molar fraction of SDS present on the mixed micelles. The observed transitions were proven reversible by the dilution of the detergent, which allowed us to perform a thermodynamic analysis of the process. Further analysis were conducted by analyzing the effect of SDS on the spectrum 's shape. Changes were subtle, but by using the spectral phasor approach [4], we could identify new transitions and obtain additional information about intermediate species in the folding mechanism.

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Structural and biophysical characterization of a novel nematode lipid binding protein of *Dioctophyme renale*

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The giant kidney worm, *Dioctophyme renale*, is a debilitating and potentially lethal parasite that typically inhabits and destroys, the host's right kidney, and may also be found in ectopic sites. It is circumglobally distributed, mainly in wild carnivores that live close to rivers and is increasingly regarded as a threat to other domestic animals and humans. Previous results from our laboratory identified two major proteins present in the pseudocelomic fluid (PF), one of 17 kDa (P17), that has a prosthetic heme group and is possibly related to oxygen transport, and another of 44 kDa (P44), that binds lipids and is secreted. Both proteins would play a crucial role in the biochemistry of the parasite because lacking the metabolic pathways for the syntheses of the heme group, fatty acids, and cholesterol, this organism must obtain these compounds from its host. The aim of this work is to carry out a structural and biophysical characterization of P44, emphasizing its possible biological functions. Therefore, the protein was purified from PF by SEC and then delipidated by HPLC. Purity was checked by SDS-PAGE followed by Western blotting. Glycosylated residues were evidenced using a Schiff based dyeing and by an enzymatic deglycosylation assay. P44 presents both far and near UV CD spectra consistent with a well folded protein. Additionally, denaturation curves were studied by fluorescence, showing high stability towards denaturing agents such urea and guanidinium chloride. To assess function, fluorescence displacement experiments were carried out. Affinity for oleic acid (Kdapp 6.9 μ M) and cholesterol (Kdapp 27.6 μ M) was measured by their ability to displace DAUDA or ANS probes, respectively. In summary, P44 emerges as a novel nematode lipid binding protein likely involved in the modulation of the host's immune response.

The GM₁ ganglioside induces toxic prefibrillar α -synuclein amyloid oligomers

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Parkinson's disease (PD) is an age-related disorder characterized by the presence of amyloid deposits of the 140-amino-acid protein AS (α -synuclein). AS fibrillation, the process of native soluble proteins misfolding into insoluble fibrils comprising cross- β -sheets, involving transient prefibrillar species with different biophysical features. *In vitro* evidence suggests that pre-fibrillar species (oligomeric intermediates), rather than mature amyloid fibrils, are likely to be the primary pathogenic agents in neurodegenerative diseases.

We previously demonstrated that oligoGM₁ (the oligosaccharide soluble portion of GM₁ ganglioside) stimulated the formation of amyloid fibrils as compared to GM₁ ganglioside which reduces AS aggregation. The morphological analysis showed that AS fibrils are lower in height in the presence of oligoGM₁ compared to AS fibrils and we visualized structures compatible with AS oligomers in the presence of GM₁.

In this report, by using dot blot and atomic force microscopy (AFM), we demonstrated that GM₁ stimulates the formation of oligomeric structures of AS *in vitro*.

Furthermore, we verified that, in the presence of GM₁, aggregation kinetics (followed by Thio T fluorescence) is lower respect to oligoGM₁. Through this analysis, we confirmed that oligoGM₁ accelerates the fibrillation of AS.

Finally, cytotoxicity assay with cell culture of human SH-SY5Y neuroblastoma cells, demonstrated that oligomers obtained in the presence of GM₁ have a higher cytotoxicity than AS fibers formed both in the absence or presence of oligoGM₁.

The results obtained contribute to the hypothesis recently emerged that demonstrate that the extent of fibrillary amyloid plaque deposition not correlate with neurodegenerative disease pathogenesis.

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The QLQ-SNH complex from the intrinsically disorder proteins GRF and GIF

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Formation of complexes between transcription factors and transcriptional co-regulators, play an essential role in the control of gene expression. **Growth-regulating factors (GRF)** and **GRF-interacting factors (GIF)**, are part of a complex regulatory module that affects key biological processes such as cellular proliferation, leaf longevity and organ development. The GRF family shares a conserved architecture, predicted to be mostly disordered, featuring two N-terminal domains **QLQ** and WRC. GIF proteins are also mostly disordered and present a short, conserved domain called **SNH** in its N-terminal region. The QLQ domain contains a conserved QX_3LX_2Q sequence and mediates GRF-GIF complex formation by interacting with SNH domain of GIFs, whereas the WRC is a putative zinc finger domain responsible for target DNA recognition. Moreover, QLQ domain is also present in essential core ATPase subunits of the chromatin remodeling complex of the BRAHMA (BRM) family. Despite the importance of these proteins in plant development, there is no information on the physical interaction between GRF-GIF and BRM. In this work, we present a structural characterization of the QLQ:SNH complex, studying the interaction between SNH domains of GIF1 and GIF2 with the QLQ domains of GRF1 and BRM from *Arabidopsis thaliana*, applying CD and NMR techniques.

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The Spf1p P5A-ATPase “arm-like” domain is not essential for ATP hydrolysis but its deletion impairs autophosphorylation

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The P5A-ATPases are primary active transporters present in the ER of all eukaryotic cells. In humans, mutations in the P5-ATPases are associated with neurodegenerative diseases. Recently, it was reported that P5A-ATPases possess a unique ATP-dependent helix dislocase activity allowing the extraction of membrane proteins that are mistargeted or misincorporated in the ER. Structurally, the P5A-ATPases exhibit domains without homology in other members of the P-ATPase family and whose function is unknown. One of these distinctive regions comprises an extended insertion which was called the “arm-like” domain connecting the phosphorylation domain (P) with transmembrane segment M5, near the peptidyl-substrate binding pocket. We used site-directed mutagenesis to obtain a mutant of the yeast P5A-ATPase Spf1 lacking the arm domain (Δ armSpf1). Purified preparations of the mutant protein hydrolyzed ATP at maximal rates of 50% of that of the wild type enzyme. When incubated with ATP Δ armSpf1 produced a lower level of the catalytic phosphoenzyme in amounts proportionate to the ATPase activity. These results indicate that the presence of the arm domain is necessary to attain the “phosphorylation-ready” conformation of the protein.

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Understanding the reversibility of formate/carbon dioxide redox reaction catalyzed by formate dehydrogenases.

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Formate dehydrogenase (Fdh) is an enzyme whose physiological role is catalyzing the oxidation of formate (HCOO^-) to carbon dioxide (CO_2) to obtain reducing equivalents required for several metabolic routes in a living cell. Fdhs are also able to reduce CO_2 to HCOO^- under a reducing environment, either in solution (with a physiological or artificial electron donor) or adsorbed to an electrode under reducing overpotentials. In this work, we present the isolation and biochemical/kinetic characterization of Fdh from *Thiobacillus sp.* (TspFdh), a metal-free, O_2 -tolerant Fdh. TspFdh is a monomeric protein of ~45 kDa that uses NAD^+ as the redox cofactor. The catalytic (bi-substrate) mechanism for NAD-dependent Fdhs (NAD-Fdh) implies that both HCOO^- and NAD^+ must interact in the enzyme active site for hydride transfer to occur, releasing CO_2 and NADH as products. It was reported elsewhere that TspFdh catalyzes the reverse reaction more efficiently than other NAD-Fdhs, therefore, we investigated the reason behind this difference. Kinetic assays using several concentrations of both formate and NAD^+ showed that TspFdh follows a sequential rather than a Ping-Pong (substituted) mechanism. Previous reports pointed out this, and also showed through inhibition studies that NAD-Fdhs from plants would follow a compulsory ordered mechanism with a negligible accumulation of ternary complex (Theorell-Chance), while NAD-Fdhs from prokaryotes fit well to a rapid-equilibrium sequentially random mechanism. The latter means that HCOO^- and NAD^+ have improved affinity for Fdh- NAD^+ and Fdh- HCOO^- complexes, respectively, compared to free enzyme. A variable temperature study of the kinetic isotope effect (KIE) showed that in TspFdh would occur H-tunneling, which is detectable in the low-temperature limit. Nevertheless, we only used protio- ^1H - and ^2H -formate, so we were unable to determine the intrinsic KIE. NAD-Fdhs from *Candida boidinii* and other sources do not present detectable tunneling. This observation together with studies being performed in Mo/W-dependent Fdhs might be important to understand why some Fdhs catalyze more efficiently the reverse reaction.

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Bidirectional and synergistic allosteric regulation by metabolites of the kinase PDK1

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Phosphoinositide-dependent protein kinase 1 (PDK1) is a master AGC kinase of the PI3K signalling pathway that phosphorylates at least other 23 AGC kinases, being PKB/Akt the most relevant substrate for growth and cell survival, and therefore a potential drug target for cancer treatment. Over the years, our laboratory used a chemical and structural biology approach to study and characterize in detail the mechanism of regulation of the catalytic domain of PDK1. We demonstrated an allosteric regulation from a regulatory site to the active site, as well as the existence of the reverse process. This bidirectional allosteric mechanism of regulation between both pockets can therefore be modulated by small molecules that bind to their specific orthosteric site and either enhance or inhibit interactions at the allosteric site. Considering this, it is not surprising that while the pharmaceutical industry has been developing compounds that bind at the ATP-binding site of kinases, they unwillingly developed drugs that affect protein kinase-protein interactions. We now provide further evidence of the bidirectional system using H/D exchange - MS experiments and show how this is a powerful technique for describing combinatorial coupling effects of a cooperative ligand pair binding at noncontiguous sites. On the other hand, could metabolites bind at the active site of protein kinases and physiologically regulate the formation of protein kinase complexes? Here I present the crystal structure of the catalytic domain of PDK1 in complex with a metabolite bound to the ATP-binding site and compare the structure of the complex and allosteric effects of this metabolite to the crystal structures and allosteric effects of the metabolites Adenine and Adenosine. The findings open the possibility that the physiological regulation of the kinase complexes may be modulated by metabolites and implies that the metabolic state of cells could directly feedback to the regulation of cell signalling.

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Effect of ATP on the synergistic liquid-liquid phase separation of α -synuclein and tau

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Alzheimer's (AD) and Parkinson's (PD) diseases are the most common neurodegenerative disorders associated with cognitive and motor deficits, respectively. The presence of hyperphosphorylated tau and AS amyloid fibrils are the histopathological hallmarks of AD and PD, respectively. The high comorbidity, overlap of clinical symptoms, and co-deposition of aggregates in these proteinopathies suggest that both proteins share common processes that would trigger their cytotoxic aggregations.

Liquid-liquid phase separation (LLPS) of amyloidogenic proteins has been recently suggested as a key player in disease pathogenesis. In these condensates, the components are at much higher concentrations than those in the cytoplasm and can be dynamically exchanged with molecules in the environment. During maturation, the coacervates may become less viscoelastic resulting in the formation of amorphous or amyloid aggregates. Therefore, evaluating conditions that affect the state of protein condensates are of crucial relevance.

In this work, we studied the synergistic phase separation of AS and tau under conditions in which each individual protein shows a low propensity to form liquid droplets. In addition, we explored the influence of ATP, which is dysregulated under pathological conditions, in the phase separation behavior of the proteins. The formation of liquid droplets was followed by fluorescence microscopy and differential interference contrast (DIC). Whereas liquid droplets containing both AS and tau were observed at concentrations of 100 μ M and 1 μ M, respectively, no condensates were formed when the proteins were incubated individually under the same conditions. The addition of ATP at physiologically relevant concentrations inhibits the phase transition of the proteins. Our findings are important for understanding factors that might influence comorbidity in neurological diseases.

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Testing divalent cations as essential activators of the ATPase activity and effect of ssRNA on the catalytic cycle of Zika Virus NS3 helicase

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Zika Virus non structural protein 3 (NS3h) is a molecular motor that couples translocation along single stranded and unwinding of double-stranded RNA with the catalysis of the hydrolysis of nucleoside triphosphates (NTPs)[1]. ATPase activity of NS3h is dependent on the presence of magnesium, which is an essential activator.

In this work we study the effect of single-stranded RNA on the ATPase activity of NS3h and investigated the ability of different divalent cations to replace the role of magnesium.

ATP substrate curves were obtained at different concentrations of homopolyribonucleotide poly(A). ATPase activity of NS3h was enhanced by the presence of RNA, we propose and fit to the experimental data a kinetic model that describes such effect.

In order to determine the ability of different divalent cations to replace magnesium as an essential activator on the catalysis of ATP hydrolysis by NS3h we performed ATPase activity measurements in media containing CaCl_2 , SrCl_2 , MnSO_4 or FeSO_4 in the absence of Mg^{2+} .

Activity was observed in the presence of both MnSO_4 and CaCl_2 and was non detectable in the case of SrCl_2 and FeSO_4 .

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AlphaFold helps improve Pfam models and annotations of multisubunit tethering complexes subunits from the CATCHR family. A case study.

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AlphaFold DB is a recently launched online resource developed by DeepMind in conjunction with the EMBL-EBI, based on results of AlphaFold 2.0, an AI system that predicts highly accurate 3D protein structures from the amino acid sequence for the human proteome and 20 other key organisms. In Pfam, a protein classification database based at the EMBL-EBI, we use these predictions to refine domain boundaries, improve our models, protein coverage and annotations, widely used in the scientific field. From some partial 3D structures and AlphaFold predictions, we improved Pfam entries from the complexes associated with tethering containing helical rods (CATCHR) family, one of the three groups of multisubunit tethering complexes (MTCs) that mediate the interaction between transport vesicles and their target membranes. This is one of the key processes of vesicular trafficking toward different parts of eukaryotic cells, essential to carry out many cellular functions. CATCHR includes the exocyst, conserved oligomeric Golgi (COG), Golgi-associated retrograde protein (GARP and its alternative endosome-associated recycling protein (EARP) complex), and DSL1 complexes, widely conserved from plants to humans and protists. Subunits of these complexes are evolutionary related and share a particular structure consisting of helical bundles arranged in tandem towards the C-terminus and a coiled-coil (CC) region at the N-terminus. Therefore, combining structures and AlphaFold predictions allowed us to build new Pfam families for these subunits and update the existing ones to provide the scientific community with better models to help the understanding of protein interactions and support the drug design field to find effective treatments for human diseases.

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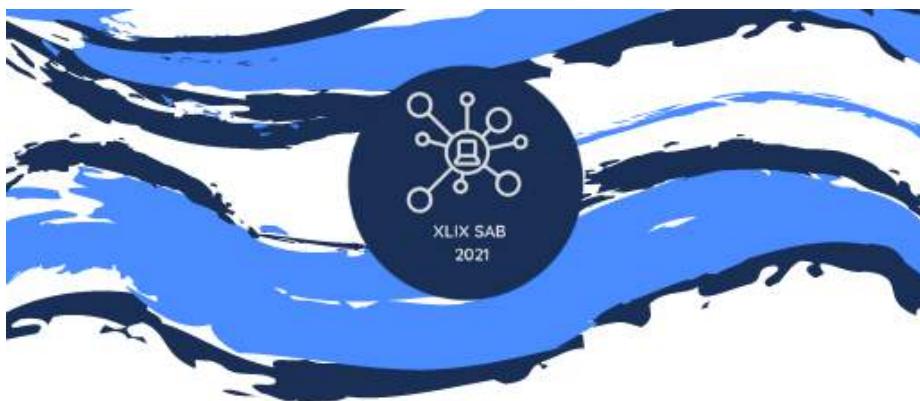
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