

La Plata - 2018



XLVII Reunión Anual de la Sociedad Argentina de Biofísica

Libro de Resúmenes

5 al 7 de Diciembre 2018
Facultad de Ciencias Médicas de La Plata - UNLP

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M. Soledad Celej, Juan Pablo Acierno

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Mario Raúl Ramos

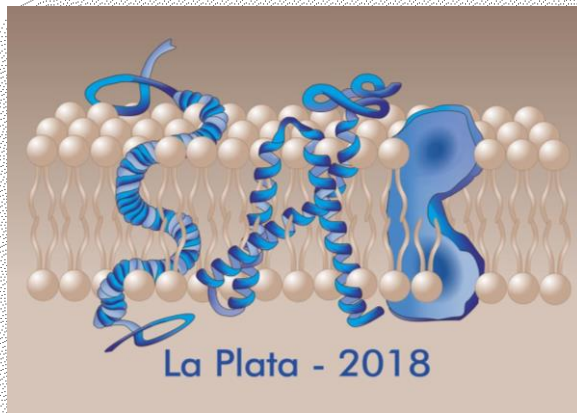
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Sociedad Argentina de Biofísica

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XLVII Reunión Anual SAB

5 – 7 Diciembre 2018

La Plata, Argentina

XLVII Annual Meeting SAB

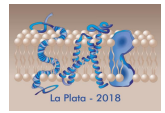
5 – 7 December 2018

La Plata, Argentina



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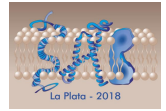
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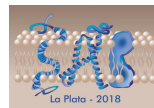
Welcoming words by the organizing committee

Dear members, participants and friends,

As members of the local organizing committee, we are pleased to welcome you to La Plata and to the XLVII Annual Meeting of the Argentine Society of Biophysics (SAB).

The SAB, founded in 1972 when there were not still societies of biophysics in Latin America and officially recognized as member of IUPAB, has steadily maintained annual meetings from its origins according to a permanent policy of promoting communication and cooperation in the scientific community. In every meeting SAB recognizes the scientific work of young investigators through granting the *Jorge Ponce Hornos* Award to the best poster presentation. Also, since three consecutive years SAB supports the “Meetings of young biophysics” organized by a group of supporting members and Ph.D. candidates in order to promote the collaboration and empowerment of the professional relationships of our young people and to strengthen ties of comradeship and friendship. For these reasons, it has been an honour to organize this Meeting and we feel deeply encouraged because of the great attendance of the members, investigators, fellows and students.

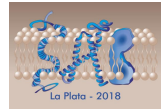
The Meeting will commence with a tribute to the recently deceased Prof. Dr. Rodolfo R. Brenner, an unquestioned mentor of the Argentine Biochemistry and Biophysics. Afterward, during three days, we will enjoy a scientific programme that includes seven Plenary Conferences and eight Symposia, with distinguished national



and international speakers, in which we endeavour to have representatives from all the regions of Argentina as well as investigators in different stages of their career. Apart from having a Symposium of young investigators organized by fellows, works have been selected among over 150 contributions received for the poster sessions, for oral communications within each Symposium.

The cultural, university and scientific imprint of the La Plata city transforms it into an ideal scenario for scientific meetings. The urban landscape is characterized by a planned sketch with large green areas that invite to go all over. Emblematic buildings such as the Museum of Natural Sciences, the Cathedral or the Observatory are unquestionable symbols. In particular, our Meeting will be held in the new building of the Integrated Hospital of the School of Medical Sciences, in front of Paseo del Bosque.

In order to carry out the XLVII Annual Meeting, we have the financial aid of the National Agency of Scientific and Technological Promotion (ANPCyT), of the National Council of Scientific and Technological Research (CONICET), of the University of La Plata (UNLP), of the School of Medical Sciences of UNLP, of the Exact Sciences Foundation of UNLP, and of SAB. We have also received the collaboration of sponsors, such as Avanti Polar Lipids and JENCK. We would like to thank the coordinators of the Symposia, Conferences, and Poster Sessions, as well as the Jury of the Jorge Ponce Hornos Award. We have a special gratitude to those speakers who so generously have financed their own costs of transportation from their cities and countries of origin to be present today among us. Finally, we would like to express our sincere thanks to Mario Raúl Ramos, graphic designer of INIBIOLP for his valuable collaboration during the organization of the present Meeting, as well as Rosana del Cid, English translator of INIBIOLP, for her assistance during the meeting.

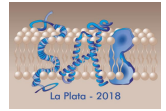


Lastly, we must remember the dramatic situation of our scientific and technological system, currently going through budget cuts, personnel reduction, failure to fulfil prior commitments in subsidies for research and international cooperation. This disheartening situation has been crowned by the recent and regrettable degradation of the Ministry of Science, Technology and Productive Innovation to another secretariat of the Ministry of Education, underestimating the role and importance played by the scientific and technological development of a country at the moment of shaping its future. In this regard, we would like to recall the words by Dr. Bernardo Alberto Houssay, Argentine, winner of the Nobel Prize in Physiology and Medicine in 1947 and founder of CONICET:

“Some people think that scientific investigation is a luxury or an interesting but dispensable entertainment. What a serious mistake! It is an urgent, immediate and unavoidable necessity. Rich countries are as such because they devote money into the scientific-technological development, and poor countries continue being this way because they don’t. Science is not expensive, expensive is the ignorance”.

We really appreciate your presence and hope you make the most of and enjoy the Meeting and our city.

Organizing Committee
XVLII Annual Meeting SAB



Scientific programme

Wednesday, December 5

8:30 – 10:30 h *Registration*

10:30 – 11:00 h **Opening Ceremony**

In memoriam Dr. Rodolfo R. Brenner

Horacio A. Garda, Instituto de Investigaciones Bioquímicas de La Plata (INIBIOLP), La Plata, Argentina.

11:00 – 12:00 h **Confraternity Conference SBE-SAB**

Chair: Lía I. Pietrasanta

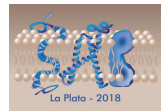
Structural basis and energy landscape for the Ca²⁺-gating and calmodulation of the Kv7.2 K⁺ channel. Óscar Millet, Center for Cooperative Research in Bioscience (CICbioGUNE), Bilbao, Spain.

12:00 – 13:30 h **Symposium 1 - Biomembranes: structure, dynamics and function**

Chairs: C. Fernando García & Nadia S. Chiaramoni

Permeability of red blood cell membranes to hydrogen peroxide. Matías N. Möller, Instituto de Química Biológica, Universidad de la República, Montevideo, Uruguay.

Structural and functional studies of bacterial model membranes and peptide interaction. Fernando G. Dupuy, Instituto Superior de



Investigaciones Biológicas (INSIBIO), San Miguel de Tucumán, Argentina.

New tools for the structural characterization of membrane systems.

Rafael Oliveira, Centro de Investigaciones en Química Biológica de Córdoba (CIQUIBIC), Córdoba, Argentina.

On the antibacterial action of aurein 1.2 and maculatin 1.1 peptides over complex bilayers with differential glycolipid content. A multidisciplinary study using computational and experimental methods. Galo E. Balatti, Instituto de Física de Buenos Aires (IFIBA), Buenos Aires, Argentina.

13:30 – 15:00 h *Lunch*

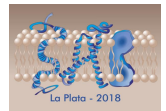
15:00 – 16:30 h **Symposium 2 - Proteins: structure, dynamics and function**

Chairs: Betina Córscico & Mauricio P. Sica

Dissecting the molecular assembly mechanism of a viral RNA polymerase complex. Gonzalo de Prat Gay, Instituto de Investigaciones Bioquímicas de Buenos Aires (IIBBA), Fundación Instituto Leloir (FIL), Buenos Aires, Argentina.

The singular periplasmic flagella from Leptospira: from structure to cellular function (toward integrative Structural Biology). Alejandro Buschiazzi, Instituto Pasteur de Montevideo, Montevideo, Uruguay.

Highly selective mechanisms to transport molybdate and tungstate in sulfate reducing bacteria. M. Gabriela Rivas, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Santa Fe, Argentina.



Study of enterocyte FABPs' protein-protein interactions in Caco-2 cells. Natalia M. Bottasso Arias, Instituto de Investigaciones Bioquímicas de La Plata (INIBIOLP), Universidad Nacional de La Plata, La Plata, Argentina.

16:30 – 17:30 h **Gregorio Weber Conference**

Chair: José María Delfino

Nicotinic receptors as therapeutic drug targets: Molecular bases of function, dysfunction and drug modulation. Cecilia B. Bouzat, Instituto de Investigaciones Bioquímicas de Bahía Blanca (INIBIBB), Bahía Blanca, Argentina.

17:30 – 19:30 h **Poster Session A (odd ID) – with Coffee Break**

19:30 – 21:00 h **Welcome Reception Cocktail**

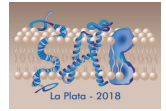
Thursday, December 6

8:30 – 9:00 h **Registration**

9:00 – 10:00 h **Plenary Lecture A**

Chair: M. Alejandra Tricerrí

Structure and function of serum amyloid A in lipid transport and inflammation: new insights into an ancient protein. Olga Gursky, Department of Physiology and Biophysics, Boston University School of Medicine, Boston, USA.



10:00 – 10:30 h *Coffee break*

10:30 – 12:00 h **Symposium 3 – Mathematical models, bioinformatics and computational biophysics**

Chairs: Marcelo D. Costabel & Gisela R. Franchini

Protein structural divergence leave footprints the evolutionary information. Cristina Marino Buslje, Instituto de Investigaciones Bioquímicas de Buenos Aires (IIBBA), Fundación Instituto Leloir (FIL), Buenos Aires, Argentina.

Application of in silico screening for the discovery of new treatments for neglected infectious diseases. Alan Talevi, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, La Plata, Argentina.

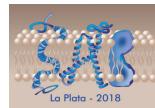
Peptide-lipid membrane interaction in relation to lipid variables: A molecular dynamics approach. M. Florencia Martini, Instituto Química y Metabolismo del Fármaco (IQUIMEFA), Buenos Aires, Argentina.

Ligand binding rates in heme proteins from markov state models and molecular dynamics simulations. Mauro Bringas, Instituto de Química, Física de los Materiales, Medioambiente y Energía (INQUIMAE), Buenos Aires, Argentina.

12:00 – 13:30 h **Symposium 4 - Young Researchers**

Chairs: Macarena Siri & M. Florencia González Lizarraga

Analysis of single cell dynamics and their relationship with the cell cycle and cell fate in mouse embryonic stem cells. Federico Sevlever, Instituto de Fisiología, Biología Molecular y Neurociencias (IFIBYNE), Buenos Aires, Argentina.



Single image deconvolution with super-resolution using the SUPPOSE algorithm. Micaela Toscani, Departamento de Física, Facultad de Ingeniería, Universidad de Buenos Aires, Buenos Aires, Argentina.

In vivo systems to study class II bacteriocins toxicity and immunity. Natalia Ríos Colombo, Instituto Superior de Investigaciones Biológicas (INSIBIO), San Miguel de Tucumán, Argentina.

Single Molecules Studies of the Dynamics of NS3h Helicase from the Dengue Virus. Fernando Amrein, Instituto de Química y Fisicoquímica Biológicas (IQUIFIB), Universidad de Buenos Aires, Buenos Aires, Argentina.

13:30 – 15:00 h Lunch

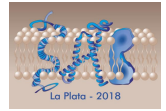
15:00 – 16:30 h Symposium 5 – Intermolecular interactions

Chairs: Lisandro J. Falomir Lockhart & Horacio A. Garda

Molecular determinants that regulate the efficiency of protein N-glycosylation. Julio J. Caramelo, Instituto de Investigaciones Bioquímicas de Buenos Aires (IIBBA), Fundación Instituto Leloir (FIL), Buenos Aires, Argentina.

Exploiting the therapeutic potential of ready-to-use drugs: Repurposing antibiotics against amyloid aggregation in neurodegenerative diseases. S. Benjamín Socías, Instituto de Investigación en Medicina Molecular y Celular Aplicada (IMMCA), San Miguel de Tucumán, Argentina.

Photosensitized modification of proteins: from oxidative stress to biocatalysis application. Claudio D. Borsarelli, Instituto de



Bionanotecnología del NOA (INBIONATEC), Universidad Nacional de Santiago del Estero, Santiago del Estero, Argentina.

Biophysical characterization of the interaction between SNH and QLQ protein domains. Marco C. Miotto, Instituto de Biología Molecular y Celular de Rosario (IBR), Rosario, Argentina

16:30 – 17:30 h **Plenary Lecture B**

Chair: Vanesa S. Herlax

Sticholysins, beyond their ability to form pores in the membranes, are attractive components for the design of nanobiomedical devices.

M. Eliana Lanio, Centro para Estudios de Proteínas, Universidad de La Habana, La Habana, Cuba.

17:30 – 19:30 h **Poster Session B (even ID) – with Coffee Break**

19:30 – 21:00 h **SAB Assembly Meeting**

Friday, December 7

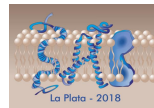
8:30 – 9:00 h *Registration*

9:00 – 10:00 h **Plenary Lecture C**

Chair: Sabina M. Maté

Langmuir monolayers as cell membrane models to investigate molecular-level interactions with pharmaceutical drugs. Osvaldo N.

Oliveira Jr., Instituto de Física de São Carlos, Universidade de São Paulo, Sao Carlos, Brazil.



10:00 – 10:30 h *Coffee break*

10:30 – 12:00 h **Symposium 6 - New technologies**

Chairs: Natalia Wilke & M. Soledad Celej

Cell adhesion studies using electrochemical sensors. Diego A. Pallarola, Instituto de Nanosistemas (INS), Universidad Nacional de San Martín (UNSAM), San Martín, Argentina.

Nanomechanical properties and molecular recognition assessed by Force Spectroscopy. María Elena Vela, Instituto de Investigaciones Físico-químicas Teóricas y Aplicadas (INIFTA), Universidad Nacional de La Plata, La Plata, Argentina.

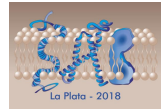
Photothermic ablation of pathogenic bacteria through the combined application of NIR irradiation and nanomaterials as photothermal agents. E. Inés Yslas, Facultad de Ciencias Exactas, Físicoquímicas y Naturales, Universidad Nacional de Río Cuarto (UNR), Río Cuarto, Argentina.

Protein scaffold library for nanotechnological applications based on BLS protein engineering. Santiago Sosa, Instituto de Investigaciones Bioquímicas de Buenos Aires (IIBBA), Fundación Instituto Leloir (FIL), Buenos Aires, Argentina.

12:00 – 13:30 h **Symposium 7 - Channels, transporters and receptors**

Chairs: Cecilia B. Bouzat & Gabriela Amodeo

Expanding our knowledge about the structure and function of the serotonin type 3 receptor. Jeremías Corradi, Instituto de



Investigaciones Bioquímicas de Bahía Blanca (INIBIBB), Bahía Blanca, Argentina.

Arachidonic acid modulation of BK (Slo1) channels: Role of the $\beta 1$ accessory subunit. Pedro Martín, Instituto de Estudios Inmunológicos y Fisiopatológicos (IIFP), Universidad Nacional de La Plata, La Plata, Argentina.

Mechanism of primary aldosteronism induction by mutations in the $Na^+,K^+-ATPase$. Pablo Artigas, Department of Cell Physiology and Molecular Biophysics, Texas Tech University Health Sciences Center, Lubbock (TX), USA.

Modeling arginine peptides' adsorption to membrane pores: A molecular theory approach. Pedro G. Ramírez, Instituto de Matemática Aplicada San Luis (IMASL), UNSL-CONICET, San Luis, Argentina.

13:30 – 15:00 h *Lunch*

15:00 – 16:30 h **Symposium 8 - Microscopy and spectroscopy**

Chairs: Lorena Sigaut & Luis A. Bagatolli

Sensors and actuators to understand 3D organization of living matter. Hernán E. Grecco, Instituto de Física de Buenos Aires (IFIBA), Buenos Aires, Argentina.

Laurdan spectroscopy in a bi-photonic microscope for the study of biological membrane heterogeneity. Susana A. Sánchez Donoso, Facultad de Ciencias Químicas, Universidad de Concepción, Concepción, Chile.



Fluorescence Correlation Spectroscopy experiments using single-wavelength calcium dyes. Silvina M. Ponce Dawson, Instituto de Física de Buenos Aires (IFIBA), Buenos Aires, Argentina.

A spectroscopic and kinetic study of the singlet oxygen mediated oxidation of human and bovine serum albumin. M. Beatriz Espeche Turbay, Instituto de Bionanotecnología del NOA (INBIONATEC), Universidad Nacional de Santiago del Estero, Santiago del Estero, Argentina.

16:30 – 17:30 h Plenary Lecture D

Chair: Mario R. Ermácora

Using crystallography to redefine the function of a biological molecule: plasma retinol-binding protein. Hugo L. Mónaco, Department of Biotechnology, University of Verona, Verona, Italy.

17:30 – 18:00 h Coffee break

18:00 – 18:30 h Closing Ceremony and Ponce Hornos Awards

Chairs: Lía I. Pietrasanta & Horacio A. Garda

18:30 – 19:30 h Closing Conference

Chair: Gerardo D. Fidelio

On sphingolipids and tigers. Félix Goñi, BIOFISIKA Basque Centre for Biophysics, Universidad del País Vasco, Bilbao, Spain.

Plenary Lectures

XLVII SAB 2018

Langmuir monolayers as cell membrane models to investigate molecular-level interactions with pharmaceutical drugs

Oliveira Jr ON^a

a - São Carlos Institute of Physics, University of São Paulo, Brazil

The design of new pharmaceutical drugs requires detailed understanding of their mode of action, particularly in cases of drug resistance as for the super-resistant bacteria. Novel drugs, including antimicrobial peptides, are being sought which can cause disruption on the bacteria membrane, either via pores or through detergent-like mechanisms. In this presentation, an overview will be given of cell membrane models based on Langmuir monolayers and vesicles, with which the mode of action of pharmaceutical drugs can be probed at the molecular level. Monolayer composition is varied to mimic distinct types of bacteria membranes, e.g. including lipopolysaccharides to account for the outer layer of Gram-negative bacteria. The interactions between the monolayer-forming molecules and the drugs are investigated with surface pressure isotherms, Brewster angle microscopy and polarization-modulated infrared reflection absorption spectroscopy (PM-IRRAS). Upon combining these methods with leakage experiments in vesicles, one may determine the chemical groups responsible for the drug-monolayer interactions and distinguish between pore formation and detergent mechanisms. Most importantly, this type of study allows one to correlate molecular-level interactions in cell membranes with drug bactericide activity.

Contact: chu@ifsc.usp.br

Acknowledgments

FAPESP, CNPq and CAPES (Brazil)

On sphingolipids and tigers

Goñi FM^a, Alonso A^a

a - Instituto Biofisika (CSIC, UPV/EHU) and Universidad del País Vasco, 48940 Leioa, Spain.

Some of the simplest sphingolipids, namely sphingosine, ceramide and their phosphorylated compounds [sphingosine 1-phosphate (Sph-1-P) and ceramide 1-phosphate (Cer-1-P)], are potent metabolic regulators. Each of these lipids modifies in marked and specific ways the physical properties of the cell membranes, in what can be the basis for some of their physiological actions. The present paper is an overview of the mechanisms by which these sphingolipid signals, sphingosine and ceramide, in particular, are able to modify the properties of cell membranes. Moreover the properties of ceramide, sphingomyelin and cerebroside will be comparatively discussed, in parallel with the behaviour of a fierce free tiger, a fierce caged tiger, and a tamed tiger.

Sticholysins, beyond their ability to form pores in the membranes, are attractive components for the design of nanobiomedical devices

Lanio M^a, Laborde R^a, Cruz-Leal Y^a, Lopetegui I^a, Alvarez C^a, Pazos F^a, Luzardo M^a, Fernández A^b, Oliver L^b, Mesa C^b, Abreu-Butin L^c, Longo-Maugéri I^c, Nogueira C^d, Grubaugh D^d, Higgins D^d, Fernández L^b

a - Center for Protein Studies, Faculty of Biology, University of Havana

b - Center for Molecular Immunology, Havana, Cuba

c - Department of Microbiology, Immunology and Parasitology, Federal University of São Paulo, Brazil,

d - Department of Microbiology and Immunology, Harvard Medical School, Harvard University, USA

Sticholysins I and II (StI/II, Sts), two pore-forming proteins (PFPs) produced by the sea anemone *Stichodactyla helianthus*, are highly hemolytic cysteineless proteins exhibiting a preference for sphingomyelin-containing membranes. They are able to form oligomer pores of diameter 2 nm and to permeabilize membranes from different cells in nM range. StI binds and permeabilizes with higher efficiency sterol-containing membranes independently of their ability to form domains and probably due to molecular heterogeneity, fluidity, and negative curvature of the bilayer induced by sterols. In this work we also explored the Sts potential use to improve the antigen-specific cytotoxic T CD8+ lymphocytes (CTLs) response. We studied the enhancement of CTLs response by liposomes co-encapsulating Sts with ovalbumin as model antigen (Lp/OVA/Sts). These liposomal formulations induced a robust OVA-specific CD8+ T-cell response *in vivo* and *in vitro* significantly enhanced activation of the SIINFEKL-specific B3Z CD8+ T cells as a consequence of antigen cross-presentation by macrophages, but not by dendritic cells (DCs). Unexpectedly, Lp/OVA/Sts-induced activation was inhibited by lysosomes proteases inhibitors, but not proteasome inhibitor indicating that Sts induce antigen cross-presentation by vacuolar pathway. The formulations Lp/OVA/Sts also conferred a higher protection to mice challenged with OVA-expressing tumor cells, which was strongly affected by CD8+ T-cells depletion. Interestingly, free-Sts were able of inducing activation of DCs and it was dependent of TLR-4 and MyD88, suggesting that the effect of these proteins on the cellular immune response could be beyond their pore-forming ability. The antigen-specific CTLs immune response enhanced by immunization of WT mice with Lp/OVA/StII was significant reduced in TLR-4 knockout mice. Our results suggest the potentialities of Sts encapsulated into liposomes as adjuvant for enhancing effective CTLs mediated immune responses.

Acknowledgments

FAPESP, CNPq, CAPES, IFS, DRLAC, Project of Lab UH-CIM

Structural insights into enigmatic functions of serum amyloid A in health and disease

Jayaraman S^a, Frame N^a, Gursky O^a

a - Department of Physiology and Biophysics, Boston University School of Medicine, Boston MA USA

Our research addresses central unresolved questions in normal functions and pathological misfolding of a plasma protein better known for its role in disease than for its beneficial action in innate immunity and lipid homeostasis. Serum amyloid A (SAA, 12 kDa) is an enigmatic biomarker of inflammation and a protein precursor of amyloid A (AA) amyloidosis, a major life-threatening complication of chronic inflammation. SAA has been highly conserved since Cambrian period and must have played a vital yet unknown role. In acute infection, inflammation or injury, plasma levels of SAA raise up to 1,000-fold reaching >1 mg/ml within hours and then drop. The advantage for survival of this dramatic but transient increase is unknown. However, the rapid and major commitment of liver to the biosynthesis of SAA, its lipophilic character, and the evolutionally conserved aspects of its structure and function emerging from our work strongly support the importance of SAA - lipid interactions. Our biochemical studies suggest that the vital primordial function of SAA is to clear cell membrane debris from the injured sites. Our structural and biophysical studies suggest strongly that SAA performs this function by binding and solubilizing lipids via an unique concave hydrophobic surface formed by its two amphipathic alpha-helices. We propose that the curvature of this site, which is maintained via a 100% conserved GPGG motif, explains preferential binding of SAA to its major plasma carrier, high-density lipoprotein (or “good cholesterol”), versus larger less curved lipoproteins. Finally, our results reveal that SAA generates substrates and removes reaction products from secretory phospholipase A2, an acute-phase reactant upregulated simultaneously with SAA. Together, these results explain why SAA has been highly evolutionally conserved for 500 million years, why is it upregulated concomitantly with sPLA2 in inflammation, and provide *raison d’être* for SAA.

Acknowledgments

Collaboration with Drs. Marcus Fandrich and Christian Haupt (Ulm, Germany) is gratefully acknowledged. This work was supported, in part, by the National Institutes of Health grants GM067260 and HL007969.

Using crystallography to redefine the function of a biological molecule: plasma retinol-binding protein

Mónaco HL^a

a - Biocrystallography Laboratory, Department of Biotechnology, University of Verona

RBP4 (plasma retinol-binding protein) is the 21 kDa transporter of all-trans retinol that circulates in plasma as a moderately tight 1:1 molar complex of the vitamin with the protein (*D.S. Goodman. Plasma retinol-binding protein. In "The Retinoids"; Sporn, Roberts & Goodman, Eds. 1984 vol 2, pp41-88. Academic Press, NY*). Many years ago we published the structure of RBP4 holo and believed to be apo at 2.5 Å resolution (*G. Zanotti, et al, J. Mol. Biol. 1993* **230**, 613-624) and wrote at the end of the abstract "In the case of the unliganded form, the central cavity that is occupied by the vitamin in human crystalline holo RBP, is filled by electron density that, at the present resolution, we interpret as solvent." The crystals had been prepared by microdialysis using protein purified from human plasma and, since they were still kept in our laboratory, we tested them at the ESRF a couple of years ago. We collected a full data set of both the liganded and unliganded crystals at a resolution of 1.5 Å and 2.0 Å respectively and were able to refine the unliganded form using about 20,000 reflections instead of the 10,000 used in the original paper. We identified a fatty acid in the ligand-binding site of the protein believed to be apo. We then prepared crystals of RBP4 purified from human urine and amniotic fluid, two sources that contain non-fluorescent RBP4, i.e. not bound to retinol, and for that reason believed to be the apo form of the protein. In every case we found a fatty acid in the central cavity of the RBP4 molecule, a result that we confirmed by GC-MS analysis. This result changes substantially our perception of this protein that has been considered to be specific for retinol from its discovery about 50 years ago and is a good example of how simply increasing the quality of the diffraction data can change our understanding of the function of a protein (*M. Perduca et al. BBA-Molecular and Cell Biology of Lipids 2018* **1863** 458-466).

Nicotinic receptors as therapeutic drug targets: Molecular bases of function, dysfunction and drug modulation

Bouzat C^a

a - INIBIBB- UNS/CONICET- Bahía Blanca- Argentina

Nicotinic acetylcholine receptors (nAChR) are pentameric ligand-gated ion channels widely expressed in neuronal and non-neuronal cells in both vertebrates and invertebrates. In humans, dysfunction of nAChRs is associated with neurological, muscular and immune disorders. The nAChR operates as a molecular machine that transduces the binding of ACh into an electrical signal. Its molecular design has been tuned to function as a near perfect on-off switch that responds to ACh with the efficiency and speed required for proper cell function. We have combined mutagenesis, patch-clamp recordings, single-channel kinetic analysis and *in silico* studies to unravel the molecular mechanisms and structures underlying nAChR activation and modulation as well as to identify compounds with potential therapeutic use. For the muscle nAChR, a key protagonist in muscle contraction, we have postulated mechanisms that describe its activation and modulation, deciphered how mutations lead to congenital myasthenic syndromes, and explored worm receptors as antiparasitic drug targets. We have also focused on $\alpha 7$ nAChR, which is the homomeric member of the family and is involved in cognition, attention, memory and inflammation. We have revealed unique aspects of $\alpha 7$ activation as well as mechanisms and sites of action of positive allosteric modulators, which are promising therapeutic tools for schizophrenia and Alzheimer's disease. We have also identified the molecular function of novel heteromeric nAChRs containing the $\alpha 7$ subunit. Overall, our studies have allowed an integrated description of the nAChR, providing information of its molecular function in health and disease states and guiding rational therapy.

Structural basis and energy landscape for the Ca²⁺-gating and calmodulation of the Kv7.2 K⁺ channel

Bernardo-Seisdedos G^{a,b}, Viadero EN^a, Gomis-Perez C^a, Malo C^a, Villarroel A^a, **Millet O^b**

a - Instituto Biofisika (CSIC, UPV/EHU), campus of the University of Basque Country, 48940, Leioa, Spain

b - Protein Stability and Inherited Disease Laboratory, CIC bioGUNE, Bizkaia Technology Park, Building 800, 48170, Derio, Spain

Kv7.2 (KCNQ2) channel is the principal molecular component of the slow voltage-gated non-inactivating K⁺ M-current, a key controller of the neuronal excitability. To investigate the calmodulin-mediated Ca²⁺ gating of the channel, we used NMR spectroscopy to structurally and dynamically describe the association of helices *hA* and *hB* of Kv7.2 with calmodulin (CaM), as a function of Ca²⁺ concentration. The structures of the CaM/Kv7.2-hAB complex at three different calcification states are here reported. In the presence of a basal cytosolic Ca²⁺ concentration (10-100 nM) only the N-lobe of calmodulin is Ca²⁺-loaded and the complex (representative for the open channel) exhibits collective dynamics in the millisecond timescale towards a lowly populated excited state (1.5%) that corresponds to the inactive state of the channel. In response to a chemical or electrical signal, intracellular Ca²⁺ levels rise up to 1-10 μM, triggering Ca²⁺ association to the C-lobe. The associated conformational rearrangement is the key biological signal that shifts populations to the closed/inactive channel. This reorientation affects C-lobe of CaM and both helices in Kv7.2, allosterically transducing the information from the Ca²⁺ binding site to the trans-membrane region of the channel.

Symposia

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New tools for the structural characterization of membrane systems

Oliveira RG^a

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

New emerging techniques are allowing the experimental determination of structural parameters in membrane systems at the South Cone. The first one is a complementation of classical reflectivity in a Brewster angle microscope (BAM). By contrast matching we experimentally determine the refractive index of Langmuir monolayers. The refractive index and reflectivity allow us to calculate the thickness of the films as a function of surface pressure. In order to evaluate what kind of thickness is actually measured, the results from BAM were compared to the ones determined by GIXOS (Grazing Incidence X-ray Off Specular Scattering). The last is a new setup that substitutes the specular X-ray reflectivity on the determination of the perpendicular structure of the monolayer. This setup is currently working at the Brazilian Synchrotron, rendering the first results which will be more meaningful at the future synchrotron Sirius. The comparison of GIXOS and BAM thicknesses, among other measurements in bilayers and multilayers, (SAXS and XRD) allows us to determine that the BAM setup measures the whole thickness of the monolayer. Additionally, the grazing incidence setup is allows measuring the first X-ray diffractions at wide angle. At Sirius, with an X-ray beam 104 more powerful, the new data at grazing incidence will be obtained faster, allowing kinetic acquisition and full modeling of the data over extended scattering vector range. For the next step, we are considering to couple the grazing incidence setup to an X-ray fluorescence detector over the monolayer; this will allow mapping of the vertical distribution of the heavier elements from Z=15. Finally, the Laboratorio Argentino de Haces de Neutrones (LAHN) is planning for the second round of instrumentation a Neutron Reflectometer and a Small Angle Neutron Scattering instrument. Altogether, these efforts will cover different systems from monolayer to bi- and multi-layers in a set of variable environmental conditions.

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On the antibacterial action of aurein 1.2 and maculatin 1.1 peptides over complex bilayers with differential glycolipid content. A multidisciplinary study using computational and experimental methods

Balatti GE^a, Szymanowski F^b, Hugo A^b, Ambroggio E^c, Gomez Zavaglia A^b, Pérez PF^b, Fidelio GD^c, Martini MF^d, Pickholz MA^a

a - Instituto de Física de Buenos Aires (IFIBA), Argentina.

b - Centro de Investigación y Desarrollo en Criotecología de Alimentos (CIDCA-UNLP)

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

d - Instituto de Química y Metabolismo del Fármaco. Facultad de Farmacia y Bioquímica (UBA-CONICET)

The antimicrobial peptides (AMPs) are key molecules of the innate immune system that exert their action mainly against bacteria. It was suggested that AMPs act by increasing the permeability of the membrane after an initial peptide-lipid interaction. Among AMPs, the aurein and the maculatin exhibit a well-proven lytic activity and, previously, we shed light about the molecular mechanism by which they damage different biomembranes by combined computational and experimental approaches. We also found that the *Lactobacillus delbrueckii* subsp. *lactis* (CIDCA133) and the *L. delbrueckii* subsp. *bulgaricus* (CIDCA331) strains of lactic bacteria show differential susceptibility to the action of several AMPs, including aurein and maculatin.

Considering that bilayer composition is a key aspect of the early peptide-lipid interaction and that the resistant strain shows high amounts of glycolipids, we defined a set of different model membranes and carried out extensive Molecular Dynamics (MD) within the MARTINI coarse-grain (CG) force field. Since both peptides are helicoidal AMPs but they seem to act by different molecular mechanisms, the aim was to understand how the lipid composition is related with the resistance to the action of that kind of peptides.

The results show a capability of both peptides to affect membrane bilayers, but the different membranes are differentially affected. In effect, we found a strong correlation between the proportion of glycolipids in the membrane and the ability to keep the membrane integrity against the AMP action. In addition, and as we pointed in previous works, maculatin can induce membrane curvature in the bilayers, despite the glycolipid presence. Each model membrane shows differential molecular behavior with the AMP molecules, and the use of CG simulations supported by experimental techniques can help to elucidate the specific molecular factors that allow certain bilayer configurations to resist or diminish the effects of AMPs against them.

Structural and functional studies of bacterial model membranes and peptide interaction

Medina Amado C^a, Garnica M^a, De Marco N^a, Oliveira RG^b, **Dupuy F^a**

a - Instituto Superior de Investigaciones Biológicas (INSIBIO) CONICET/UNT and Instituto de Química Biológica "Dr Bernabé Bloj", Facultad de Bioquímica, Química y Farmacia, UNT. San Miguel de Tucumán, Argentina

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

Novel antimicrobial therapies are needed as a result of the increasing number of microbials resistant to ordinary antibiotics. In this regard, bacterial membranes represent an important molecular target for new antimicrobials like amphipathic peptides. The bacterial lipid bilayer is typically composed by a higher amount of anionic lipid, compared to eukaryotic membranes. Also, nonbilayer structures can be formed by some of the main bacterial lipids, like phosphatidylethanolamine (PE) or even phosphatidylglycerol (PG) and cardiolipin (CL), when negative charge is screened. In this work, bacterial model membranes were studied by means of x ray diffraction techniques in order to assess structural rearrangements produced by interaction with antimicrobial peptides. In addition, functional and molecular properties as determined by infrared, fluorescence spectroscopy and Langmuir monolayers were investigated. The results indicate that bilayer composition can modulate the mechanisms by which peptides interact and perturb membrane properties in different ways. Thus, there is none a single mechanism of action of peptide antimicrobial activity, but it rather depends on the intrinsic properties of each particular membrane.

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Arachidonic acid modulation of BK (Slo1) channels: Role of the $\beta 1$ accessory subunit

Moncada M^a, Ducca G^a, Orsi F^a, Castillo K^b, Milesi V^a, **Martín P^a**

a - IIFP, CONICET-UNLP, La Plata, Argentina.

b - Centro Interdisciplinario de Neurociencias de Valparaíso (CINV), Chile

Arachidonic acid (AA) is a polyunsaturated fatty acid involved in modulation of several ion channels activity. Previously, we reported that 10 μM AA activates the high conductance Ca^{2+} - and voltage-dependent K^{+} channel (BK) in human vascular smooth muscle cells where the α subunit of BK is expressed together with the accessory $\beta 1$ -subunit ($\beta 1$) [1]. In this work, we studied in depth the action mechanism of AA using the patch-clamp technique on BK channel heterologously expressed with $\beta 1$. 10 μM AA activated BK only in presence of $\beta 1$, changing the voltage dependence of activation (left shift on G-V curve, $\Delta V_{1/2} = -55.2 \text{ mV} \pm 4.4$; $n=3$; $p<0.05$). We also demonstrated that the modulation of the channel by AA is direct, without involving its metabolites since activation persisted in the presence of AA metabolic enzymes blockers (Indomethacin, CDC and 17-ODYA to block the COX, LOX and CYP450 enzymes, respectively). Considering that activation by AA requires the presence of $\beta 1$, which modulates the Ca^{2+} and the voltage sensor, and the intrinsic opening of the channel, we analyzed whether AA acts changing the $\beta 1$ modulation of these processes. By measuring the gating currents, we evaluated if the voltage sensor is affected by AA, observing that it produces a significant left shift in the Q-V curve ($\Delta V_{1/2} = -17.2 \pm 8.1 \text{ mV}$, $n=5$, $p<0.05$). We also studied the effect of AA on the intrinsic channel opening probability (NPoi). The results showed that AA increases NPoi in all tested cells (control: NPoi = 0.0013 ± 0.0008 ; AA: NPoi = 0.0245 ± 0.0051 ; $n=4$; $p<0.05$). Finally, the AA-induced BK channel activation was independent of the intracellular Ca^{2+} concentration ($\Delta V_{1/2} = -59.8 \text{ mV} \pm 4.8$ and $-67.5 \text{ mV} \pm 8.8$ at 3 nM and 1 μM Ca^{2+} , respectively, $n=5-6$, $p<0.05$). These results indicated that BK activation by AA depends on the presence of $\beta 1$ -subunit involving changes in both the voltage sensor activation and in the intrinsic opening of the channel.

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Expanding our knowledge about the structure and function of the serotonin type 3 receptor

Corradi J^a

a - Instituto de Investigaciones Bioquímicas de Bahía Blanca (INIBIBB) - UNS/CONICET - Bahía Blanca-Argentina.

Serotonin (5-hydroxytryptamine: 5-HT) is a monoamine with diverse and important functions in nearly every living organism in the Earth. 5-HT₃ receptors are the only members of the 5-HT receptors subfamily (5-HT₁₋₇) that belong to the Cys-loop receptor family. They are ligand-gated ion channels that mediate fast synaptic responses in the central and peripheral nervous system and are implicated in many physiological and pathological processes, such as cognition, pain, vomiting reflex, depression, anxiety, among others. Five subunits have been identified in humans (A-E), where the A is the only capable of forming homomeric receptors (5-HT_{3A}), and the B-E are accessory subunits that only form receptors by combining with A. Since its discovery, the 5-HT_{3A} receptor has been widely studied through electrophysiological methods limited to the acquisition of macroscopic currents due to its very low conductance. By incorporation of a triple mutation in the intracellular loop we obtained a high-conductance receptor (5-HT_{3AHC}). Using this model receptor, we were able to record single-channel events which, in combination with macroscopic currents, kinetics analysis and *in silico* studies, allowed us to reveal the mechanisms for receptor activation by full and partial agonists, and to identify sites and mechanisms of modulation. Our results also showed that the AHC subunit is a valuable tool for the elucidation of the stoichiometry of heteromeric receptors. All this information provides new clues for understanding about 5-HT₃ receptors implication in human health and for the designing of more selective drugs.

Mechanism of primary aldosteronism induction by mutations in the Na⁺,K⁺-ATPase

Artigas P^a, Meyer DJ^a

a - Department of Cell Physiology and Molecular Biophysics Texas Tech University Health Sciences Center

Primary hyperaldosteronism, also known as Conn's syndrome, is the most common cause of secondary hypertension and is frequently triggered by a unilateral aldosterone-producing adenoma in the adrenal gland. These adenomas carry somatic mutations to various genes encoding channels and primary-active transporters, including ATP1A1 which encodes the catalytic α 1-subunit of the Na/K pump, the P-type ATPase that builds and maintains the ionic gradients used for excitability and secondary active transport. All reported ion-channel mutations increase inward currents through the mutated channels (a so-called "gain-of-function"), and therefore are proposed to elevate aldosterone-production by depolarizing the resting voltage, leading to higher Ca²⁺-channel activity and increased [Ca²⁺]_i. Observation of abnormal inward currents through the first four ATP1A1 mutants characterized led to the proposal that Na/K pump mutations induce hyperaldosteronism by a similar depolarization mechanism. I will present and discuss recent evidence from our laboratory in support of an alternative hypothesis: That Na/K pump mutations induce hyperaldosteronism through a loss-of-function mechanism.

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Modeling arginine peptides' adsorption to membrane pores: A molecular theory approach

Ramírez PG^a, Vila JA^a, Szleifer I^b, Longo GS^c, Del Pópolo MG^d

a - Instituto de Matemática Aplicada San Luis (IMASL), UNSL-CONICET, San Luis, Argentina.

b - Department of Biomedical Engineering, Northwestern University, Evanston, IL, USA.

c - Instituto de Investigaciones Fisicoquímicas Teóricas y Aplicadas (INIFTA), UNLP-CONICET, La Plata, Argentina.

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

Experiments and molecular simulations suggest that the membrane translocation mechanism of arginine-rich cell penetrating peptides (ARCPPs) across lipid bilayers involves the formation of trans-membrane hydrophilic pores. However, there are still many unanswered questions regarding the role of relevant peptide and membrane properties on translocation efficiency, such as membrane charge and acidity, peptide length and conformational flexibility, etc., as to state that there is clear consensus on how ARCPPs work under physiological conditions. In the present work, we develop a Molecular Theory (MT) to systematically investigate the binding of arginine-rich peptides to lipid bilayers bearing a cylindrical pore. The MT accounts for the acid-base equilibrium of all titratable species, the electrostatic and steric interactions as well as entropic effects, while also incorporating specific molecular information of the peptides, including size, shape, conformation, protonation state, and charge distribution. The state of protonation of lipids in the membrane is not assumed *a priori* but predicted locally depending on the interplay between molecular organization and the aforementioned physico-chemical effects. We present a methodical investigation of the effect of pore size, peptide concentration, and chain length, on the extent of peptide adsorption and insertion into the pore. Our results suggest that membrane adsorption plays a key role on peptide translocation. For peptides shorter than ARG₉, adsorption increases significantly with chain length, but saturates for longer peptides. However, such behavior only occurs at relatively low peptide concentrations as increasing peptide concentration favors adsorption of the shorter molecules. Peptide inclusion into the pore shows a non-monotonic dependence on the pore radius. We also observe that to favor peptide adsorption, the pore surface becomes more negatively charged than the rest of the membrane surface.

Contact: pgerramirez@gmail.com

Protein scaffold library for nanotechnological applications based on BLS protein engineering

Sosa S^a, Rossi AH^a, Violi IL^b, Szalai AM^b, Bonomi HR^a, Stefani FD^b, Goldbaum FA^a

a - Fundación Instituto Leloir and IIBBA-CONICET

b - Centro de investigaciones en Bionanociencia (CIBION), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)

Self-assembly of biological macromolecules, such as proteins, lipids and DNA, is a key aspect of life. Protein nanoparticles (PNPs) are a special subset of supramolecular protein assemblies. The PNP hallmark is a symmetric, repetitive structure, that can have enhanced stability (e.g. chemical, mechanical or thermal) and can be highly immunogenic. Engineering oligomeric protein self-assembly is emerging as an interesting way to fabricate nanostructures with well-defined geometries, stoichiometry and functions.

BLS (*Brucella* Lumazine Synthase) is a highly stable and immunogenic homodecamer that can be regarded as a dimer of head-to-head oriented homopentamers. Therefore, we decided to engineer it in order to develop a versatile scaffold. We have designed two pentameric mutants that can bind to each other to form an heterodecamer. Our results show that this heterodecameric structure can be used as an asymmetric particle. As a proof of concept a bifunctional BLS particle bearing Alexa Fluor 488 fluorophore molecules on one side and sialic acid binding domains on the other was used for labelling murine and human cells (flow cytometry and confocal microscopy). We have also introduced reactive cysteines in different positions of the wild type BLS and its pentameric forms in order to evaluate different applications. We have some promising results (absorbance spectroscopy, DLS and SEM) that show that BLS can be used as a scaffold of Gold Nanoparticles oligomers. On the other side, we have shown that this structure can be used for multichromoforic FRET studies (smFRET). Finally, bioinformatics studies suggest that BLS can be used as a protein cage of small molecules. *In silico* analysis show that this protein has a cavity (9,5 Å radius) that can be engineered to bind fluorophores or drugs.

In conclusion, we have developed a library of rational designed mutants of BLS that can be used in different fields, such as biotechnology and nanotechnology.

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Exploiting the therapeutic potential of ready-to-use drugs: Repurposing antibiotics against amyloid aggregation in neurodegenerative diseases

Socias SB^a, González Lizarraga MF^a, Avila CL^a, Vera CC^a, Ploper D^a, Acuña L^a, Del-Bel E^a, Raisman-Vozari R^a, Chehín R^a

a - IMMCA (Instituto de Investigación en Medicina Molecular y Celular Aplicada)

Neurodegenerative diseases are chronic and progressive disorders that affect specific regions of the brain, causing gradual disability and suffering that results in a complete inability of patients to perform daily functions. Amyloid aggregation of specific proteins is the most common biological event that is responsible for neuronal death and neurodegeneration in various neurodegenerative diseases.

Therapeutic agents capable of interfering with the abnormal aggregation are required, but traditional drug discovery has fallen short. The exploration of new uses for approved drugs provides a useful alternative to fill the gap between the increasing incidence of neurodegenerative diseases and the longterm assessment of classical drug discovery technologies. Drug re-profiling is currently the quickest possible transition from bench to bedside. In this way, experimental evidence shows that some antibiotic compounds exert neuroprotective action through anti-aggregating activity on disease-associated proteins. The finding that many antibiotics can cross the blood-brain barrier and have been used for several decades without serious toxic effects makes them excellent candidates for therapeutic switching towards neurological disorders. The present review is, to our knowledge, the first extensive evaluation and analysis of the anti-amyloidogenic effect of different antibiotics on well-known disease-associated proteins. In addition, we propose a common structural signature derived from the antiaggregant antibiotic molecules that could be relevant to rational drug discovery

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Molecular determinants that regulate N-glycosylation efficiency

Couto PM^a, Caramelo JJ^a

a - Instituto de Investigaciones Bioquímicas de Buenos Aires (IIBBA-CONICET) y Fundación Instituto Leloir

N-glycosylation is one of the most common a drastic protein modification in eukaryotes. Nearly 25 % of these proteins are modified with a high mannose oligosaccharide. These bulky and hydrophilic moieties fulfill several roles, from molecular recognition to protein folding quality control. N-glycans are covalently attached to the lateral chain of asparagine residues displayed in the “sequon” context Asn-X-Ser/Thr ($X \neq \text{Pro}$). The presence of a sequon is necessary but no sufficient for its occupancy. For this reason, a glycoprotein may be present as a mixture with partially occupied sites, a property called N-glycosylation macroheterogeneity. Factors affecting sequon occupancy are poorly understood. For instance, the particular sequence of a sequon and its flanking residues clearly modulate its use. Nevertheless, identical sequons can be occupied very differently, depending on their global context. Here, we explore how protein stability affects sequon occupancy. By generating a family a proteins with different stability, displaying one sequon at the time in different positions, we found that protein stability is a major factor that contributes to this phenomenon. In this scenario, sequon occupancy may work as a proxy to gauge thermodynamic stability in vivo.

Photosensitized modification of proteins: from oxidative stress to biocatalysis application

Borsarelli CD^a

a - Instituto de Bionanotecnología del NOA (INBIONATEC), CONICET, Universidad Nacional de Santiago del Estero (UNSE), RN 9, Km 1125, 4206 Santiago del Estero, Argentina.

Solar light interaction with biological molecules plays several vital roles including photosynthesis, photosignalling, and circadian rhythm among others. Photon absorption produces excited states that depending on several reaction conditions driving to different photochemical pathways with biological consequences. Despite the direct absorption of solar UVB photons by proteins is highly minimized by the filtering of the ozone layer, the later does not avoid the occurrence of photosensitized processes, where UVA and visible photons are absorbed by other intrinsic or extrinsic chromophores inducing secondary reactive intermediates targeting protein residues.

Here, we present some examples of photosensitized reactions involving organic and inorganic sensitizers with several proteins (e.g. albumins, lysozyme, synuclein, lipase, etc.) using visible light. Mechanistic aspects are discussed, e.g. involvement of molecular oxygen, charge-transfer reactions, and the results are analyzed in terms of the effect of the protein milieu on the molecular distribution and photophysical properties of the sensitizer, protein oxidation and crosslinking effects, etc. The impact of the photosensitized reactions is presented on the protein structure and functionality in an oxidative stress picture, and also for technological applications to obtain immobilized insoluble biocatalyst suitable for biodiesel production.

Contact: cdborsarelli@gmail.com

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Biophysical characterization of the interaction between SNH and QLQ protein domains

Miotto MC^a, Crespo R^a, Tabares LC^b, Rasia RM^a

a - Instituto de Biología Molecular y Celular de Rosario, IBR-CONICET-UNR, Rosario, Argentina

b - Institute for Integrative Biology of the Cell (I2BC), Department of Biochemistry, Biophysics and Structural Biology, Université Paris-Saclay, CEA, CNRS UMR 9198, F-91198 Gif-sur-Yvette, France

Plant development is a process that is finely regulated by the action of various molecular mechanisms. One of these mechanisms comprises the activation or repression of the expression of certain genes, through the action of specific proteins. The Growth-Regulating Factors (GRF) - GRF-Interacting Factors (GIF) system is formed by two families of activators and transcriptional coactivators conserved in terrestrial plants. A range of studies have demonstrated the function of the combined action of these proteins on plant development, but there is no biophysical evidence of such protein-protein interaction so far.

Here we present the first biophysical approach to unveil *Arabidopsis thaliana* GRF and GIF protein interaction at a structural level. Since the proteins show a high degree of intrinsic disorder throughout the sequence, we designed protein constructs with the regions genetically mapped as essential for the interaction between GRF and GIF, the QLQ and SNH domains, respectively. We characterized the isolated constructs and their complexes using circular dichroism, fluorescence and nuclear magnetic resonance spectroscopies. The SNH domain adopts unstable helical conformations, that are stabilized upon complex formation. NMR paramagnetic relaxation enhancement shows that SNH forms two parallel helices in the complex. The QLQ:SNH complex stoichiometries are variable and depend on the partners involved. Altogether our data puts forward an unexpected level of complexity in this interaction network.

Keywords: QLQ, SNH, protein-protein interaction, CD, NMR

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Fluorescence Correlation Spectroscopy experiments using single-wavelength calcium dyes

Villarruel C^a, Sigaut L^b, **Ponce Dawson S^b**

a - Instituto de Investigaciones Biotecnológicas, UNSaM-CONICET

b - Departamento de Física, FCEN-UBA and IFIBA, CONICET-UBA

Fluorescence Correlation Spectroscopy (FCS) is an optical technique that is commonly used to estimate diffusion coefficients and reaction rates in various systems. In FCS the fluorescence coming from a small volume is collected. Determining the timescales over which the fluorescence fluctuations decay it is possible to quantify the processes that affect the transport of the observed molecules across the observation volume. To this end the autocorrelation function (ACF) of the fluctuations about the mean fluorescence are computed. In the case of single-wavelength calcium dyes fluorescence fluctuations are both due to the diffusive transport of the observed molecules and to the reactions with the calcium ions present in the sample. Namely, these dyes increase their fluorescence intensity several orders of magnitude upon calcium binding. In this talk I will describe how the ACF should be interpreted in such a case. Comparing with experiments performed in solution, I will show that it is possible to infer from these experiments the free diffusion coefficient of the calcium ions and of other species that affect their transport even if they are not fluorescent. Results on experiments performed in intact cells will also be presented.

Laurdan spectroscopy in a bi-photonic microscope for the study of biological membrane heterogeneity

Sánchez SA^a, Gunther G^b

a - Departamento de Polímeros. Facultad de Ciencias Químicas. Universidad de Concepción, Concepción

b - Departamento de Química Orgánica y Físico Química, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago. Chile

Laurdan (6-lauroyl,1-2-dimethylamino naphthalene) is a fluorescent probe designed and synthesized in 1979 by Gregorio Weber to study dipolar relaxation of excited states. The dipole moment existing in the naphthalene group of the Laurdan molecule, changes upon excitation and causes the reorientation of the surrounding solvent dipoles, with the concomitant red shift of the probe's emission spectrum. The emission spectrum of Laurdan immersed in a lipid bilayer is centered at 440 or 490 nm if the lipids are in gel or liquid state respectively. The Generalized Polarization function ($GP = [(I_{440} - I_{490}) / (I_{440} + I_{490})]$) was defined in 1990 as a way of measuring these wavelength displacements in cuvette. The development of two photon excitation allowed the measurements of Laurdan GP images and the spatial visualization of lipid domains having different fluidities in model and real systems. After this, the use of Laurdan has been reinforced by combining its fine sensitivity for sensing small changes in water content in the bilayer with microscopy methodologies. Laurdan-Fluorescence correlation Spectroscopy (FCS) allowed the identification of nanometer mobile lipid domains in vivo. Laurdan-FLIM-phasor helped to differentiate between changes in the membrane due to polarity or to dipolar relaxation and lately the use of Laurdan spectral phasor has allowed the identification and quantification of small changes in membrane fluidity due to external stimulus. In this work we show the use of the traditional and the combined techniques to study changes in membrane fluidity in red blood cells and nucleated cells.

Contact: susanchez@udec.cl

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Sensors and actuators to understand 3D organization of living matter

Grecco HE^a

a - Laboratorio de Electrónica Cuántica, Departamento de Física, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires & IFIBA, CONICET

Cellular function emerges from the concerted action of nanometer-sized molecules. From their mobility and interaction, micrometer-sized patterns are formed. In turn, these patterns modulate the same interactions that create them, connecting spatial and temporal scales. Understanding how cellular function emerges from such simultaneous upward and downward causation requires going beyond the phenotype to quantify cellular processes with molecular resolution. In this talk I will describe our efforts towards understanding spatial organization of living matter. Achieving this goal requires novel imaging and analysis techniques that are able to cope with the variance of biological systems.

Contact: hgrecco@df.uba.ar

A spectroscopic and kinetic study of the singlet oxygen mediated oxidation of human and bovine serum albumin

Dorado RD^a, **Espeche Turbay MB**^a, Borsarelli CD^a

a - Instituto de Bionanotecnología del NOA (INBIONATEC), CONICET, Universidad Nacional de Santiago del Estero (UNSE), RN 9, Km 1125, 4206 Santiago del Estero, Argentina.

Singlet oxygen ($^1\text{O}_2$) is a very harmful non-radical species that reacts with electron-rich biological molecules. Due to their intrinsic abundance in the biological milieu, proteins are the main targets of action of $^1\text{O}_2$, causing structure changes, covalent and non-covalent aggregation, and enzymatic activity losses. Serum albumins (SAs), are fundamental for the maintenance of the oncotic pressure of the plasma and the transporting of small vital molecules and drugs.

As well as the functionality of both SA are depending on the protein structure, in this report we evaluated the impact of the $^1\text{O}_2$ oxidation on the human (HSA) and bovine (BSA) SAs, which show an amino acid similarity of about 77%, but differing mainly in the number of Trp and Tyr residues, two of the oxidizable residues by $^1\text{O}_2$ besides Cys, Met and His. The ruthenium (II) tris(2,2'-bipyridine) cation (Rubpy, 20 μM) was used as photosensitizer for generation of $^1\text{O}_2$, since Rubpy does not bind to the SAs (10-100 μM). Photolysis was performed using a 1 W blue LED (443 ± 27 nm) and the oxidation kinetics was monitored by both absorption and fluorescence spectroscopy. Post-irradiation the sensitizer was separated by ion exchange chromatography, and the modified proteins were analyzed by several spectroscopies, laser particle electrophoresis (Z potential), protein electrophoresis (PAGE); carbonyl content and pseudo-esterase activity determination through biochemical techniques. Results indicated the oxidative degradation of Trp residues with the production of carbonylic fluorescent products. The primary structure of the SA was retained, despite changes of the superficial electrical potential and hydrophobicity, and the reduction of the pseudo-esterase activity. Albeit both SAs share similar globular structure and amino acid sequence, the present results indicates subtle differences in the impact of the $^1\text{O}_2$ -mediated protein oxidation.

Contact: beaespeche@gmail.com

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Application of in silico screening for the discovery of new treatments for neglected infectious diseases

Bellera CL^a, Sbaraglini ML^a, Alberca LN^a, Dietrich R^a, Gavernet L^a, Carrillo C^b, Soto CA^c, **Talevi A^a**

a - Laboratory of Bioactive Research and Development (LIDeB), Department of Biological Sciences, Faculty of Exact Sciences, Universidad Nacional de La Plata (UNLP) - Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)

b - Institute of Sciences and Technology Dr César Milstein (ICT Milstein), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina

c - Department of Microbiology, Parasitology and Immunology, School of Medicine, Institute of Microbiology and Medical Parasitology, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Universidad de Buenos Aires (UBA)

Neglected diseases are infectious conditions characterized by a lack of proportion between the disease burden and the investment on new diagnostic tools and therapeutic solutions. In silico screening involves the use of computational models to choose which compounds from a [digital] chemical library will be submitted to experimental testing. It has been proposed as an efficient strategy to search for new drugs against neglected conditions, especially when applied to systematic drug repurposing, i.e. to propose additional medical uses for already known medications. Drug repurposing provides innovative therapies in a cost- and time-efficient manner, since the new therapeutic indications are built on available pharmacokinetic, safety and manufacturing data.

Here, we will present some examples of combined structure- and ligand-based virtual screening in the search of new drugs for trypanosomatid-caused conditions with a focus on cruzipain inhibition and putrescine uptake inhibition, which has led to the discovery of antibiotic, antihypertensive and antihistaminic drugs with trypanocidal activity. In some cases, our repurposed candidates were advanced to acute and chronic mice models of Chagas disease, with positive results.

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Ligand binding rates in hemeproteins from markov state models and molecular dynamics simulations

Bringas M^a, Lombardi L^b, Luque FJ^c, Estrín DA^a, Capece L^a

a - Departamento de Química Inorgánica, Analítica y Química Física - F.C.E.N. - U.B.A. (Argentina)

b - Instituto de Cálculo - F.C.E.N. - U.B.A.

c - Campus de la alimentació - Universidad de Barcelona

Obtaining kinetic information from molecular dynamics (MD) simulations is a challenging task, specially when the characteristic timescale of the processes of interest is beyond our computational resources. Markov state models allow us to estimate kinetic properties of biologically relevant processes in which multiple free energy minima are involved.

We present an application of these methodologies to heme proteins, aiming to understand the physico-chemical contributions to small ligand migration through channels and their implication in binding rates order of magnitude. We will present results from two truncated proteins from *Mycobacterium tuberculosis*, trHbN and trHbO. The first one is relevant for ·NO detoxification in its ferrous form, and is capable to discriminate the two necessary ligands for ·NO dioxygenation. Ligand selectivity (it binds ·NO about 100fold faster than it binds O₂) has been attributed to conformational changes in particular residues promoted by the uptake of O₂ (“the PHE gate hypothesis”) and to water molecules retained in the proximity of the heme iron by polar residues. The second one binds both O₂ and ·NO with a 10⁵ M⁻¹ s⁻¹ rate, becoming practically irrelevant in ·NO oxidation. For trHbO, the hypothesis of a frustrated channel that difficults ligand uptake is widely accepted.

In this type of methodology, a process is interpreted as a network of free energy minima that allows to reach the product states from the reactant. Here we use multiple short MD trajectories to characterize transitions between neighboring minima, and we combine those transition rates to extract information regarding the complete network. This allows us to evaluate quantitatively (yet comparatively) the effect of the protein matrix in ligand diffusion towards the active site. Our results suggest that retained water in the distal cavity strongly accounts for trHbN ligand selectivity. We were also able to capture ·NO uptake rate differences between trHbN and trHbO.

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Peptide-lipid membrane interaction in relation to lipid variables: A molecular dynamics approach

Martini MF^a

a - Cátedra de Química Medicinal, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires (UBA), Junín 956, Ciudad Autónoma de Buenos Aires (1113), Argentina CONICET- Universidad de Buenos Aires. Instituto de la Química y Metabolismo del Fármaco (IQIMEFA). Ciudad Autónoma de Buenos Aires, Argentina

In most studies of peptide-lipid membrane interaction, the main protagonist of the interaction is the peptide. This is related to the classical image of a cell as a compartmentalized system in which the membrane is a selective barrier of the containment of cellular material. However, the enormous variety of lipids, associated with the multiple states of aggregation or microdomains that can be formed in them, makes this a complex surface, which deserves attention when the system variables are adjusted.

In this case, we present two systems of interaction of peptides with lipid membranes, where the lipid composition and the phase state of the membrane substantially modify the quality and the mechanism of interaction.

In one of the cases, we have studied, from a molecular approach, the interaction of two cationic antimicrobial peptides with membranes of different composition. The variations in the lipid composition studied are related to the differences in two bacterial strains that present a different susceptibility to the action of the mentioned peptides. This structural difference allowed us to evaluate advanced instances of the interaction, in which the difference of lipids was fundamental to demonstrate the presence or absence of destabilizing structures of the membrane, which in this case is the formation of pores.

In the second case, a highly investigated peptide, such as melittin, was studied in its interaction with the lipid membrane model of eukaryotic cells, to evaluate from a molecular approach, the beginnings and the possibilities of interaction with this type of membranes. In this particular case, the phase state of the membrane was fundamental, when analyzing the interaction. The studies in the ripple phase of the membrane, allow us to evaluate the first steps of the interaction of melittin with this type of membranes.

Both cases, through molecular dynamics simulations, validated with experimental data, allow us to reach a molecular un

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Protein structural divergence leave footprints the evolutionary information

Buslje CM^a

a - Laboratorio de Bioinformática Estructural. Fundación Instituto Leloir

The analysis of evolutionary information in a protein family, such as conservation and covariation, is computed from multiple sequence alignments of distant homologous sequences. Although high structural differences between proteins can be expected in such divergent alignments, most works linking evolutionary and structural information use a single structure ignoring the structural variability within protein families.

We found that inter-residue contacts and solvent accessibility undergo large variations in protein families. As a consequence, the mean solvent accessibility of multiple structures correlates better with the conservation pattern than the solvent accessibility of a single structure. Also, high covariation scores tend to reveal residue contacts that are conserved in the family, rather than conformer specific ones.

We elucidated the relevance of considering the structural divergence and concluded that the use of comprehensive structural information allows a more accurate interpretation of the evolutionary information computed from sequence alignments. Therefore, considering structural divergence would lead to a better understanding of protein function, dynamics, and evolution.

Cell adhesion studies using electrochemical sensors

Pallarola D^a

a - Instituto de Nanosistemas, Universidad Nacional de General San Martín, Av. 25 de Mayo y Francia, San Martín, 1650, Argentina

Cell-cell and cell-extracellular matrix recognition and adhesion are central processes of the cell's sensory machinery and are mediated by transmembrane adhesion receptors of the integrin family. These processes play a crucial role in most fundamental cellular events including motility, proliferation, differentiation, and apoptosis.

The creation of new types of sensitive conductive surfaces capable of mimicking the nanoscale order of the extracellular matrix and with the ability to detect and transduce cell adhesion in real time is of great interest in the field of biomedical research. Electrical-impedance-based sensors are a powerful tool to investigate how cells interact with their environment. These devices are based on impedance measurements using weak and non-invasive AC signals and provide a quantitative description of the cell adhesion process through monitoring changes in the impedance signal in a label-free, instantaneous and non-destructive manner.

Our approach was based on the controlled positioning of binding sites for cells through a quasi-hexagonal arrangement of gold nanoparticles deposited on an indium tin oxide microelectrode. The resulting sensors exhibit an ordered pattern of particles that provides not only a precise spatial distribution of adhesive ligands, but an adequate transduction of surface events into electrical signals. The use of transparent electrodes allows for simultaneous and real-time monitoring of cell adhesion using electrochemical and optical microscopy techniques. Particularly, these sensors were applied to study cell-cell and cell-substrate interactions of epithelial cells on surfaces coated with cyclic pentapeptide ligands with different binding affinities to integrins $\alpha v\beta 3$ and $\alpha 5\beta 1$.

Contact: dpallarola@unsam.edu.ar

Nanomechanical properties and molecular recognition assessed by Force Spectroscopy

Daza Millone MA^a, Vázquez R^b, Maté S^b, Vela ME^b

a - Instituto de Investigaciones Fisicoquímicas Teóricas y Aplicadas (INIFTA), CCT- La Plata, CONICET. Universidad Nacional de La Plata. Sucursal 4 Casilla de Correo 16, La Plata (1900) Argentina

b - Instituto de Investigaciones Bioquímicas de La Plata (INIBIOLP), CCT- La Plata, CONICET. Facultad de Ciencias Médicas. Universidad Nacional de La Plata. 60 y 120, La Plata (1900) Argentina

The characterization of biological membranes involves many issues, from their composition at the molecular level to their morphological and structural aspects. The use of model biomembranes has allowed numerous advances in the study of interactions with molecules, the identification of phases and in the quantification of their nanomechanical properties. The Atomic Force Microscopy (AFM) has turned out to be one of the most powerful techniques in the investigation of these subjects and in particular the Force Spectroscopy (FS) offers valuable information in the nanometric scale allowing to monitor processes in physiological medium and in real time.¹

In this work, we present results from Surface Plasmon Resonance (SPR) and AFM of a ternary lipid mixture (DOPC/ 16:0-SM/Cho) that exhibits phase coexistence, i.e. a liquid-ordered (Lo) phase enriched in sphingomyelin (SM) and cholesterol (Cho) which is segregated from the liquid-disordered (Ld) phase composed mainly of DOPC. This ternary lipid mixture mimics lipid raft-like domains.² Supported lipid bilayers (SLBs) were formed by vesicle fusion either on mica or on dithiothreitol (DTT) self-assembled monolayers on Au following previous procedures.³ The presence of different lipid phases was characterized by topographic AFM images on mica substrates and by FS on mica and Au/DTT surfaces. FS gives information about the nanomechanical properties and distribution of lipid phases in SLBs and is used in this work to confirm the lipid bilayer formation on a non-flat surface like polycrystalline Au. The nanomechanical properties of SLBs formed on polycrystalline Au were comparable to the ones obtained on a flat mica substrate. The combination of these techniques corroborates the adequate formation of SLBs on Au chips opening a great variety of studies concerning interactions of model biomembranes with biomolecules, surfactants and nanomaterials among other systems by SPR.

Contact: mevela@inifta.unlp.edu.ar

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Dissecting the molecular assembly mechanism of a viral RNA polymerase complex

de Prat Gay G^a

a - Fundación Instituto Leloir, Av Patricias Argentinas 435, (1405), Buenos Aires, Argentina

Infection by respiratory syncytial virus (RSV) is the major cause of lower respiratory disease in infants. RSV is highly contagious, stable on surfaces, and can mutate rapidly, hampering the development of effective vaccines and antivirals. For this reason, we aim at understanding the chemical and molecular details behind RSV replication and transcription, centered around the assembly of the RNA polymerase complex (RPC). This complex is composed of the RNA dependent RNA polymerase (L), the nucleocapsid protein (N), the phosphoprotein cofactor (P) and the transcription anti-terminator (M2-1). The P tetramer shows disorder-order transitions that are essential for its role as scaffold of the RPC, and tightly interacts with the M2-1 tetramer. The latter interacts cooperatively with RNA, where RNA and P compete for binding M2-1. The N protein forms RNA bound decameric rings which constitutes the nucleocapsid assembly unit. These interact stoichiometrically with P, with contributions of both N- and C-terminal disordered domains of P to binding. Transfected N and P are detected in the cytosol, but when co-transfected they form spherical granules of irregular sizes. M2-1 alone or co-transfected with P is dispersed in the cytosol but in the presence of N it is driven into the granules, indicating N as the responsible for this peculiar localization. M2-1 is present only in pneumoviruses and its interaction with P is a highly specific antiviral target. We aim at building a hierarchical thermodynamic, kinetic and structural picture as well as the in-cell assembly of the RPC. The idea of antivirals directed to the assembly of the RPC should complement those under development or new ones, to attain high specificity and potency. RSV shares architectural and functional features with paramyxoviruses and filoviruses, which include pathogens such as measles, mumps, parainfluenza and Ebola.

Contact: gpg@leloir.org.ar

Highly selective mechanisms to transport molybdate and tungstate in sulfate reducing bacteria

Rivas MG^a, Otrelo-Cardoso AR^b, Nair RR^b, Correia MAS^b, Correia Cordeiro RS^b, Santos-Silva T^b

a - Department of Physics, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral and CONICET, Santa Fe, 3000, Argentina

b - UCIBIO/REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516, Caparica, Portugal

Molybdenum (Mo) and tungsten (W) are chemically analogous elements that are found in the environment. Tungsten (W) is considered the twin element of Mo, but is not a universal element being limited to bacteria and archaea. In biological systems, Mo and W are incorporated into the active site of enzymes through binding to a pyranopterin moiety. The Mo and W enzymes have important physiological roles, including the catalysis of reactions involved in the metabolism of carbon, nitrogen and sulfur.¹ These elements are incorporated into the cell as their soluble oxoanions molybdate and tungstate through specific transport systems known as Tup/WtpABC and ModABC, respectively. These systems are composed of a periplasmic protein that binds the metal (Mod/Wtp/TupA), a membrane protein which works as a channel (Mod/Wtp/TupB) and a cytoplasmic protein (Mod/Wtp/TupC) responsible for the hydrolysis of ATP, thus generating the energy needed to transport the oxoanions to the cell. Due to the high similarity between Mo and W, one of the major challenges of biology is to develop systems to differentiate them. The first selection gate for these metals is the component A of the transport system. Although ModA, TupA and WtpA differ in their primary sequence and in the coordination chemistry of the oxoanion, all three proteins can bind both molybdate and tungstate (with different affinity binding constants).²⁻⁴

The subject of the present work is to study the component A of molybdate and tungstate transport system in the sulfate reducer *Desulfovibrio alaskensis* G20. Functional and structural studies were combined to understand the uptake mechanisms and their specificity for the two anions molybdate and tungstate.

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The singular periplasmic flagella from *Leptospira*: from structure to cellular function (toward integrative Structural Biology)

San Martin F^a, Trajtenberg F^a, Gibson K^b, Mechaly A^a, Brady M^b, Wunder E^c, Larrieux N^a, Picardeau M^d, Liu J^e, Ko A^c, Sindelar C^b, **Buschiazzo A^a**

a - Laboratory of Molecular and Structural Microbiology, Institut Pasteur de Montevideo, Uruguay

b - Molecular Biophysics & Biochemistry Dept, Yale University, USA

c - School of Public Health, Yale University, USA

d - Biology of Spirochetes Unit, Institut Pasteur, France

e - Microbial Pathogenesis Dept., Yale University, USA

The bacterial Spirochaete phylum comprises several species that cause deadly diseases (e.g. Lyme disease, syphilis and leptospirosis). Spirochetes swim very efficiently relying upon flagellar appendages. Even though this motility is common to all bacterial species, Spirochetal flagella are uniquely confined within the periplasmic space, between the outer and inner cell membranes. This is relevant during infection because: (1) it hides flagella from host immune responses; and (2) it allows flagella to associate tightly to the cell body, powering a screw-like ‘drilling’ motion of the whole organism, critical during infection for penetrating host tissues. Despite its relevance in disease, most aspects of spirochete motility remain mysterious, including the composition and 3D structure of the molecular machinery that drives it. To address this knowledge gap, we study the genus *Leptospira*, which harbors the simplest spirochetal flagellar apparatus, and causes the broadest distributed zoonotic disease worldwide. Undertaking an integrative approach combining genetics and protein biochemistry, with top-down cryo-electron microscopy of the entire filament, and bottom-up X-ray crystallography of constituent parts, we have made several findings. *Leptospira* flagellar filaments have a complex composition, comprising six distinct proteins instead of the paradigmatic flagellin-only filaments of well-studied bacteria. Four flagellin-like FlaBs, two sheath FlaA isoforms, and two completely novel sheath proteins (FcpA and FcpB) are all expressed within leptospiral filaments. The crystal structures of FcpA and FcpB, essential for *Leptospira* motility, have been solved, revealing novel protein folds. Using 3D cryo-electron microscopy approaches, a strikingly asymmetric architecture of the entire filament was uncovered. A conceptual model of the assembly is proposed, explaining the functional relevance of such a complex and asymmetric flagellar filament to drive Spirochete propulsion

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Study of enterocyte FABPs' protein-protein interactions in Caco-2 cells

Bottasso Arias N^a, Rodriguez Sawicki L^b, Corsico B^a

a - INIBIOLP, CCT-La Plata, CONICET, Facultad de Ciencias Médicas, UNLP, 60 y 120, La Plata

b - Fac. de Ciencias Exactas, UNLP

Two isoforms of Fatty Acid Binding Proteins (FABPs) are abundantly expressed within intestinal epithelial cells: FABP1 and FABP2. Together they represent 1-2% of total proteins present within the enterocytes. Both proteins are associated with intracellular dietary lipid transport and trafficking towards diverse cell fates, although their specific functions are not well understood and are thought to differ. Using the Caco-2 enterocyte cell model, we undertook protein-protein interaction studies to determine FABP1 and FABP2 protein partners.

Firstly, to evaluate FABP1 interactions with other proteins present in Caco-2 cell lysate we applied the Far Western blot technique followed by MS analysis. We identified two candidate proteins: Heat Shock Protein 60 and calreticulin. On the other side, Co-immunoprecipitation (Co-IP) of FABP1 followed by MS showed interaction with Creatin kinase B.

Secondly, FABP2-protein interactions were analyzed by CoIP. We tested the interaction between FABP2 and a member of the PPAR (Peroxisome Proliferator Activated Receptors) transcription factors family, PPAR γ .

Thirdly, we examined PPAR γ expression levels in an FABP1 ablated Caco-2 cell line generated in our lab applying an antisense technique. The results showed a concomitant decrease in PPAR γ levels in FABP1 knock down (kd) cells relative to controls as well as some PPARs downstream genes.

In FABP1 kd cells and controls we performed a luciferase reporter assay with a PPAR response element in frame with the luciferase gene. When FABP1 kd cells were treated with oleic acid we observed no increase in luciferase response compared to the vehicle treatment, opposed to an increase in control cells.

In summary, our work shows for the first time the interaction of FABPs with different proteins expressed in Caco-2 enterocytes. Particularly, interaction with PPARs may be indicative of the role of enterocyte FABPs mediating gene expression. We will conduct further experiments to test this hypothesis.

In vivo systems to study class II bacteriocins toxicity and immunity

Ríos Colombo NS^a, Chalón MC^a, Galván AE^a, Navarro SA^a, Lanza L^a, Barraza DE^a, Acuña L^a, Fernandez de Ullivarri M^a, Minahk CJ^a, Bellomio A^a

a - Instituto Superior de Investigaciones Biológicas - INSIBIO (CONICET-UNT). Instituto de Química Biológica "Dr. Bernabé Bloj". Facultad de Bioquímica, Química y Farmacia. Universidad Nacional de Tucumán (FBQF-UNT), Batalla de Chacabuco 461, San Miguel de Tucumán (T4000ILI). Tucumán, Argentina

Class II bacteriocins are membrane-active peptides that act over a narrow spectrum of bacterial targets and have a great potential application as antibiotics in medical sciences. They act on the cytoplasmic membrane dissipating the transmembrane potential by forming pores. There is solid evidence that membrane receptor proteins are necessary for their function, however the precise role of this receptor and the nature of the pore remain elusive. The most accepted model suggest that bacteriocins bind the receptor to change its conformation, creating a channel that remains open. Nonetheless, several studies support a second model in which the bacteriocin is able to disrupt the membrane itself and the receptor might act just as an anchor allowing the subsequent bacteriocin insertion to form the pore. In order to reveal whether or not the pore structure involves the specific receptor, we designed chimeric peptides fusing the membrane protein EtpM with different class II bacteriocins. We chose *E. coli* as a receptor-free expression host. The fusion EtpM-bacteriocin anchors each bacteriocin to the membrane and kills the expressing host cell, even in the absence of the specific receptor. These results are in line with the second model in which the pore is formed through a receptor-independent interaction with the lipid bilayer. The effect of these interactions was also analyzed, through a fluorophore that changes its fluorescence intensity according to transmembrane potential.

On the other hand, an immunity protein protects the producer strain against its own bacteriocin. For antimicrobials under investigation for clinical applications, the potential emergence of resistant pathogens and the study of immune mechanisms are a primary concern. Though no direct in vitro interaction bacteriocin-immunity has been reported before, by using an in vivo system, we present evidence that this binding might occur, not in aqueous solution but in a membrane inserted conformation

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Single image deconvolution with super-resolution using the SUPPOSE algorithm

Toscani M^a, Martínez S^b, Martínez O^a

a - Laboratorio de Fotónica, Departamento de Física FIUBA - CONICET

b - IMAS, Departamento de Matemática FCEyN UBA - CONICET

We present the results of super-resolution deconvolution of fluorescent intracellular images using the SUPPOSE algorithm. The image is acquired using a standard fluorescence microscope and a CMOs low noise high dynamic range camera. The algorithm relies in assuming that the image source can be described by an incoherent superposition of point sources and a precise measurement of the microscope point spread function (PSF). The deconvolution problem is converted into finding the number of sources and the position of the sources. The retrieval is performed using a genetic algorithm that finds an adequate fit to the measured signal. The method is particularly fit for sparse data, such as encountered in typical intracellular experiments, where only a minor fraction of the explored volume has fluorescent markers. The technique retrieves by construction only positive values for the spatial density, avoiding the need for nonlinear constraints found in prior deconvolution techniques such as Tikhonov-Miller and Richardson-Lucy. The method automatically subtracts the background from the image. Based on the measured point spread function with the information of the quality of the fit and the information on the noise figure of the camera as a function of the read signal, the method provides a predictor of the uncertainty in the reconstruction both in lateral resolution and amplitude. A fivefold increase in resolution is shown both by inverting a synthesized artificial image and using known beads clusters. The algorithm was applied to reconstructing images from bovine pulmonary artery endothelial cells with fluorescent labels for the F-actin, microtubules and mitochondria. The algorithm is used for the reconstruction requires the precise measurements of the PSF and the noise figure of the camera. It can be applied to reconstruct the image with super-resolution down to $\lambda/10$ and also to increase the resolution using a low magnification for wide field objective.

Single Molecules Studies of the Dynamics of NS3h Helicase from the Dengue Virus

Amrein F^a, Gebhard LG^b, Arunajadai S^c, Kaplan A^d, Gamarnik AV^b, Bustamante C^e, Kaufman SB^a

a - Instituto de Química y Físicoquímica Biológicas y Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires-CONICET

b - Fundación Instituto Leloir-CONICET

c - Cota Inc

d - Faculty of Biology, Technion—Israel Institute of Technology

e - Department of Physics, Departments of Chemistry, and Molecular and Cell Biology and Howard Hughes Medical Institute, University of California, Berkeley, California 94720

Dengue virus (DENV) NS3h protein is an RNA-helicase that catalyzes the hydrolysis of ATP and couples the free energy of this reaction to the translocation on single strands and to unwind double stranded RNA. Binding and hydrolysis of ATP are essential for helicase function because they are the energy source of the mechanical work. These processes are stochastic in nature although show patterns with a certain periodicity which is manifested both in step-size and dwell times.

The former comprising discrete movements of the protein along the RNA strand, and the latter corresponding to periods during which the motor carries chemical transformations (ATP binding, ADP release, ATP hydrolysis) and no mechanical movement. We performed single molecule experiments at different [ATP] using optical tweezers to measure length variation between extremes of a dsRNA as a result of the unwinding activity of NS3h

Pairwise Distances of the points in the molecular trajectories (PWD) and Fast Fourier Transform (FFT) were used to extract information from experimental data to characterize step-sizes and dwell times. From the analysis of these results we obtained the following conclusions 1) the mechanical step-size value strongly correlates with the NS3h site-size value for the RNA (10 to 11 nt) and, 2) the unwinding dwell times values followed an inverse relation with the ATP concentration, indicating that binding of ATP takes place during the dwell.

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Poster Sessions

XLVII SAB 2018

Antioxidant properties of resveratrol on DNA components photooxidation

Neyra Recky JR^a, Tosato MG^a, Serrano MP^a, Thomas AH^a, Dántola ML^a, Lorente C^a
a - Instituto de Investigaciones Fisicoquímicas Teóricas y Aplicadas, UNLP (INIFTA), La Plata, Argentina.

A large number of physicochemical changes were described in photodynamically treated DNA molecules, including conformational changes and cleavage. These changes are a consequence of the photooxidation of the nucleotides, especially those that contain guanine bases.

Pterin (Ptr) is a natural compound that under UV-A radiation is photoactive, and photoinduces the degradation of biomolecules.¹ It was previously demonstrated that Ptr is able to damage DNA and nucleotides by both type I and type II mechanisms. Moreover, under UV-A irradiation in the presence of Ptr, a supercoiled pUC18 plasmid suffers a fast relaxation followed by linearization (evidencing breaks) at longer irradiation times.²

Resveratrol (RSV) is a natural polyphenolic compound³, which can be found in different plant species. The therapeutic potential of RSV has been widely studied, due to its antiviral, cardioprotective, anticancer, antiinflammatory and antioxidant properties. However, not much is known about its antioxidant ability in radiation mediated processes. The main goal of this work is evaluate if RSV can prevent the photosensitized oxidation of DNA.

Aqueous solutions of nucleotides and Ptr (pH 6, room temperature) were exposed to UV-A radiation ($\lambda_{ex}=365$ nm) during different times in presence and absence of RSV. The photochemical reaction was studied by UV-Vis spectrophotometry, HPLC, fluorescence spectroscopy, mass spectrometry and laser flash photolysis.

Results indicate that the photoinduced damage of nucleotides is reduced in the presence of RSV, noticing that, under certain concentrations, the photodamage process is inhibited. The mechanistic study indicates that, although RSV is capable to deactivate the triplet excited state of Ptr, the inhibition of the photosensitized process is due to the deactivation of nucleotide radicals.

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A model of the transport of flexible cargoes along microtubules

Fernandez Casafuz A^a, Bruno L^a, Levi V^b

a - Departamento de Física, FCEyN, UBA - IFIBA, CONICET

b - Departamento de Química Biológica, FCEyN, UBA - IQUBICEN, CONICET

Intracellular organization depends on molecular motors that transport organelles and other cargoes along microtubules and actin filaments. In order to understand the behavior of molecular motors during intracellular transport several models have been presented, mainly dealing with the motion of spherical rigid organelles. It has been determined that the transport usually requires the conjoint action of multiples copies of motors and that their spatial distribution may affect the transport. For this reason, it is important to further explore the action of molecular motors when driving cargoes with different geometries and variable elasticity. In this work, we use numerical simulations to analyze the motion of an elongated and flexible organelle driven by several copies of molecular motors along a 1D track in the sein of a very viscous cytoplasm. The model assumes an internal degree of freedom for the organelle that allows it to deform in a damped elastic potential. In the simulations, motors undergo unidimensional diffusion on the cargo, attach to a linear track, step on it and detach with different probabilities. The system is numerically integrated to obtain the organelle trajectory and length variations during the transport, as well as the distribution of active motors along the organelle. These results are then compared with experimental observations of mitochondria during active motion in *Xenopus Laevis melanophores*.

Effects of α -hemolysin on human erythrocytes. Part 1. Regulation of extracellular ATP and cell volume

Lauri N^a, Leal Denis MF^a, Alvarez C^a, Lefevre S^b, González-Lebrero RM^a, Espelt MV^a, Ostuni M^b, Herlax V^c, Schwarzbaum P^a

a - IQUIFIB-Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, UBA. Junin 956, 1113 Ciudad de Buenos Aires

b - Inserm- Institut national de la santé et de la recherche médicale

c - INIBIOLP, CCT-La Plata, CONICET, Facultad de Ciencias Médicas, UNLP, 60 y 120, La Plata

The hemolytic toxin α -hemolysin (HlyA) is a virulence factor produced by several strains of *Escherichia coli*. It is involved in urinary tract infections, peritonitis, meningitis and septicemia.

We studied the regulation of extracellular ATP (ATPe), cell volume and hemolysis in human erythrocytes exposed to HlyA.

HlyA induced an increase of [ATPe] (3-36 fold) and hemolysis (1-44 fold), which was compatible with simultaneous lytic and non-lytic ATP release. Elevated [ATPe] was partially counteracted by ATPe hydrolysis driven by ectoATPase activity, and intracellular ATPase activity of hemolyzed cells.

A quantitative analysis showed that lytic and non-lytic ATP exit mainly governed ATPe kinetics, while ATPe hydrolysis was important at late times of toxin exposure.

Several features of HlyA-induced ATP release from erythrocytes were characterized: 1- the un-acylated toxin ProHlyA and the deletion mutant protein HlyA Δ 914-936 were unable to induce ATP release or hemolysis, suggesting that acylation of HlyA and the Δ 914-936 segment binding erythrocyte glycoporphins were important for toxin activity; 2- treatment of ATP loaded liposomes with HlyA caused low ATP release, so that the lipid inserted toxin did not permeate ATP; 3- carbenoxolone, a pannexin 1 inhibitor, partially inhibited (43-67%) ATP exit, implying a role for this protein as an ATP conduit; 4- HlyA induced a 1.5 fold swelling, while blocking this swelling reduced ATP release by 77 %. Reciprocally, blocking ATPe activation of P2X receptors reduced HlyA dependent swelling by 60-80%. Thus a catalytic loop was identified where swelling activated ATP release, and subsequent [ATPe] increments activated further swelling.

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Interacción entre las integrinas $\alpha 5\beta 1$ y $\alpha \nu\beta 3$ durante la adhesión celular

Díaz C^a, Missirlis D^b, Neubauer S^c, Rechenmacher F^c, Kessler H^c, Spatz J^b

a - Instituto de Investigaciones Físicoquímicas Teóricas y Aplicadas (INIFTA) (CCT CONICET La Plata, UNLP y CIC)

b - Max Planck Institute for Medical Research

c - Institute for Advanced Study and Center for Integrated Protein Science (CIPSM), Technische Universität München

Las principales integrinas de adhesión a fibronectina (FN), $\alpha 5\beta 1$ y $\alpha \nu\beta 3$, exhiben un comportamiento cooperativo durante la adhesión celular, migración y mecanosensado, a través de mecanismos aún no completamente resueltos. Se utilizaron superficies nanoestructuradas con propiedades mecánicas ajustables y ligandos peptidomiméticos diseñados para unirse selectivamente a diferentes integrinas. Mediante el empleo de estas superficies hemos encontrado que las adhesiones focales formadas por células endoteliales sobre superficies selectivas a $\alpha 5\beta 1$, rápidamente reclutan a las integrinas $\alpha \nu\beta 3$ pero no vice-versa. Al bloquear a la integrina $\alpha \nu\beta 3$, se impide la formación de adhesiones focales y la adhesión celular sobre los sustratos $\alpha 5\beta 1$ -selectivos, indicando un mecanismo dependiente del anclaje extracelular y destacando la necesidad de la unión de la integrina $\alpha \nu\beta 3$ para la eficiente adhesión celular. El reclutamiento de las integrinas $\alpha \nu\beta 3$ ocurre independientemente de las propiedades mecánicas del sustrato, por encima del límite que soporta la formación de adhesiones focales. Estudios mecanísticos revelan la necesidad de ligandos solubles para $\alpha \nu\beta 3$ presentes en el serum para permitir el reclutamiento de integrinas y se ha excluído a FN exógena o endógena como posible ligando responsable. Nuestros resultados ponen en evidencia un mecanismo novedoso de cooperación entre integrinas y el rol crítico de las integrinas $\alpha \nu\beta 3$ para promover la adhesión celular.

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Antifungal activity of arginine based-surfactants against yeasts

Fait ME^a, Mylek S^a, **Grillo P**^a, Cotabarren J^a, Prieto ED^b, Garrote G^c, Vázquez R^d, Maté S^d, Morcelle S^a, Bakás L^a

a - CIPROVE, Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas, UNLP, 47 y 115, La Plata

b - INIFTA-CONICET, Fac. de Cs. Exactas, UNLP. Suc. 4-C.C. 16. (1900) La Plata, Argentina. Fac. de Ingeniería, UNLP, 1 y 47 (1900) La Plata, Argentina

c - Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA-UNLP-CONICET)

d - INIBIOLP, CCT-La Plata, CONICET, Facultad de Ciencias Médicas, UNLP, 60 y 120, La Plata

The antifungal activity of two novel arginine-based compounds (Bz-Arg-NHC₁₀ and Bz-Arg-NHC₁₂), which were synthesized by an enzymatic strategy, was studied against different *Candida* species, using two clinical isolates (*C. albicans* and *C. tropicalis*) and the strain *Candida albicans* ATCC 64548 from the American Type Culture Collection (ATCC, Manassas, USA) as indicator microorganisms. Cetrimide (alkyl trimethyl ammonium bromide mixture), a commercial cationic disinfectant was used for comparison. Minimum Inhibitory Concentrations (MIC) were calculated from DO measurements after 24 h of exposure to the compounds. Results were corroborated using the resazurin assay. Minimum Fungicidal Concentrations were also obtained. The three surfactants proved antifungal activity against all isolates, revealing their potential use as effective disinfectants. The effect of surfactants on pathogens survival was analyzed in Sabouraud Dextrose Broth added with Bz-Arg- NHC₁₀ or Bz-Arg-NHC₁₂ at different concentrations. Viability curves showed a decrease of the number of viable microorganism after 24 h of incubation when compared to that of the control in absence of the surfactant. Experiments in lipid monolayers composed by dioleoylphosphatidylcholine (PC), phosphatidylethanolamine (PE) and ergosterol (ERG) confirmed that both surfactants can insert in the fungal membranes, suggesting it as a possible target for the antifungal activity. Morphological changes in yeasts exposed to the surfactants were analyzed by AFM. Finally, antiadhesive activity was studied pretreating polystyrene microplates with different surfactant concentrations. Overall, the evidence from this preliminary study supports the use of these arginine-based surfactants as an alternative to commercial cationic surfactants, especially for their use as additives in topical formulations and for superficial disinfection.

Keywords: arginine-based surfactants; antifungal activity; *Candida*; lipid monolayer; AFM

Control of cell adhesion by thermal annealing of natural polyelectrolyte multilayers: mimicking the extracellular matrix with surfaces with gradients in their physicochemical properties

Muzzio NE^a, Pasquale MA^b, Pietrasanta LI^a, Moya SE^c, Azzaroni O^b

a - Instituto de Física de Buenos Aires (IFIBA, UBA-CONICET)

b - Instituto de Investigaciones Fisicoquímicas Teóricas y Aplicadas (INIFTA, UNLP-CONICET)

c - Soft Matter Nanotechnology Group, CIC biomaGUNE

Cell adhesion plays a key role in many physiological and pathological processes, and is a fundamental issue in the design of new materials for cell biology studies and medical devices. Moreover, materials with gradients in their physicochemical properties better mimic the complexity and functionalities of the extracellular matrix. Polyelectrolyte multilayers (PEMs) assembled by the layer by layer technique are a versatile approach to functionalize a biomaterial surface. Though PEMs assembled from natural polyelectrolytes are very appealing for biological and medical applications, they display poor cell adhesion as they are soft materials.

We present a novel strategy for the enhancement of cell adhesion on natural PEMs based on thermal annealing. Poly-L-lysine and alginate multilayers are assembled and heated at 37°C for three days. The changes in the physicochemical properties of the PEMs are assessed by means of the quartz crystal microbalance, atomic force microscopy, zeta potential and contact angle. The adhesion of different immortalized cell lines on nonannealed and annealed PEMs is studied.

After the annealing the films become more compact and smoother, with an increase in the Young's modulus of one order of magnitude. Furthermore, PEMs become more hydrophobic and the surface charge decreases from around zero for nonannealed PEMs to -14 mV for annealed ones. The protein deposition on PEMs significantly changes as well. Cell spreading and focal contact formation is remarkably improved on the annealed PEMs.

Based on the impact on PEM properties and cell adhesion caused by the thermal annealing, a temperature gradient is applied to induce a spatial variation of PEM properties resulting in a gradient on cell adhesion. Thermal annealing offers a friendly and simple method for enhancing cell adhesion without changing the composition and biocompatibility of PEMs, with potential applications in cell studies and tissue engineering.

Cytosolic Ca²⁺ dynamics in normal and pathological human cells

Vigil MA^a, Ontiveros M^a, Rinaldi D^a, Espelt MV^a, Mangialavori I^a, Rey O^b, Rossi JP^a, Ferreira-Gomes M^a

a - Instituto de Química y Fisicoquímica Biológicas y Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires-CONICET

b - Instituto de Inmunología, Genética y Metabolismo (INIGEM)

Calcium homeostasis is highly regulated in cells. Free cytosolic Ca²⁺ ([Ca²⁺]_c), which acts as a second messenger, is crucial for a wide range of biological functions. All cells must maintain a low concentration of [Ca²⁺]_c (~100 nM) to maintain viability, while using their increase as a versatile signaling pathway. Prolonged intracellular elevation of Ca²⁺ can trigger cell death. Evidence shows that the calcium homeostasis is altered in cancer cells and the alteration is involved in tumor onset, angiogenesis, progression and metastasis. In this work, we characterized the dynamics of [Ca²⁺]_c for different neoplastic cell lines SW480, CaCo and HepG2, and compare them with the normal dynamics of HEK293T cells.

The dynamics of [Ca²⁺]_c were examined by the Ca²⁺ release from endoplasmic reticulum (ER) in the different cell lines. The cells were loaded with fluo4 or fura2 to measure [Ca²⁺]_c and followed the fluorescence in real time before and after the addition of thapsigargin, an inhibitor of the sarcoplasmic reticulum calcium pump.

The dynamics of [Ca²⁺]_c in HEK293T cells showed a maximum peak followed by an exponential decrease to the basal level. On the other hand, in the colon carcinoma cells, SW480 and CaCo, the peaks returned to a higher steady-state. In HepG2 cells, no peaks were observed but an exponential increase to a maximal steady-state.

Then, we studied the effect of quercetin on the dynamics of [Ca²⁺]_c. Quercetin is a flavonoid with antitumoral properties, which inhibits calcium channels and pumps, altering the intracellular calcium homeostasis. We observed that quercetin altered the dynamics of [Ca²⁺]_c in SW480, CaCo, and HepG2 cells, whereas in HEK293T cells did not change. Our results suggest that quercetin could affect the calcium homeostasis in pathogenic cells by inhibition of Ca²⁺ transport systems.

This work reveals that the study of dynamics of [Ca²⁺]_c could be used for characterizing differences in calcium homeostasis in pathogenic cells.

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Effects of α -hemolysin on human erythrocytes. Part 2. Morphology, rheology, adhesion and signaling

Lauri N^a, Leal Denis MF^a, Lefevre S^b, Alvarez C^a, Enrique N^c, Rinaldi D^a, Vecchio L^c, Stringa P^c, Muñoz-Garay C^d, Milesi V^c, Ostuni M^b, Herlax V^e, Schwarzbaum P^a

a - IQUIFIB-Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, UBA. Junin 956, 1113 Ciudad de Buenos Aires

b - Inserm- Institut national de la santé et de la recherche médicale

c - IIFP-Instituto de Estudios Inmunológicos y Fisiopatológicos. Facultad de Ciencias Exactas. UNLP

d - Instituto de Ciencias Físicas, UNAM, Cuernavaca, Morelos, México

e - INIBIOLP, CCT-La Plata, CONICET, Facultad de Ciencias Médicas, UNLP, 60 y 120, La Plata

α -hemolysin (HlyA) of *E. coli* irreversibly binds to human erythrocytes (RBCs) and trigger changes in extracellular ATP and cell volume, ultimately leading to hemolysis.

Here we analyzed the capacity of HlyA treated RBCs to release ATP, change shape, adhere, deform and/or aggregate. Cells were treated with HlyA (HlyA-RBCs) or with the un-acylated protoxin ProHlyA (Pro-RBCs), and compared to control conditions (c-RBCs).

HlyA-RBCs showed an acute 1.3-2.2 fold increase of Ca^{2+}_i , increased crenation followed by swelling, and externalization of phosphatidylserine. Perfusion of HlyA-RBCs through adhesion platforms showed high adhesion to activated HMEC cells at low flow (0.2 dyn/cm²), although higher flows induced rapid detachment. Except for crenation, none of these changes occur in Pro-RBCs.

Deformability changes were analyzed by ektacytometry as a function of osmolarity or shear stress. In osmoscan curves, c- and Pro-RBCs deformed optimally in isosmolar conditions, while with HlyA-RBCs the toxin time-dependently shifted the curve to the right, suggesting increased sphericity. HlyA-RBCs displayed highly reduced elongation under shear stress, and very low aggregation in syllectograms. RBCs were then perfused through a rat mesentery, where intravascular levels of ATP and free hemoglobin (to estimate lytic ATP release) were measured in perfusate samples. Results showed that HlyA-RBCs, unlike c- and Pro-RBCs, increased intravascular ATP 83-fold, and hemolysis -56-fold, with parallel increments in arterial bed pressure. Non-lytic ATP release of HlyA-RBCs was the major component explaining intravascular ATP.

Overall results showed that HlyA-RBCs displayed activated ATP release, high but weak adhesivity, low deformability and aggregability and high sphericity.

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Mechanical properties of in-vivo intermediate filaments and their interplay with microtubules and microfilaments

Smoler M^a, Pallavicini C^b, Levi V^a, Bruno L^b

a - Departamento de Química Biológica, FCEyN, UBA - IQUBICEN, CONICET

b - Departamento de Física, FCEyN, UBA - IFIBA, CONICET

Intermediate filaments (IFs) are one of the main components of the eukaryotic cytoskeleton. IFs are crosslinked with the stiffer microtubules and actin networks, contributing to the viscoelasticity of the cytoplasm and cell mechanical integrity. IFs are composed of several members of a large family of cytoskeletal proteins, including nuclear lamins, which contributes to the structural integrity of the nucleus. IFs mechanical properties have been obtained mostly in in-vitro assays, however, key aspects of the organization of these filaments in the intracellular environment remain elusive. In order to explore mechanical properties of IFs in living cells, we transfected BHK and MEF 3T3 cells with a vimentin-GFP plasmid and obtained images of the IFs networks using confocal microscopy. We recovered the coordinates of individual fluorescent filaments with sub-pixel precision from these images, using an algorithm developed in our lab. By performing a Fourier analysis of the IFs shapes we determined the persistence length of these filaments (the length of the filament over which thermal bending becomes appreciable and a measure of the filament rigidity - it is proportional to the flexural rigidity of the polymer-) and found a value of $2.1\mu\text{m}$, in the order of the in-vitro values. We also analyzed the effect of microtubules and microfilaments networks on the stiffness of IFs in living cells. Whereas microtubules depletion induced by nocodazol did not affect the persistence length of IFs, vinblastin treatment -a drug that stabilizes MTs dynamics- resulted in a 2-fold increase of IFs apparent stiffness. On the other hand, actin subtle depolymerization, driven by latrunculin B, also enhanced IFs apparent stiffness. These results suggest a high environmental dependence of the IFs mechanical properties. This work contributes to the comprehension of the mechanical behavior of the cytoskeletal filaments to get a better insight into cell mechanics and organization.

Processes controlling the regulation of extracellular ATP in Caco-2 cells

Bazzi Z^a, Alvarez C^a, Marginedas-Freixa I^b, Ostuni M^b, Schwarzbaum P^a

a - IQUIFIB-Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, UBA, Junin 956, 1113 Ciudad de Buenos Aires

b - Inserm- Institut national de la santé et de la recherche médicale

In most eukaryotic cells, intracellular ATP can be released by mechanical and oxidative stress, calcium influx and/or exposure to toxins. These treatments mimic several conditions to which epithelial cells lining the human intestine are exposed. In the intestinal lumen, extracellular ATP (ATPe) can activate purinergic receptors, which can modulate ATP release. Additionally, ATPe can be hydrolyzed by ectonucleotidases located on the apical domain of the epithelium.

To gain insight into ATPe regulation of the intestine, we analyzed the effects of several stimuli on ATP efflux, purinergic activation and [ATPe] hydrolysis of the human intestinal Caco-2 cell line, a model of epithelial enterocytes.

Real time luminometry was used to measure ATPe kinetics and ATPase activity.

In the absence of stimuli, Caco-2 cells displayed a stable [ATPe] at approx. 20 ± 5 nM, suggesting negligible ATP release. Addition of exogenous ATP ($0.5\text{--}8 \mu\text{M}$) led to an acute [ATPe] increase, followed by a non linear decay caused by ectoATPase activity. This was a linear function of [ATP], with slope (K_{ATP}) being 0.044 min^{-1} .

Next, we determined the effect of calcium influx and the mechanical perturbation on ATP release. All these stimuli led to similar ATPe kinetics, with variable degrees of [ATPe] increase to a maximum (2-6 fold over basal levels), followed by an acute exponential decay. Under mechanical stress in the presence of various blockers, ATP release exhibited exocytotic and conductive components, the latter being mediated by Pannexin1. Blockage of P2X purinergic receptors reduced ATP release by 57-66%.

Results shows that upon activation of ATP release, the accumulated ATPe activated purinergic receptors that promoted further activation of ATP exit. Simultaneously, EctoATPase activity partially compensated these increases in [ATPe]. Thus, ATPe kinetics of Caco-2 cells resulted from the dynamic balance between ATP release, purinergic activation and ATPe hydrolysis.

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The cell as a gel: materials for a conceptual discussion

Bagatolli L^a

a - Instituto de Investigación Médica Mercedes y Martín Ferreyra

We established that oscillating ATP levels during glycolytic oscillations in yeasts are tightly coupled with the *dynamic state of intracellular water*, being this phenomenon scale independent. Specifically, we demonstrated that an optimum extent of dipolar relaxation of intracellular water, modulated by *optimal levels of ATP and an optimally organized actin network* is crucial to the emergence of the oscillations⁽¹⁻⁴⁾, supporting the view of a highly coherent and ordered cellular interior with properties similar to a responsive coascervate.

We also found a strong coupling among temporal oscillations of thermodynamic variables such as temperature, heat flux and volume and the activity of intracellular metabolites⁽⁵⁾. These results are interpreted in light of a recently proposed theoretical formalism⁽⁶⁾ in which isentropic thermodynamic systems can display coupled oscillations in all extensive and intensive variables, reminiscent of adiabatic waves. This interpretation is in line with the view of the cellular interior as a structured near equilibrium system, where energy inputs can be low and sustain regular oscillatory regimes, challenge the notion that biological processes are essentially dissipative.

All these results, which are difficult to conceptualize using the canonical cell model based in mass action kinetics and dilute solution theory, can be mechanistically depicted using the Association-Induction Hypothesis, proposed by G. N. Ling (AIH)⁽⁷⁾. The AIH, which is based in statistical mechanical principles of colloidal systems, offers a appropriate frame to explain our results, challenging the canonical view of the cell.

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Measurement of plasma membrane Ca^{2+} pump activity following cytoplasmic Ca^{2+} kinetics

Saffioti NA^a, Rinaldi D^a, Dalghi M^b, Rossi RC^a, Rossi JP^a, Ferreira-Gomes M^a

a - Instituto de Química y Fisicoquímica Biológicas (UBA-CONICET)

b - Institute of Biochemistry and Molecular Medicine, and Swiss National Centre of Competence in Research, NCCR TransCure, University of Bern

The store operated Ca^{2+} entry (SOCE) is a cell response triggered by endoplasmic reticulum Ca^{2+} depletion. Under this stimulus, the ORAI subunits in the plasma membrane aggregate to form the SOCE channels that allow extracellular Ca^{2+} to entry into the cytoplasm. The SOCE response has been used in living cells to study the mechanisms of Ca^{2+} extrusion following the kinetics of cytoplasmic Ca^{2+} . Although there have been proposed different methods to assess the experimental data, nowadays there is no consensus in the literature about how to interpret the changes in Ca^{2+} kinetics and how these changes are related to alterations in different Ca^{2+} control mechanisms. In order to investigate the Ca^{2+} transport by the plasma membrane Ca^{2+} pump (PMCA) in living cells, we studied the Ca^{2+} kinetics elicited by SOCE in conditions where PMCA is the main mechanism of Ca^{2+} extrusion. HEK-293T cell line was transfected transiently with PMCA4 and incubated in mediums with different Ca^{2+} concentrations. Cytoplasmic Ca^{2+} was measured in real time by loading cells with fluorescent Ca^{2+} indicators. Our results showed that cytoplasmic Ca^{2+} kinetics had a transient increase, followed by a slow decrease until a stationary level was reached. The cytoplasmic Ca^{2+} levels depended on the extracellular Ca^{2+} concentration and PMCA expression. The experimental data was analyzed by means of an empirical equation that allows to describe the data in terms of the balance between Ca^{2+} uptake and release by the cells. Then we compared this method to others found in the literature. At the same time, the results were interpreted in terms of a mathematical model of cytoplasmic Ca^{2+} kinetics. This analysis allowed us to relate the value of parameters obtained by different methods with the PMCA Ca^{2+} transport activity.

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Activation/deactivation of Carnitine Palmitoyltransferase 1A: An insight from molecular dynamics simulations

Frigini EN^a, Barrera E^a, Pantano S^a, Porasso RD^a

a - IMASL - CONICET

Carnitine Palmitoyltransferase 1A (CPT1A) is an enzyme that is anchored in the outer mitochondrial membrane (OMM). This enzyme controls the passage of fatty acids into the mitochondria and then enters the process of β -oxidation of a long-chain fatty acids. The CPT1A is inhibited by malonyl-CoA, the first intermediate of the synthesis of fatty acids, and responds to: (i) the curvature of the OMM and (ii) lipid characteristics for its activation and deactivation. The experimental results on the enzyme show that its N-terminal domain adopts two states: activated or deactivated. The purpose of this study was to elucidate a mechanism of regulation of the enzyme CPT1A by simulations of molecular dynamics (MD). The lipids used for bilayer models were palmitoyl-oleoyl phosphatidylcholine (POPC) and palmitoyl-oleoyl phosphatidylethanolamine (POPE) in the presence/absence of the enzyme. Four simulations of MD were carried out: (i) lipid bilayer mixtures (without restraint), (ii) lipid bilayer mixtures (with restraint on POPE), (iii) lipid bilayer mixtures + enzyme (without restraint) and (iv) lipid bilayer mixtures + enzyme (with restraint on POPE). Our results showed that the bilayer with restraint have a greater curvature with respect to the bilayer without restraint. The results of this study show the influence of bilayer curvature, inducing the state of deactivation when the bilayer is flat (without restraint) and activating CPT1A when the bilayer is curved (with restraint)

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Description of membrane heterogeneities in plasma membrane of *Saccharomyces cerevisiae*

Genovese DM^a, Wilke N^a

a - CIQUIBIC (CONICET - UNC), Centro de Investigaciones en Química Biológica de Córdoba, Haya de la Torre y Medina Allende, Ciudad Universitaria, 5000, Córdoba, Argentina

The plasma membrane is a highly specialized organelle composed of a wide variety of lipid and proteins, which also vary spatially and temporally. The lateral interactions between the components are generally not ideal, leading to the presence of transient heterogeneities with variable stability. Our general objective is to characterize in plasma membrane of *Saccharomyces cerevisiae*, the inhomogeneous distribution of species, as well as their characteristics and regulation. The plasma membrane of *Saccharomyces cerevisiae*, unlike that of mammals, shows long-time heterogeneities of micrometric size, simplifying this study.

We have succeeded in marking the plasma membrane of wild type strains of *Saccharomyces cerevisiae* in exponential growth phase, using different molecular probes. We used DiIc12, NBD-Chol and FM4-64 whose partition depends on the degree of disorder of the coexisting phases; and Laurdan, whose emission spectrum gives information regarding the fluidity of the environment in which it is located. The presence of heterogeneities was detected with all the mentioned probes, being more marked the distribution of NBD-cholesterol. Yeast marked with both, NBD-Chol and FM4-64, or NBD-Chol and DiIc12, showed colocalization in the most fluorescent regions.

The emission spectra of Laurdan showed two maximum, indicating that in the analyzed ROIs (400nm x 400nm) two regions with different fluidity.

Finally, the maximum in the spectra of FM4-64 incorporated to all yeast membranes was more blue-shifted compared to the probe in homogeneous media (ethanol, or chloroform), and more red-shifted compared to DPPC vesicles, thus sensing an ordered medium, but not as rigid as in the DPPC membranes.

We conclude that plasma membranes are heterogeneous, with compact regions and less ordered regions coexisting in the micrometer scale, and with an average fluidity higher than DPPC vesicles.

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Dexamethasone stabilize the expanded phase of DMPC monolayer

Cámara CI^a, Wilke N^b, Yudi LM^a

a - INFIQC. Departamento de Físicoquímica. Facultad de Ciencias Químicas (Universidad Nacional de Córdoba). X5000HUA Córdoba. Argentina

b - Centro de Investigaciones en Química Biológica de Córdoba (CIQUIBIC), Departamento de Química Biológica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Haya de la Torre y Medina Allende, Ciudad Universitaria, X5000HUA, Córdoba, Argentina

Dexamethasone (DEX) is a synthetic glucocorticoid with a chemical structure similar to the steroids hormones. Dex is mostly used as analgesic and immunosupresant, especially in inflammation treatments (swelling, redness and pain), arthritis, skin disorder, severe allergies and asthma. Due to its hydrophobic nature and low solubility in water, DEX has toxic effects when administrated during long periods. Its toxic effects may be related with the lipophilic center, conferring a high capacity of partition in cellular membranes.

In this context, the present work pretends to evaluate how DEX modifies the properties of membrane models composed of dimyristoylphosphatidylcholine (DMPC).

We studied the capacity of DEX to penetrate pre-formed membranes and from these experiments we obtained the DEX concentration for saturation at 15 mN/m and the cut off value for this drug at 0,22mM. Besides, we studied the surface tension vs mean molecular area (Langmuir isotherm) for co-spreaded DMPC:DEX mixture at several molar ratios. These monolayers were visualized with Brewster Angle Microscopy, and the effect of DEX on the phase state and the reflectivity of the monolayer was evaluated at 15°C.

From the Gibbs adsorption experiments, we obtained a DEX saturation concentration of (0.22 ± 0.08) mM when the monolayer was pre-formed at 15mN/m. DEX has a cut off value of 45mN/m, proposed higher value to that comparable to cellular membranes. This suggests that DEX can penetrate cellular bilayer. Regarding the Langmuir isotherms, a molar fraction of DEX higher than 50% stabilized the expanded phase of the DMPC monolayer blurring the LE/LC phase transition. This was in agreement with a decrease in reflectivity as DEX molar fraction increased at surface pressure higher than 30mN/m.

Conclusion: Dex penetrates DMPC pre-formed monolayers up to surface pressure lower than 45 mN/m and stabilizes the expanded phase in pre-mixed monolayers.

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Effect of Cholesterol and Xanthone in the hydration states of lipid esters and ethers by fluorescent probe.

Pérez HA^a, Rosas AS^a, Cejas JdP^a, Frías MA^a, Disalvo EA^a

a - CIBAAL Centro de Investigación en Biofísica Aplicada y Alimentos CONICET UNSE

Cell membranes influence many cellular functions and are involved in most cellular communication processes. An important biophysical parameter at the molecular level is the ordering degree of the membrane determining the movement speed of the molecules (lateral diffusion, water permeability, etc.) can be modified by cholesterol and structurally analogues compound such xanthenes. However, those properties can also be related with the organization of water molecules in the interphase. The main hydration centers in a phosphatidylcholine membrane are the carbonyl and the phosphate groups, in addition to water occluded between the hydrocarbon chains.

In this work, the effect of cholesterol and xanthone on the hydration degrees of vesicles (MLV) composed by ester and ether lipids on the same chain length below and above the transition temperature was studied using LAURDAN as a fluorescent probe sensitive to polarity. The chemical structures of these lipids differ only with respect to the type of bond (carboxylic ester, ether) between the glycerol and the hydrophobic chain attached to the primary hydroxyl of glycerol, i.e., ether Pc lacks of a hydration center.

The results show that the GP_{ex} values are lower for the ether compared to the ester, indicating a higher hydration at the membrane interface in the absence of carbonyl groups, both below and above the transition temperature (T_m). On the other hand, the presence of cholesterol and xanthone decrease the GP_{ex} values in both lipids being more noticeable in the ether PC compared to the ester PC. Therefore, we conclude that the absence of carbonyl groups modifies the dipolar relaxation of the immediate environment of the marker. Therefore, the dehydrating effects of cholesterol could be a consequence of the different ability of reorientation of lipid dipoles in the glycerol region and of water molecules in the other hydration sites. The differences between the effects of xanthone are discussed on structural base.

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Evidence of tryglyceride-phase incorporation into artificial bilayers for studying lipid droplets biogenesis

Caruso B^a, Mangiarotti A^b, Wilke N^b, Perillo MA^a

a - IIBYT (CONICET - ICTA y Cátedra de Química Biológica, FCFEYN, Universidad Nacional de Córdoba)

b - CIQUIBIC, Dto. Química Biológica, Facultad de Ciencias Químicas, CONICET, Universidad Nacional de Córdoba, Córdoba, Argentina.

Lipid Droplets (LD) are intracellular structures consisting on an apolar lipid core - composed mainly of triglycerides (TG) and steryl esters- which is surrounded by a phospholipid and protein monolayer. LDs originate in the ER bilayer, where TG synthesis concludes. The mechanisms underlying TG nucleation, size maturation and budding-off from the ER membrane are a matter of current investigations and the role of dewetting from cytosolic-bilayer interface appears to play a critical role. In order to contrast the nano-sized "blisters" of TG that some authors predict¹, here we formed free-standing bilayers by transferring films of a monolayer of mixed phosphatidylcholine(EPC)/TG in coexistence with TG microlenses (*i.e.* an excluded TG phase floating in the surface). These membranes were characterized by adding them the solvatochromic fluorescent probe Nile Red (NR) and observing them under spectral confocal microscope. Such bilayers exhibit fluorescence emission spectra comparable of bilayers of vesicles with similar composition (POPC and TO). By comparison with literature data and fluorescence spectra of EPC and TG monolayers, the peaks could be assigned to different phases, namely 1) PC membranes ($\lambda^{em}max=630\text{ nm}$) bilayer and bilayer) and 2) TG isotropic phase ($\lambda^{em}max=570\text{ nm}$). No microscopic structures could be observed at $\lambda^{em}max=570\text{ nm}$. Diffusion of NR under this TG phase was characterized using FRAP analysis yielding values ($D=2\ \mu m^2s$) typical of model bilayer membranes, suggesting that the probe is diffusing in a 2D structure. This system appears appropriate for describing which is the distribution of the TG phase, that is, homogeneously among the intrabilayer space or in nanoscopic "blisters", by evaluating diffusion times obtained by FCS and FRAP.

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Interacción de péptidos transmembrana y bicapas estudiada por Dinámica Molecular

Rosetti CM^a, Montich G^a

a - Centro de Investigaciones en Química Biológica de Córdoba (CIQUIBIC), Departamento de Química Biológica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Haya de la Torre y Medina Allende, Ciudad Universitaria, X5000HUA, Córdoba, Argentina

Se caracterizaron, para sistemas modelo de bicapa y péptido con distinto desajuste hidrofóbico, la deformación en función de la composición de la membrana. Este problema tiene relevancia considerando que los cambios de composición entre diferentes membranas celulares, podrían modular la adaptación proteína-bicapa según su localización, por ejemplo, en distintos dominios lipídicos u organelas. Se realizaron simulaciones de dinámica molecular, con el modelo de grano grueso (GG) Martini como campo de fuerza. Los péptidos son secuencias de distinto número de leucinas y alaninas alternadas, con dos triptofanos en cada extremo (péptidos WALP). Los distintos grados de desajuste hidrofóbico se obtuvieron variando el número de leucinas y alaninas. Se analizaron péptidos con 15, 17, 19, 21, 23, 25, 27, 29, 31 y 33 aminoácidos, en presencia de membranas con distintos módulos de flexión (Kb) e inclinación (Kt). Se estudiaron las curvas de inclinación del péptido y perfil de espesor de la membrana en función del desajuste hidrofóbico. Se observó que los péptidos largos (desajuste negativo) se inclinan respecto a la normal, con una mínima deformación de la membrana, como ha sido descrito en otros trabajos. Nuestros resultados muestran que el ángulo de inclinación depende claramente de la composición de la bicapa, disminuyendo en membranas más rígidas. En sistemas con péptidos cortos (desajuste positivo) la inclinación respecto a la membrana varía ligeramente, o se mantiene constante, para distintos largos de péptido. Este ángulo de inclinación depende del tipo de membrana. Por otro lado, la reducción del espesor hidrofóbico en el entorno del péptido es el mismo en todas las bicapas y compensa apenas un 40% del desajuste. Este resultado indica que hay otros modos de adaptación péptido-membrana: la extensión de la alfa hélice en las distintas condiciones no varía, y se están evaluando otros factores como la orientación de los triptofanos y la exposición al agua.

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Lipid monolayers at the liquid/liquid interface as an experimental model to understand miniemulsions

Mottola M^a, Perillo MA^a

a - Instituto de Investigaciones Biológicas y Tecnológicas (IIByT), CONICET - Cátedra de Química Biológica, Departamento de Química, Facultad de Ciencias Exactas, Físicas y Naturales, FCEyN, Universidad Nacional de Córdoba

Lipid miniemulsions (ME) are oil in water dispersions stabilized by an interfacial layer of a surfactant and are systems commonly used to encapsulate, maintain and release molecules of pharmacological interest. Since each of those droplets can be regarded as an individual batch reactor, a whole variety of polymerizations reactions can be performed starting from ME to obtaining a polymer based colloidal system, consisting on a liquid-core and a polymeric-shell structure (Liquid Core Capsules, LCC).

In this context, Langmuir monomolecular films (LF) at the liquid-liquid interface can be used as experimental models to investigate the dynamic behavior of surfactants at the oil-water interface in ME. Previously, we have used this technique to study the composition and thermodynamic behavior of monomolecular layers of Egg L- α -phosphatidylcholine (EPC) at the Vaseline/water (VAS/W) interface to be further translated to a ME formulation. Based on these previous results, the aim of the present work is to synthesize ME composed of VAS, EPC and a cationic surfactant (CTAB) to use them as a template for silica polymerization allowing the obtention of LCC capable to encapsulate hydrophobic drugs such as Diazepam and Dexamethasone.

We found that both compounds have a complex surface behavior and display a favorable penetration into a previously formed EPC monolayer at VAS/W interface, achieving an equilibrium surface pressure $\pi_e \approx 25$ mN/m at 20 μ M in the subphase. Furthermore, LCC were characterized by DLS, ζ -potential and TEM microscopy. They presented a mean diameter of ≈ 200 nm and were stable at least for one month in suspension. Also, the average encapsulation efficiency for both drugs was determined between 40% and 60%. Their encapsulation in LCC would serve as a method to increase their concentration in aqueous fluids as well as a sustained releasing source.

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Study of Antimicrobial Action of Aguaribay Essential Oil on *Staphylococcus aureus*

Cutro AC^a, Castelli MV^b, **Rosales M**^{c,d}, López SN^b, Hollmann A^a, Rodriguez S^d

a - CIBAAL Centro de Investigación en Biofísica Aplicada y Alimentos CONICET UNSE

b - Facultad de Ciencias Bioquímicas y Farmacéuticas- FBioF-UNR- CONICET

c - Facultad de Agronomía y Agroindustrias FAyA UNSE

d - Facultad de Agronomía y Agroindustrias FAyA UNSE-CONICET

Microorganisms have developed resistance to many antibiotics and as a result, immense clinical problems in the treatment of infectious disease have been emerged. A key strategy to control antibiotic resistance is to stimulate the development of novel antibacterials to continue treating resistant infections. In particular, the antimicrobial activities of extracts and plant essential oil have formed the basis of many alternative medicines and natural therapies. Essential oils of plant origin are among the best known substances to have attracted attention in recent years for you several uses like as potential pest control agents, food preserving, antibacterial, antioxidant and antifungal. The genus *Schinus* (Anacardiaceae) is a native species from the north-west region of Argentina. It has been reported many traditional uses for *S. areira* L. like as a purgative, diuretic, parasiticide, insecticide, vulnerary, and topical disinfectant, and for the treatment of rheumatism, stomach upsets, menstrual disorders, bronchitis and conjunctivitis, treatment of colds, as an expectorant, among others. In this work, the essential oil (EO) chemical composition of *S. areira* from Northwest of Argentina has been determined and its antimicrobial potential. Bioautography assay has been shown, that *S. areira* EO has antibacterial activity on *S. aureus*, producing inhibitory effect at 25, 50 and 100 μg . The minimum inhibitory concentration was 62.5 $\mu\text{g}\cdot\text{mL}^{-1}$, showing that at 256 $\mu\text{g}\cdot\text{mL}^{-1}$ a bacteriolytic action. Further studies on the interaction of *S. aureus* EO with artificial membranes, such as phosphatidylcholine and phosphatidylglycerol liposomes (PC:PG; 5:1 MLVs), indicates that its interaction, produce change in electrical properties of the membrane, producing an increase in Zeta potential of 6 mV. This could indicate that interaction with membrane could be one of target for its antimicrobial mechanism of action.

The castling between hopanoids and sterols in membranes

Mangiarotti A^a, Genovese DM^a, Puentes EN^a, Naumann C^b, Wilke N^a

a - Centro de Investigaciones en Química Biológica de Córdoba (CIQUIBIC), Departamento de Química Biológica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Haya de la Torre y Medina Allende, Ciudad Universitaria, X5000HUA, Córdoba, Argentina

b - Department of Chemistry and Chemical Biology, Indiana University-Purdue University

In biological membranes, lipids play multiple roles and thereby influence cell processes, either individually or collectively. The sterols are lipids that have the property of regulating dynamics and maintaining membranes in a microfluid state. While cholesterol is the major sterol in vertebrates, ergosterol plays a key role in fungi, and stigmasterol and sitosterol are the major constituents of sterol profiles of plants species. Hopanoids are pentacyclic compounds that are proposed to be sterol surrogates of primitive bacteria.

It has been proposed that the sterol modulation of the lipid order emerged as a critical evolutionary step of biological membranes allowing the cells to control their fluidity without compromising their membrane integrity. Although prokaryotes lack sterols, it has been demonstrated that hopanoids can form liquid-ordered (lo) phases in model membranes. In this manner, the capacity of membranes to form lo domains, and thus compartmentalize, may be originated before the earth oxygenation and the emergence of sterols and eukaryote organisms.

Despite having proved that lo phase may be present in the membrane of organisms of different kingdoms, there still is a lack of systematic studies in which the differences and similitudes between the different sterols and hopanoids are evaluated together. In view of this, we have particular interest in performing a systematic analysis of the effect of sterols and hopanoids on the properties of model membranes. The first study consisted on evaluating the interfacial and diffusional properties of films composed of pure sterols and the hopanoid diplopterol. Later, we described binary and ternary lipid mixtures with sterols or hopanoids in monolayers and bilayers. This evaluates if the different compounds promote the formation of a liquid-ordered phase based on its distinctive characteristics: retaining of the fluidity while increasing the order of the hydrocarbon chains and decreasing the permeability.

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The GABAergic insecticide Fipronil interacts with membrane lipids: a Langmuir film study

Felsztyna I^a, Sánchez-Borzone ME^a, García DA^a

a - IIBYT (CONICET-UNC), Departamento de Química, Cátedra de Química Biológica, Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba

The GABA_A receptor (GABA-R) is the main inhibitory receptor of the Central Nervous System and is a membrane intrinsic protein whose activity may be affected by physical changes in the membrane. It possesses binding sites for drugs other than the neurotransmitter GABA, including convulsant agents which behave as channel blockers. The latter constitutes the action site of widely used neurotoxic pesticides, including fipronil, an insecticide with low toxicity for humans. It was previously demonstrated that many lipophilic compounds that regulate GABA-R function interact with membrane lipids causing changes in their physical properties and, consequently, non-specific receptor modulation cannot be discarded. Taking into account that the insecticide fipronil is highly lipophilic, we focused our study on its membrane interaction using dpPC monolayers. This work constitutes a part of a major project oriented to the development of bioinsecticides with high selectivity and low resistance. dpPC surface pressure versus area isotherms measured in the presence or absence of fipronil (0,25 to 25 μM in the subphase) showed that the insecticide was able to expand the LE phase of the phospholipidic interface in a concentration dependent manner, until the film saturation, diminishing the monolayer stability. The dpPC phase transition between LE and LC phases tends to disappear in the presence of fipronil. Compressibility modulus determination shows that all concentrations of fipronil modify the membrane elasticity. Furthermore, this compound can easily penetrate on the membrane according to their ability to incorporate into the monomolecular film at different surface pressures showing a $\pi_{\text{cutoff}} > 40$ mN/m. These results suggest that the insecticidal activity of fipronil could involve the interaction, not only with GABA-R, but also with lipid molecules causing changes in the physical properties of the membrane.

Contact: ivanfelsztyna@gmail.com

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The membranotropic behavior of semiochemicals, possible implications in this way of actions

Rodriguez S^a, Pinto A^b, **Hollmann A^c**

a - Instituto de Ciencias Químicas, FyA, Universidad Nacional de Santiago del Estero

b - INBIONATEC, CONICET, National University of Santiago del Estero. 4206, RN 9 Km 1125, Santiago del Estero, Argentina

c - Laboratorio de Compuestos Bioactivos, Centro de Investigación en Biofísica Aplicada y Alimentos CIBAAL- CONICET, Universidad Nacional de Santiago del Estero

Insects play an important role in agriculture and human health. Insect communication is mainly based on chemosensation due to the small body size of insects, which limits their ability to produce or perceive auditory and visual signals, especially over large distances. In this context, unraveling the chemical language of insects has been the subject of intense research in the field of chemical ecology for the past five decades. Chemicals involved in insect communication, called semiochemicals, are volatiles or semi-volatiles compounds that allow to insects to find a mate, besides the oviposition site in reproduction and food sources. Actually, insect olfaction mechanism is subject to study, but systematic analyses of the role of neural membranes are scarce. In the present work, we evaluated the interactions of α -pinene, benzaldehyde, eugenol, and grandlure, among others, with a DPPC lipid membrane model using surface pressure experiments and Monte Carlo computational analysis. The results obtained allowed to confirm, that these semiochemicals are able to interact with model lipids membranes. Furthermore, Monte Carlo analysis strongly suggests that semiochemicals after the interaction with the membrane are grouped in clusters that implied a high concentration of drug in restricted spatial domains of the membrane. Overall, the obtained results allowed us to propose and discuss a plausible membranotropic mechanism of interaction between semiochemicals and insect neural membrane.

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Alteration on glycated erythrocyte membrane by *in vitro* action of propofol

Batista da Silva MV^a, Castellini H^b, Alet N^c, Riquelme B^d, Alet A^a

a - Área Física ; Facultad de Ciencias Bioquímicas y Farmacéuticas ; Universidad Nacional de Rosario

b - Departamento de física ; Facultad de Ciencias Exactas, Ingeniería y Agrimensura ; Universidad Nacional de Rosario

c - Facultad de ciencias médicas ; Universidad Nacional de Rosario

d - Grupo de Física Biomédica ; Instituto de Física de Rosário ; CONICET

When blood is at rest, erythrocytes (RBC) aggregates forming *rouleaux*. However, in pathological conditions (diabetes) the RBCs anionic charge can be reduced by glycation, reducing cell repulsion and favoring RBC aggregation as well as changes in viscoelastic parameters. This effect can be increased in surgical procedures when the patient is under anesthetic drugs, such as propofol.

Blood samples from healthy donors were collected by venipuncture in sterile vials containing EDTA and washed two times with PBS. The RBCs were glycated *in vitro* with glucose solutions of 0.25%, 0,5% and 1% in PBS. After washed, the RBCs were resuspended in autologous plasma (40%) and diluted with PBS+propofol 4 $\mu\text{g}/\text{mL}$ (1:1) for propofol treatment (37°C, 30 min).

Viscoelastic parameters were studied using an Erythrocyte Rheometer: δ (the phase shift between deformation and applied shear stress), DI (Erythrocyte Deformability Index), μ (Elastic Modulus), η (Surface Viscosity of Membrane), G' (Dynamics Elasticity), G'' (Dynamics Lost), η' (Viscous component of Dynamics Viscosity), and η'' (Elastic component of Dynamics Viscosity). For digital image analysis the isolated cell coefficient (CCA) was calculated.

The samples glycated and treated with propofol were altered in comparison with the ones only glycated. The DI increased with the glucose concentration. The elastic modulus changed in a very small ration. The viscosity increased correlatively with the glucose concentration. For the G' , G'' , η' and η'' parameters, the values did not show significant differences.

The elastic modulus presented a tendency to decrease while increasing glucose concentration, perhaps causing hardening of the RBC cytoskeleton. When treated with propofol, the elastic modulus further diminished. Therefore, Propofol may could further harden the cytoskeleton. This hardening may be retarding the response between the applied cutting tension and its deformation.

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Biomembrane damage by a lipophilic photosensitizer

Vignoni M^a, Urrutia MN^a, Junqueira HC^b, Greer A^{c,d}, Reis A^e, Baptista MS^b, Itri R^f, Thomas AH^a

a - INIFTA, UNLP, CCT La Plata-CONICET, CC 16, Suc. 4, (1900) La Plata, Argentina

b - Departamento de Bioquímica, Instituto de Química, USP, São Paulo, Brasil

c - Department of Chemistry, Brooklyn College, CUNY, New York 11210, United States

d - Ph.D. Program in Chemistry, Graduate Center of CUNY, New York 10016, United States

e - ICETA/REQUIMTE/LAQV, University of Porto, 4169-007 Porto, Portugal

f - Instituto de Física, USP, São Paulo, Brasil

Pterins are natural products that can photosensitize the oxidation of DNA, proteins and phospholipids.¹⁻³ Recently, a new series of lipophilic pterins were synthesized and their photophysical properties investigated.⁴ These decyl-pterins led to efficient intercalation in large unilamellar vesicles and produce, under UVA irradiation, singlet molecular oxygen, a highly oxidative species that react with polyunsaturated fatty acids (PUFAs) to form hydroperoxides. Here, we demonstrate that the association of 4-(decyloxy)pteridin-2-amine (*O*-decyl-Ptr) to lipid membranes is key to its ability to trigger phospholipid oxidation in unilamellar vesicles of phosphatidylcholine rich in PUFAs used as model biomembranes. Our results show that *O*-decyl-Ptr is at least one order of magnitude more efficient photosensitizer of lipids than pterin (Ptr), the unsubstituted derivative of the pterin family, which is more hydrophilic and freely passes across lipid membranes. Lipid peroxidation photosensitized by *O*-decyl-Ptr was detected by the formation of conjugated dienes and oxidized lipids, such as hydroxy and hydroperoxides derivatives. These primary products undergo a rapid conversion into short-chain secondary products by cleavage of the fatty acid chains some of which are due to subsequent photosensitized reactions. As a consequence, a fast increase in membrane permeability is observed. Therefore, lipid oxidation induced by *O*-decyl-Ptr could promote cell photodamage due to the biomembrane integrity loss which, in turn, may trigger cell death.

Contact: mvignoni@inifta.unlp.edu.ar

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Cholesterol, a very good ally of nicotinic receptors

Fabiani C^a, Peñalva DA^a, Corradi J^a, Antollini S^a

a - Instituto de Investigaciones Bioquímicas de Bahía Blanca (INIBIBB-CONICET)

The muscle nicotinic acetylcholine receptor (AChR) has an extracellular domain which contains neurotransmitter-binding sites and a transmembrane domain that forms the ion channel pore and exhibits extensive contacts with the surrounding lipids. The AChR is present in high-density clusters in the muscle cell membrane where it localizes mainly in liquid-ordered (Lo) domains enriched in cholesterol and sphingolipids. We studied the relationship between AChR and cholesterol in *T. californica* AChR-rich membranes, model membranes containing purified AChR and cells expressing AChR and evaluated different experimental conditions: depletion of cholesterol by methyl- β -cyclodextrin, enrichment of cholesterol or cholesterol-hemisuccinate (symmetric or asymmetric situations), and oxidation of cholesterol using cholesterol oxidase. After having analyzed: i) membrane order by anisotropy and GP, ii) augmentation/diminution of Lo domains by GUVs formation and fluorescence microscopy, iii) AChR location in these domains by detergent treatment and SDS-PAGE, iv) AChR structural conformation by crystal violet fluorescent probe, and v) AChR functionality by electrophysiology, we can conclude that a change in the amount, distribution or oxidation of cholesterol impacts not only in the size and location of Lo domains and in the AChR preference for them, but also in AChR functionality and AChR structural conformation.

Comparative study of insect and mammal neuronal membranes: microviscosity, interfacial behavior and morphology of the transferred films

Colmano GN^a, Clop EM^b, Sánchez-Borzone ME^b, Turina AdV^b

a - Instituto de Investigaciones Biológicas y Tecnológicas (IIByT), CONICET - Cátedra de Química Biológica, Departamento de Química, Facultad de Ciencias Exactas, Físicas y Naturales, FCEfYN, Universidad Nacional de Córdoba

b - IIBYT (CONICET-UNC), Departamento de Química, Cátedra de Química Biológica, Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba

Interfacial behavior, molecular organization and composition of neuronal membranes from very different animal source are reported.

Neuronal membranes were obtained from synaptosomal membranes (SM) of bovine brain cerebral cortex and ganglionar membranes (GM) of dissected cerebral ganglia of *Triatoma infestans* nymphs (V instar).

SM and GM π -A isotherms showed a liquid expanded behaviour with a bidimensional phase transition at 36 mN/m in SM isotherm whereas GM films showed a more expanded and less cooperative transition at 17mN/m. GM films appeared to be less stable at high π ($\pi_{\text{col-SM}} = 45$ mN/m and $\pi_{\text{col-GM}} = 36$ mN/m)

A noticeable hysteresis was observed between the compression-decompression (C-D) cycle of both SM and GM isotherms. The $\Delta\Delta G$ between these two states was higher to GM than to SM reflecting the more plastic characteristic of the GM monolayer ($\Delta\Delta G_{\text{GM}}=64.3$ KJ and $\Delta\Delta G_{\text{SM}}=22.4$ KJ).

The floating monolayer was transferred to a solid support using the Langmuir Schaefer mode (LS films). Images obtained from LS films by epifluorescence microscopy allowed us to appreciate the complex topology present in natural membranes, with some structures not observed in the air-water interface. LS films of GM presented structures usually associated with protein agglomerates, that grew as p increased. In comparison, LS of SM showed a higher presence of condensed domains than observed in GM.

Microviscosity was assessed by fluorescence anisotropy of DPH (A_{DPH}) and TMA-DPH ($A_{\text{TMA-DPH}}$). The values of A_{DPH} suggest that the region of hydrocarbon chains in SM was more ordered than in GM. On the other hand, the $A_{\text{TMA-DPH}}$ values showed the opposite behavior.

The differences observed in microviscosity, interfacial behavior and morphology of LS films could be related with the composition of these natural membranes, as cholesterol content is significantly lower in GM. Electrophoretic profiles allowed to compare the proteins bands presents in vesicles membranes as well as in the prepared films.

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Design, synthesis and characterization of antifungal peptides derived from the sequence of two thistle flowers defensins

Fernandez A^a, Malbrán I^b, Vairo Cavalli S^a, Bakás L^a, Guzmán F^c, Curto LM^d, Maté S^e, Vázquez R^e

a - CIPROVE, Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas, UNLP, 47 y 115, La Plata

b - CIDEFI-Centro de Investigaciones en Fitopatologías

c - Núcleo Biotecnológico Curauma

d - IQUIFIB-Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, UBA, Junin 956, 1113 Ciudad de Buenos Aires

e - INIBIOLP, CCT-La Plata, CONICET, Facultad de Ciencias Médicas, UNLP, 60 y 120, La Plata

Plant defensins and defensin-like (DEFL) proteins are small basic, cysteine-rich proteins ubiquitously expressed in the plant kingdom and mostly involved in host defence. They present a highly variable sequence but a conserved structure. Sequence diversity appears to be correlated with differential functions exhibited (antifungal and antimicrobial, among others). Two highly conserved regions have been identified among plant defensins, which are important for their activity: the g-core located in the C-terminal region with a β hairpin structure and the α -core located in the N-terminal region. The importance of these motifs lies in the presence of positive residues which would allow the interaction with negative charges on the pathogen membrane and/or cell wall. In this work, potential antifungal peptides were designed from these regions, through the use of bioinformatic tools and using as template the sequence of two defensins (DefSm1D and DefSm2) of *Silybum marianum* previously cloned from thistle flowers. Five peptides were synthesized, from DefSm1D sequence: 3243, 3245 and 3246; and from DefSm2: 3248 and 3250. Then, peptides were purified and characterized by HPLC-RP, MALDI-TOF-MS and CD. Their activity against the phytopathogenic fungus *Fusarium graminearum*, their surface-active properties, as well as the ability to exclude the membrane-impermeable dye propidium iodide (PI) of conidia after treatment with the peptides were assessed. Peptides 3248 and 3250 were active against *F. graminearum* at micromolar concentration and produced PI uptake on conidia.

The present work contributes to the knowledge of defensins, which are defense cysteine-rich proteins present in *S. marianum* flowers, which grows in Argentina as a wild weed naturally resistance to fungal pathogens.

Effect of Cell Penetration Peptides on model membranes of different rigidities

Crosio M^a, Via M^b, Del Pópolo MG^b, Wilke N^a

a - CIQUIBIC (CONICET - UNC), Centro de Investigaciones en Química Biológica de Córdoba, Haya de la Torre y Medina Allende, Ciudad Universitaria, 5000, Córdoba, Argentina

b - Instituto de Histología y Embriología de Mendoza-CCT Mendoza

Cell Penetration Peptides (CPPs) are short sequences of mainly cationic amino acids, and are considered one of the most effective and promising vectors for delivering cargoes inside cells, such as proteins, nanoparticles, or nucleic acids. However, details on how CPPs traverse cell membranes, or how their absorption is affected by physiologically relevant parameters such as membrane composition, surface charge, and surface electrostatics remain a matter of debate.

There are two main mechanisms by which CPPs are incorporated into the cellular interior: endocytosis, a mechanism with energy cost; and diffusion through the lipid bilayer, a process called passive permeation or also direct translocation. Here we tackle this last mechanism.

The objective of this work was to test the interaction of a poly-arginine (KR9C) with lipid membranes of different composition, in order to evaluate the influence of the membrane fluidity, compressibility and rigidity. We studied the interaction of the peptide using as model membranes Langmuir monolayers, Large unilamellar vesicles (LUVs) and Giant Unilamellar vesicles (GUVs) using compositions in the liquid-disordered, liquid-ordered and solid phase states.

It was possible to observe differences in the interaction of KR9C with several membranes. On one hand, monolayer studies showed in the lipid mixtures a synergistic effect, with a higher incorporation of the peptides inside the mixed monolayers than in the pure lipid monolayers. On the other hand, differences were also observed in the interaction of the peptide with membranes of different composition. Z-potential measurements showed a lower peptide/lipid ratio for saturation for the more fluid membranes, while the more rigid membranes lysed at lower concentrations of the peptide.

Our results indicate that interaction, incorporation and concomitant effect of CPPs on membranes depends on the mechanical properties of the host membrane.

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Effect of natural terpenes on Bovine erythrocyte acetylcholinesterase (BEA) activity from bovine erythrocyte ghost membranes (BEM). Possible unspecific mechanism that tunes the BEA catalytic activity

Dutto J^a, Turina AdV^a, Perillo MA^a, Clop EM^a

a - Instituto de Investigaciones Biológicas y Tecnológicas (IIByT), CONICET - Cátedra de Química Biológica, Departamento de Química, Facultad de Ciencias Exactas, Físicas y Naturales, FCEfYN, Universidad Nacional de Córdoba

BEA is a GPI-anchored enzyme that hydrolyzes seric acetylcholine. The 'anionic' subsite in the active site determines the specificity with respect to the choline moiety through electrostatic interactions. Since a) changes on the molecular environment of GPI-anchored enzymes affect their kinetic parameters and b) monoterpenes (MT) affects biomembranes order and electrostatics according to their dipole moment modulus and orientation, here we tested the effects of MTs (1-8 cineol, CIN and camphor, CAM) on the hydrolysis of acetylthiocholine (ATC, Ellman's method) catalyzed by BEA present in BEM. The affinity of the BEA-ATC complex in the absence of MTs ($K_M=0.1$) was significantly affected by CIN which resulted a stronger inhibitor ($K_M=0.81$) than CAM ($K_M=0.11$) (both at 0.3 mM). Moreover, CIN exhibited an $IC_{50}=0.3mM$ whereas the IC_{50} of CAM was $\gg 0.6$ mM. Measurements of the fluorescence anisotropy (A) of DPH and TMA-DPH in BEM, demonstrated that both MTs affected the organization of the inner regions of the bilayer (both MTs reduced about a 10% the A_{DPH}) but not the polar head group region ($A_{TMA-DPH}$ was almost unaffected). The effect of MTs on the lateral pressure (π) and surface potential (DV) vs Area compression isotherms in Langmuir films were also studied. In the presence of CIN, the transition found in the control π -A isotherm become less cooperative and the $\pi_{collapse}$ decreased. At low π , the slopes of both isotherms (π -A and DV-A) changed; e.g. we found a $DDV\sim 20mV$ with respect to the control without CIN. At high π , CIN and control isotherms converged suggesting the CIN molecules expulsion from the film upon compression. CAM did not produce significant effects on DV, but expanded slightly the whole π -A isotherm upto the collapse point. Concluding, the inhibitory activity of CIN on BEA may be related with its effect on the membrane order and electrostatics which may be interfering unspecifically with the BEA-ATC electrostatic interaction at the active site.

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Formulation of liposomal preparations containing arginine-based surfactants

Fait ME^a, **Hermet M**^a, Caimi A^b, Pérez AP^b, Romero EL^b, Morcelle S^a, Bakás L^a

a - CIPROVE, Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas, UNLP, 47 y 115, La Plata

b - Centro de Investigación y Desarrollo en Nanomedicinas (CIDeN)

Two novel arginine-based cationic surfactants, *N*^α-benzoyl-arginine decylamide (Bz-Arg-NHC₁₀) and *N*^α-benzoyl-arginine dodecyl amide (Bz-Arg-NHC₁₂), were synthesized using papain (an endopeptidase from *Carica papaya* latex) adsorbed onto polyamide as biocatalyst. *N*^α-benzoyl-arginine ethyl ester hydrochloride (the classical substrate for the determination of cysteine and serine proteases activity) was used as arginine donor, whereas decyl- and dodecylamine were used as nucleophiles for the condensation reaction, with yields higher than 80%. The purification process was achieved by a single cationic exchange chromatographic separation, involving the use of water and ethanol as the main separation solvents. Liposomal formulations made of soy phosphatidylcholine (SPC) as phospholipids (10 mg.mL⁻¹) and Bz-Arg-NHC_n (n=10 or 12; 20-40% mol.mol⁻¹) as edge activators were prepared by the thin-film hydration method. The liposomal suspensions were sonicated (60 min with a bath-type sonicator, 80 W, 40 KHz) and extruded 10 times through two stacked 0.2 μm and 0.1 μm pore polycarbonate filters using a 100 ml Thermobarrel extruder. Size (liposomal diameter) and zeta potential were determined by dynamic light scattering and phase-analysis light scattering, respectively. The deformability values (D) of the liposomes were also calculated and phospholipids were quantified by a colorimetric phosphate microassay. For storage stability characterization, mean size, polydispersity and Z potential were measured upon storage during 3 weeks at 4°C in darkness. Based on these results, both arginine-based surfactants could be used as additives in topical formulations exerting their activity on the skin surface.

Keywords: arginine-based surfactants; liposomes; deformability; stability

Impact of sphingomyelin acyl chain (16:0 vs 24:1) on the interfacial properties of Langmuir monolayers

Vázquez R^a, Daza Millone MA^b, Pavinatto FJ^c, Fanani ML^d, Oliveira ON^c, Vela ME^b, Maté S^a

a - Instituto de Investigaciones Bioquímicas de La Plata (INIBIOLP), CCT- La Plata, CONICET. Facultad de Ciencias Médicas, Universidad Nacional de La Plata.

b - Instituto de Investigaciones Físicoquímicas Teóricas y Aplicadas (INIFTA), CCT- La Plata, CONICET. Facultad de Ciencias Exactas, Universidad Nacional de La Plata.

c - São Carlos Institute of Physics, University of São Paulo

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

Sphingomyelin (SM) and related sphingolipids are believed to have important functional roles on membrane domain formation, e.g. for the Cholesterol (Chol)-rich liquid-ordered (*Lo*) phase. Notwithstanding, N-nervonoyl-SM (24:1-SM)—the most common unsaturated SM natural species—is able to prevent phase segregation. In this study, Polarization-modulated infrared reflection-absorption spectroscopy (PM-IRRAS) was applied to investigate the conformational properties and intermolecular interactions in 16:0 vs 24:1-SM monolayers and their mixtures with 1,2-Dioleoyl-sn-glycero-3-phosphocholine (DOPC) and Chol (DOPC/SM/Chol 2:1:1). From the results we inferred that the *cis* double bond ($\Delta 15$) in 24:1-SM molecule diminishes intermolecular H-bonding and chain packing density compared to that of 16:0-SM. In ternary mixtures containing 16:0-SM, the relative intensity of the two components of the Amide I band reflected changes in the H-bonding network due to SM-Chol interactions. In contrast, the contribution of the main components of the Amide I band in DOPC/24:1-SM/Chol remained as in 24:1-SM monolayers, with a larger contribution of the non-H-bonded component. The most interesting feature in these ternary films is that the C=O stretching mode of DOPC appeared with an intensity similar to that of SM Amide I band in DOPC/16:0-SM/Chol monolayers (a two-phase [*Lo/Le*] system), whereas an extremely low intensity of the C=O band was detected in DOPC/24:1-SM/Chol monolayers (single *Le* phase). This is evidence that the unsaturation in 24:1-SM affected not only the conformational properties of acyl chains but also the orientation of the chemical groups at the air/water interface. The physical properties and overall H-bonding ability conferred by 24:1-SM may have implications in cell signaling and binding of biomolecules.

Interaction of an arginine based-surfactant with model lipid membranes

Hermet M^a, Vázquez R^b, Maté S^b, Daza Millone MA^c, Vela ME^c, Morcelle S^a, Bakás L^a

a - CIPROVE, Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas, UNLP, 47 y 115, La Plata

b - INIBIOLP (Fac. de Cs. Médicas, UNLP-CONICET)

c - INIFTA-CONICET, Fac. de Cs. Exactas, UNLP. Suc. 4-C.C. 16. (1900) La Plata, Argentina. Fac. de Ingeniería, UNLP, 1 y 47 (1900) La Plata, Argentina

The stratum corneum is a major barrier to drug penetration across the skin in transdermal delivery. To overcome this barrier, skin penetration enhancers are commonly used. Lipoaminoacids derived from arginine consist of a family of non-toxic biodegradable cationic surfactants with antifungal and antimicrobial properties. They meet four crucial requirements for the industrial development of new surfactants: low toxicity, high biodegradability, multifunctionality and use of renewal sources of raw materials for their synthesis.

The interaction of a novel arginine-based cationic surfactant, N^ω-benzoyl-arginine decyl amide (Bz-Arg-NHC₁₀) with DPPC monolayers was studied at different initial surface pressures (π_0) of the lipid films. For all the π_0 assayed, the injection of the lipoaminoacid into the subphase bulk produced a rapid increase in π reaching a maximum within the first 5 min followed by a decrease in π which stabilized after ~20 min. Bz-Arg-NHC₁₀ was able to penetrate into these lipid monolayers up to a critical pressure of 35 mN/m.

Differential scanning calorimetry was used to assess the effect of Bz-Arg-NHC₁₀ upon DPPC membranes. As the concentration of Bz-Arg-NHC₁₀ increased, the main transition temperature of DPPC slightly decreased.

AFM *in situ* experiments performed on supported DPPC bilayers on mica allowed to follow the changes induced by Bz-Arg-NHC₁₀. DPPC bilayer patches were partially removed, mainly in borders and defects for 0.05 mM Bz-Arg-NHC₁₀ solution. Increasing the concentration to 0.10 mM resulted in a complete depletion of the supported bilayers. SPR measurements allowed to quantitatively assess the DPPC removal by Bz-Arg-NHC₁₀. Experiments carried out with fully DPPC bilayers covered chips showed a net increase of the SPR signal, which can be assigned to Bz-Arg-NHC₁₀ adsorption. When patchy DPPC bilayers were formed on the substrate, a net decrease of SPR signal was obtained. This is consistent with the DPPC removal observed in AFM images.

Interaction of Antimicrobial Peptides with Model Membranes in the Presence of Cholesterol and Diplopterol

Alvares DS^a, Ruggiero Neto J^b, Wilke N^a

a - Centro de Investigaciones en Química Biológica de Córdoba (CIQUIBIC), Departamento de Química Biológica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Haya de la Torre y Medina Allende, Ciudad Universitaria, X5000HUA, Córdoba, Argentina

b - IDepartment of Physics, UNESP-IBILCE, São José do Rio Preto, SP, Brazil

The antimicrobial tetradecapeptide Polybia-MP1 (IDWKLLDAAKQIL-NH₂), extracted from the venom of the Brazilian wasp, *Polybia paulista*, displays antimicrobial and anticancer activities. Recent studies have shown that the neutralization of the N-terminus positive charge by acetylation significantly enhanced the lytic activity in mixed anionic vesicles of an analog antimicrobial peptide (Alvares; Wilke; Ruggiero Neto, BBA, 2018). We also gathered experimental evidence suggesting that a synergy between the anionic lipid phosphatidylserine and liquid ordered domains (in mixtures of cholesterol and sphingomyelin) significantly influences the lytic activity of the membrane-active helical peptide, MP1 (Alvares; Ruggiero Neto; Ambroggio, BBA, 2017). The liquid-ordered microdomains have been traditionally considered exclusive features of the eukaryotic cell being connected to the evolution of cellular complexity (Bramkamp, Microbiol. Mol. Biol. Rev., 2015). However, it was recently discovered that bacterial membranes also contain lipid ordering in a fashion similar to those found in eukaryotic membranes (Sáenz, *et al.* PNAS, 2015). It has also been proposed that hopanoids are surrogates of cholesterol, regulating membrane fluidity and order. The effect of diplopterol (an abundant hopanoid) is being studied in our group in comparison with cholesterol and other sterols. The results indicate that diplopterol forms fluid but compact membranes, with characteristics similar to the liquid-ordered phase induced by the sterols. Given these antecedents, here we study the interaction of MP1 and its acetylated analog (acMP1) with membranes in the presence of cholesterol or diplopterol. In this manner, we test the possible recognition of bacterial/mammals membranes mediated by hopanoids/sterols.

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Interactions of *de novo* designed peptides with bacterial membranes: Implications in antimicrobial activity

Maturana P^a, Martinez M^b, Espeche JC^c, Maffia P^b, Hollmann A^a

a - Laboratorio de Compuestos Bioactivos, Centro de Investigación en Biofísica Aplicada y Alimentos CIBAAL- CONICET, Universidad Nacional de Santiago del Estero

b - Laboratorio de Microbiología Molecular, IMBA, Departamento de Ciencia y Tecnología, UNQ, Argentina

c - Laboratorio de Compuestos Bioactivos, Centro de Investigación en Biofísica Aplicada y Alimentos CIBAAL - CONICET, UNSE. Facultad de Agronomía y Agroindustrias, Universidad Nacional de Santiago del Estero.

Antimicrobial peptides (AMPs) are small molecules, usually cationic, that display antimicrobial activity against a wide range of bacteria, fungi, and viruses. In a previous work by using model membranes we studied two related novel peptides, P6 that show no antimicrobial activity and P6.2 which exhibited good antibacterial activity. However, besides biophysical techniques on model membranes can provide very detailed information on the interaction of AMPs with membranes, until recently, it was unknown if any of the information predicted was relevant for AMPs activity in real bacteria. In this context, in the present work, we evaluated by using Zeta Potential, Absorbance, and fluorescent approaches the effect of both peptides in bacteria. First, Zeta Potential was applied, using *Escherichia coli* and *Staphylococcus aureus* as a bacterial model. The obtained results confirming that both peptides were able to interact with negative bacterial envelope. However, the effects on P6.2 were much more noticeable in both bacteria.

In order to get an insight into the effect that both peptides induce in the bacterial membrane, the disruption of the outer (OM) and inner (IM) membrane of *E. coli* was analyzed. For OM permeabilization study the probe 1-Nphenyl naphthylamine, only able to insert in previously damaged OM, was used. Besides both peptides were able to damage the OM, 5 times concentration of P6 was needed in order to obtain comparable results than those obtained with P6.2. Also, P6.2 exhibited a much faster damage kinetic. IM disruption was assessed following the colored product of cytosolic β -galactosidase. P6.2 not only exhibits more product formation, implying a greater disruption of the membrane but a faster kinetic.

All data put together allows postulating, in a physiologic model, that the lower affinity of P6 for bacterial envelope results in a minor final concentration of the peptide in the bacterial membrane unable to trigger the antimicrobial activity.

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Photochemical properties and membrane affinity of decyl-pterins

Sosa MJ^a, Urrutia MN^a, Walalawela N^{b,c}, Greer A^{b,c}, Thomas AH^a, Vignoni M^a

a - INIFTA, UNLP, CCT La Plata-CONICET, CC 16, Suc. 4, (1900) La Plata, Argentina

b - Department of Chemistry, Brooklyn College, CUNY, New York 11210, United States

c - Ph.D. Program in Chemistry, Graduate Center of CUNY, New York 10016, United States

Pterins are a family of heterocyclic compounds, which are present in biological systems and play different roles ranging from pigments to enzymatic cofactors for numerous redox and one-carbon transfer reactions.^{1,2} Pterins are not soluble in organic solvents and are only slightly soluble in water. Therefore, a new series of alkyl chain $[-(\text{CH}_2)_9\text{CH}_3]$ pterin conjugates have been synthesized and their photochemical and photophysical properties were studied.³ Interestingly, these properties changed in comparison with the properties of the parent compound Pterin (Ptr).⁴

In addition, the interaction of these compounds with lipid biomembranes was investigated. Size exclusion chromatography experiments showed that Ptr is not encapsulated inside the large unilamellar vesicles (LUVs) and can freely pass through the lipid membranes. On the other hand, decyl-pterins efficiently intercalate in LUVs, and therefore, their binding constants were obtained by fluorescence emission titration curves. Moreover, fluorescence spectra suggest that the fluorophore of decyl-pterins anchored to the biomembrane is in an aqueous environment.

In conclusion, decyl-chain conjugation to pterins dramatically increases their lipophilicity, while keeping their photochemical properties. This is a good indicator of their potential use as photosensitizers in biomembranes.

Contact: mjsosa@inifta.unlp.edu.ar

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Towards the self-organized criticality: lipid peroxidation effects on the dynamical evolution of the conductance process in lipid bilayer membranes

Corvalán NA^a, Caviglia AF^{a,b}, Baptista MS^c, Itri R^{a,d}, Lascano HR^{a,b}

a - Cátedra de Fisiología Vegetal, FCEfYn, Universidad Nacional de Córdoba, Córdoba, Argentina - Departamento de Física Aplicada, Instituto de Física, Universidade de São Paulo, São Paulo, Brazil.

b - Cátedra de Fisiología Vegetal, FCEfYn, Universidad Nacional de Córdoba

c - Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo

d - Departamento de Física Aplicada, Instituto de Física, Universidade de São Paulo

Reactive Oxygen Species (ROS) are by-products of the normal aerobic metabolism whose dynamics and steady-state concentration are regulated by precise mechanisms of redox balance that prevent their potentially toxic effects. Under prolonged and/or high-intensity oxidative stress conditions, large amounts of hydroperoxidized lipids (ROOH) can be accumulated in cell membranes as the first relatively stable products of the ROS-mediated process known as Lipid peroxidation (LPO). Numerous biophysical studies have shown how LPO impacts on the structure and organization of lipid membranes, but its effects on the functionality and the electrical behavior of the membrane are only partially elucidated. Taking into account the evidences about a dual concentration-dependent role of ROS as *toxic* and *signaling* molecules, the present work is focused on the study of the oxidative damage effects on the conductance process dynamics in Bilayer Lipid Membranes (BLMs), with the aim to elucidate how the electrical signaling processes can be affected under stressful conditions, how the membrane *self-organizes* to respond to external (electrical) perturbations depending on the oxidative damage severity, to finally discuss the evidence that supports the hypothesis that the conductance process dynamics could be modulated by ROS through *non-specific* mechanisms, without protein ion channels involvement.

Our results show that, oxidative damage favors the dynamical evolution of the membrane system towards a *self-organized critical state* characterized by the emergence of *long term-memory* and *scale invariance* properties of the conductance process when certain ROOH concentration and energy thresholds are overcome. This critical state appears to be strongly associated to the *ion lipid conductive pores* formation and a non-linear (non-ohmic) behavior of the electrical parameters.

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Unravel the interactions of “*de novo*” antimicrobial peptide P1 with model and bacterial membranes

Espeche JC^a, Maturana P^b, Martinez M^c, Maffia P^c, Cutro AC^b, Hollmann A^b

a - Laboratorio de Compuestos Bioactivos, Centro de Investigación en Biofísica Aplicada y Alimentos CIBAAL - CONICET, UNSE. Facultad de Agronomía y Agroindustrias, Universidad Nacional de Santiago del Estero.

b - Laboratorio de Compuestos Bioactivos, Centro de Investigación en Biofísica Aplicada y Alimentos CIBAAL - CONICET, Universidad Nacional de Santiago del Estero

c - Laboratorio de Microbiología Molecular, IMBA, Departamento de Ciencia y Tecnología, UNQ, Argentina

In the present work, we evaluate the interaction of the antimicrobial peptide 1 (P1) with model and bacterial membranes. P1 is a cationic peptide with 21 amino acids (WPKWWKWKRRWGRKKAKKRRG), designed identifying short putative active regions from AMP databases.

First, we evaluated the interaction of P1 with model membranes (i.e. liposomes of DMPC:DMPG 5:1) by using Zeta Potential. Then fluorescence quenching was applied to dissect the ability of the peptide to insert into the lipid bilayer. Both experiments confirm the interaction of P1 with this model membrane showing a depth insertion of the Trp residues in the hydrophobic core of the bilayer.

In order to evaluate the effect of the peptide in more physiologic scenery, we evaluate also by zeta potential the ability of P1 to interact with *Escherichia coli* and *Staphylococcus aureus*. In both cases, zeta potential becomes less negative after peptide incubation confirm the ability of the peptides to bound into the cell envelope. However, the effect becomes more noticeable in *S. aureus*.

Finally, the ability of the peptide to permeabilize the inner and outer membrane of *E. coli* was assessed. The results obtained confirm that P1 is able to disrupt both membranes, showing a much faster kinetics in the disruption of the outer membrane as expected.

All the data put together allows proposing a model where the insertion of the of the peptide, stabilized by Trp residues depth inserted in hydrocarbon region, promotes changes in the lipids organization following a carpet-like mechanism that results in a permeabilization of the membrane triggering the antimicrobial activity.

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Effect of the charge on the interaction of L-cysteine and its esters with monolayers of DPPC

Arias JM^a, Diaz SB^b, Ben Altabef A^a, Dupuy F^c

a - INQUINOA (CONICET-UNT) Instituto de Química del Noroeste Argentino, Ayacucho 471, San Miguel de Tucumán, CP 4000, Tucumán, Argentina

b - Instituto de Química-Física, Fac. de Bioquímica, Química y Farmacia. UNT

c - INSIBIO (CONICET-UNT) Instituto Superior de Investigaciones Biológicas, Chacabuco 461 - San Miguel de Tucumán, CP 4000, Tucuman, Argentina

The surface behavior of the aminoacid cysteine was studied by means of Langmuir monolayer technique. The effect of charge on monolayers made of cysteine and three esters derivatives with dipalmitoylphosphatidylcholine (DPPC) was investigated by preparing un-buffered and buffered (pH 4 and 8) subphases. Molecular interactions between the cysteine derivatives L-cysteine, L-cysteine ethyl ester, L-cysteine methyl ester and N-acetylcysteine with DPPC monolayers were studied measuring the change in the surface tension upon aminoacid injection in the subphase whilst keeping lipid molecular density and lateral packing controlled. The results indicate that, although the different derivatives of cysteine presented low surface activity, they were able to favourably interact with DPPC monolayers. Also, compression isotherms experiments in binary mixtures indicate that the more surface active compounds stabilized the gel phase of DPPC. The charge on cysteine and its derivatives increased the observed effects.

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Dengue and Zika virus capsids: membrane docking and oligonucleotide recruitment

Ambroggio E^a, Bagatolli L^b, Costa Navarro GS^c, Gamarnik AV^c

a - Centro de Investigaciones en Química Biológica de Córdoba (CIQUIBIC), Departamento de Química Biológica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Haya de la Torre y Medina Allende, Ciudad Universitaria, X5000HUA, Córdoba, Argentina

b - Instituto de Investigación Médica Mercedes y Martín Ferreyra

c - Fundación Instituto Leloir and IIBBA-CONICET

Flaviviridae viral capsids recruit the genomic information of virus to infective viral particles that are generated from the endoplasmic reticulum (ER). The mechanism and regulation of such processes are still not known but we hypothesize that membrane physics should be a key player. From this perspective here, we show how the capsid proteins of Dengue and Zika virus not only are able to bind ER mimicking model membranes but also to dock liposomes and at the same time interact with oligonucleic acids. These observations are results from experiments using confocal fluorescence spectral microscopy and fluorescence lifetime analysis of labelled proteins and DNA/RNA molecules when interacting with giant unilamellar vesicles and large liposomes.

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A coarse-grained simulation survey of lipid-protein interactions of several P-type ATPases

Sica M^a, Pignataro M^{Fb}, Delfino JM^b, Rossi JP^b, Mangialavori I^b

a - IEDS, Centro Atómico Bariloche, CNEA. Instituto Balseiro, Universidad Nacional de Cuyo

b - IQUIFIB-Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, UBA. Junin 956, 1113 Ciudad de Buenos Aires

Free fatty acids are known to regulate the function of several membrane proteins modulating the structure of membranes or interacting with the proteins. Specifically, there are growing evidence that oleic (OA) and linoleic (LA) acids present in membranes are natural compounds that regulate the function of Na⁺,K⁺-ATPase (1) and plasma membrane calcium ATPase (PMCA) (2). In this work we used coarse-grained simulations to compare lipid-protein interactions of several P-type ATPases. Using the Martini coarse-grained forcefield we simulated PMCA, sarcoplasmic calcium ATPase (SERCA) and Na⁺,K⁺-ATPase in membranes composed of DLPC, DOPC and OA. Protein models were prepared from PBD structures of SERCA and Na⁺,K⁺-ATPase (1t8s and 2zxe, respectively). The all-atom structure of PMCA was built by homology with SERCA using an curated alignment (3) with Modeller (autoinhibitory domain was excluded). Five independent 5- μ sec simulations were performed for each protein. Although the transmembrane architecture is shared, the lipid-protein interactions comprise a unique fingerprint which involves a range of asymmetric distributions, non-specific and specific interactions. Particularly, our simulations highlight specific interactions of OA with Na⁺,K⁺-ATPase and PMCA, absent in SERCA.

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Characterization of cooperative phenomena in pH sensing of aquaporins

Vitali VA^a, Scochera F^a, Giorgetto F^b, Canessa Fortuna A^a, Jozefkowicz C^c, Soto G^c, González-Flecha FL^a, Alleva K^a

a - IQUIFIB-Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, UBA. Junin 956, 1113 Ciudad de Buenos Aires

b - Departamento de Físico-matemática, FFYB, UBA

c - Instituto de Genética, INTA-Castelar-CONICET

Cooperative regulation of protein function is a phenomenon of central importance in many cellular processes. PIP aquaporins are tetrameric pH dependent channels and their dose-response profile of osmotic water permeability coefficient (Pf) vs intracellular proton concentration shows a sigmoidal shape, suggesting a cooperative phenomenon. We consider two time-separated stages to analyze the impact of proton concentration in water transport by PIP channels: a proton binding event, and an open-closed conformational transition. In this regard, we proposed two models where the cooperative character was alternatively attributed to one of the two involved stages. By means of this model we studied the pH response of PIP homotetramers, PIP heterotetramers (where two different paralogues PIP2 and PIP1 are assembled), and mutants PIP, expressed in the heterologous systems of *Xenopus oocyte*. Our results show that the cooperativity in PIP biological response cannot be directly attributed to a cooperative proton binding as it is usually considered, since it could also be the consequence of a cooperative conformation transition between open and closed channel states. Moreover, the decrease in the degree of positive cooperativity found when mixed populations of homo and heterotetrameric PIP channels are expressed in the plasma membrane of the same cell, would be the result of the simultaneous presence of molecular species with different levels of proton sensing. Finally, these models are also useful tools to estimate the conformational impact of mutations that alter PIP functioning. Despite the main goal of this approach is to offer a deep understanding on cooperative transport in PIP channels, we are also committed to stress that phenomenological modeling has an important epistemic value which is not in opposition with mechanistic explanations.

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Flavonoids and calcium transport through biological membranes

Ontiveros M^a, Rinaldi D^a, Marder M^a, Espelt MV^a, Vigil MA^a, Mangialavori I^a, Rossi RC^a, Rossi JP^a, Ferreira-Gomes M^a

a - Instituto de Química y Físicoquímica Biológicas y Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires-CONICET

Recently, there has been increasing interest in the research on flavonoids from plant sources because of their beneficial properties for health. Several studies have attributed them anti-oxidative, anti-inflammatory, anti-mutagenic, and anti-carcinogenic properties coupled with their capacity to modulate key cellular enzyme functions. Flavonoids exert their effect in different ways, in particular, there are flavonoids related to changes in intracellular calcium concentration, suggesting that they could affect the Ca²⁺ transport.

The present study seeks to investigate the effect of several natural flavonoids on hPMCA4 (human plasma membrane Ca²⁺-ATPase isoform 4), a P-type ATPase essential for the intracellular Ca²⁺ control in eukaryotic cells.

Results obtained with purified PMCA showed that some flavonoids inhibited the PMCA activity and that the increase in the number of -OH in the B ring enhanced the inhibition potency. The best inhibitors were quercetin and gossypin with K_i of 0.3 and 4.1 mM, respectively. The mechanism of inhibition of these flavonoids was dependent on the Mg²⁺ concentration, suggesting that the inhibitor is a flavonoid-Mg²⁺ complex. When phosphorylated intermediates (EP) were measured, quercetin led to the increase of EP, which was sensitive to ADP, whereas gossypin induced a decrease. These results suggest that gossypin could affect the ATP binding whereas quercetin could prevent the conformational change E1P → E2P.

To assess whether the effects of quercetin and gossypin on the purified hPMCA4 could occur in living cells, we monitored changes in the cytoplasmic Ca²⁺ in HeK293T cells that overexpress hPMCA4. Results are compatible with the idea that quercetin and gossypin inhibited the PMCA activity. Fluorescence microscopy images indicate that both flavonoids distributed widely in the cytoplasm of cells, suggesting that it is possible a direct interaction between these flavonoids and the cytoplasmic domains of PMCA.

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Functioning mechanism of a Cu^+ transporting ATPase from *Legionella pneumophila*: initial characterization

Placenti MA^a, Kaufman SB^a, Román EA^a, González-Flecha FL^a, González-Lebrero RM^a

a - IQUIFIB-Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, UBA. Junin 956, 1113 Ciudad de Buenos Aires

P-type ATPases are a family of membrane proteins which couple ATP hydrolysis to the transport of substrates across biological membranes. Within this family, P1B-ATPases are responsible for transition metal ions transport, playing a key role in the regulation of their intracellular concentration. Cu^+ -transporting ATPases are the most widespread and conserved members of this subfamily, being present from bacteria to human, in which mutations of these proteins are the direct cause of Menkes and Wilson diseases. Even though Cu^+ -transporting ATPases share functional and structural features with other P-type ATPases, several authors postulate that these proteins may have a unique specific mechanism. Therefore, the aim of our work is to characterize the kinetic and thermodynamic properties of the Cu^+ transporting ATPase from *Legionella pneumophila* (*LpCopA*) in order to elucidate its functional mechanism.

LpCopA was heterologously expressed in *E. Coli*, solubilized in $\text{C}_{12}\text{E}_{10}$ and purified by affinity chromatography. We first determined the optimal conditions to measure steady state ATPase activity of *LpCopA*. For that purpose, we evaluated the effect of temperature, pH, ionic strength and lipid concentration on the rate of enzyme release of phosphate from ATP. On this basis, we then performed experiments in the presence of different concentrations of the protein natural ligands ATP, Mg^{2+} and Cu^+ . Finally, we formulated kinetic models to explain the behaviours observed.

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Last but not least: flexible C-terminal domain is a key element for PIP aquaporin gating

Scochera F^a, Soto G^b, Alleva K^a

a - IQUIFIB-Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, UBA. Junin 956, 1113 Ciudad de Buenos Aires

b - INSTITUTO DE GENETICA (INTA-CONICET)

Aquaporins are tetrameric channels that facilitate the osmotic diffusion of water and small molecules across membranes. Plasma membrane intrinsic proteins (PIP) represent the larger subfamily in higher plants, and is divided into two paralogues, PIP1 and PIP2, able to form both homo and heterotetramers (Fetter et al., Plant Cell, 2004). Previous results show that BvPIP are pH sensitive channels, with PIP2-PIP1 heterotetramers pH0,5 shifted to acidic values in comparison with PIP2 homotetramer sensing (Yanef et al., PNAS 2014; Jozefkowicz et al., Biophys J 2016). A mechanistic gating model was proposed for these channels on the basis of SoPIP2;1 X-ray crystallography. This mechanism involves conformational changes in loop D, N- and C-terminal domains. However, even if this model proposes C-terminal domain as a key element of the gating mechanism, the last part of this flexible domain could not be assigned in the crystal structure due to its disorder. As the length of C-terminal domain is one of the characteristic differences between PIP1 and PIP2, we investigated if this domain is relevant for PIP gating mechanism.

We generated two PIP2 mutations : 1) the deletion of the last six C-terminal residues, mimicking the length of PIP1, and 2) the replacement of the C-terminal domain with the corresponding fragment of PIP1. We assayed the biological activity and pH regulation of PIP2 mutants expressed alone or co-expressed with PIP1. Interestingly, we found that mutations at the C-terminal domain can markedly modify PIP2 pH gating. Our results suggest a capital role of this domain in the pH gating mechanism that couldn't been resolved with structural data. These new findings shed light on the C-terminal relevance on pH gating and open the possibility of a deeper understanding of PIP gating mechanism.

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Molecular Modulation of Human $\alpha 7$ Nicotinic Receptor by Amyloid- β Peptides

Lasala M^a, Fabiani C^a, Corradi J^a, Antollini S^a, Bouzat C^a

a - Instituto de Investigaciones Bioquímicas de Bahía Blanca (INIBIBB-CONICET)

Amyloid β peptide ($A\beta$) is a key player in the development of Alzheimer disease (AD). It is the primary component of senile plaques in AD patients and is also found in soluble forms. Cholinergic activity mediated by $\alpha 7$ nicotinic receptors has been shown to be affected by $A\beta$ soluble forms. To shed light into the molecular mechanism of this effect, we explored the direct actions of oligomeric $A\beta_{1-40}$ and $A\beta_{1-42}$ on human $\alpha 7$ by fluorescence spectroscopy and single-channel recordings.

Fluorescence measurements using the conformational sensitive probe crystal violet (CrV), which shows different affinities for resting and desensitized states, revealed that $A\beta$ induces $\alpha 7$ concentration-dependent conformational changes. At 100 pM, $A\beta$ displaces CrV Kd value for the resting state towards that of the desensitized state from which $\alpha 7$ is still reactive to carbamylcholine (Carb). These observations are compatible with the induction of active/desensitized states as well as of a novel conformational state in the presence of both $A\beta$ and Carb. At 100 nM $A\beta$, $\alpha 7$ adopts a resting-state-like structure which does not respond to Carb, indicating the stabilization of $\alpha 7$ in a blocked state. In real time, we found that $A\beta$ is capable of eliciting $\alpha 7$ channel activity either in the absence or presence of the positive allosteric modulator PNU-120596. Activation by $A\beta$ is favored at picomolar or low nanomolar concentrations and is not detected at micromolar concentrations. At high $A\beta$ concentrations, the durations of the activation episodes elicited by ACh are significantly reduced, an effect compatible with slow open-channel block. We conclude that $A\beta$ directly affects $\alpha 7$ function and acts as an agonist and a negative modulator: activation of $\alpha 7$ by low $A\beta$ concentrations may be involved in beneficial physiological effects, and the reduced $\alpha 7$ activity in the presence of higher $A\beta$ concentrations may contribute to the cholinergic signaling deficit and may be involved in the initiation and development of AD.

Protective effect of the flavonoid quercetin on the inhibition of plasma membrane calcium pump by aluminum

Riesco AS^a, de Sautu M^a, Rossi JP^a, Ferreira-Gomes M^a, Mangialavori I^a

a - Instituto de Química y Físicoquímica Biológicas y Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires-CONICET

Aluminum is etiologically and epidemiologically related to several neurological disorders, including Alzheimer's, Parkinson's and amyotrophic lateral sclerosis. Exposure to high levels of this metal leads to neurofibrillary and neuronal degeneration and induces neurobehavioral deficits and morphological changes in the brain. The mechanism of aluminum neurotoxicity has been associated with several cellular phenomena, among them, a disturbance of intracellular Ca²⁺ homeostasis. Effectively, we have recently demonstrate that Al³⁺ inhibits the Ca²⁺-ATPase activity of the plasma membrane (PMCA) and sarcoplasmic reticulum (SERCA) calcium pumps [1] and produces an increase in intracellular Ca²⁺ [2]. Flavonoids are commonly found in fruits and vegetables and they are also part of human diet. It is known that flavonoids have anti-oxidant activity. They prevent metal-mediated generation of free radicals because they are capable of sequestering metal ions by chelating.

In this context, the aim of this work was to evaluate if the flavonoid quercetin protects PMCA from inhibition by aluminum and to characterize the mechanism by which it does so. Our results suggest that quercetin protects PMCA from aluminum inhibition because it has a chelating effect on this trivalent cation and the quercetin-Al³⁺ complex does not bind to PMCA. In addition, the presence of quercetin partially reverses the effect of the inhibitory effect of aluminum, suggesting that its administration is a potential strategy to reverse the effect of aluminum at the cellular level.

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The role of Na⁺ as a substitute for protons in the gastric H,K-ATPase

Valsecchi WM^a, Faraj SE^a, Cerf N^a, Rossi RC^a, Montes MR^a

a - Departamento de Química Biológica e IQUIFIB (UBA-CONICET), Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires

The gastric H,K-ATPase is responsible for the ATP-dependent exchange of intracellular protons for extracellular potassium ions. The reaction cycle is proposed to be similar to that of the Albers-Post model already used to describe P2-ATPases but the H⁺/ATP stoichiometry is still under discussion. Due to its high structural similarity with the Na,K-ATPase and the fact that protons can replace sodium as well as potassium in the reaction mechanism of the Na,K-ATPase, the role of sodium as congeners of protons has been evaluated in the H,K-ATPase. Durr *et al.* (1) presented arguments in favor of Na⁺ as surrogates for protons regarding the E2P→E1P transition and reducing the apparent affinity for Rb⁺ whereas Swartz *et al.* (2) proposed that Na⁺ acts as a K⁺ analog increasing the rate of dephosphorylation reaction in the gastric pump.

With the aim of using Na⁺ for measurements of stoichiometry, we analyzed the effects of the cation on conformational transitions, ATPase activity, and cation binding. Experiments were carried out at 25 °C in media with imidazole-HCl 25 mM, pH 7.4, using pig gastric H,K-ATPase-enriched membrane vesicles permeabilized with alameticin (3).

Results showed that Na⁺ increases the eosin fluorescence signal probably by shifting the E1↔E2 equilibrium to the E1 conformation, as it is proposed for H⁺. A similar effect was observed when increasing the ionic strength, using choline chloride, which raises the question of whether it is specific for Na⁺. Epigallocatechin-3-gallate inhibits the H,K-ATPase activity but, unlike what we found in the Na,K-ATPase, it failed to increase the affinity for Na⁺ binding and to favor the occlusion of this cation.

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A novel modulator of the glutamate-activated chloride channel with potential anthelmintic effect

Turani O^a, Castro MJ^a, Scoccia J^b, Faraoni MB^b, Gerbino DC^b, Bouzat C^a

a - Instituto de Investigaciones Bioquímicas de Bahía Blanca (INIBIBB-CONICET)

b - INQUISUR (CONICET-UNS)-Instituto de Química del Sur

Parasitic nematodes affect human health and livestock. The acquisition of resistance to current anthelmintic drugs has prompted the search for new compounds. The use of parasitic worms for drug testing is costly and difficult. The free-living nematode *Caenorhabditis elegans* has therefore emerged as a valuable platform for anthelmintic drug discovery. We have previously synthesized a small library of oxygenated tricyclic compounds and tested their anthelmintic activity by measuring rapid effects on *C. elegans*. The exposure to dibenzo[*b,e*]oxepin-11(6H)-one (Compound 1a) rapidly induced paralysis of *C. elegans* (IC₅₀ = 389 ± 50 mM). Given that Cys-loop receptors are involved in worm locomotion and are targets of antiparasitic drugs, in this work we tested the effect of Compound 1a on *C. elegans* mutant strains lacking different Cys-loop receptors. We found that a mutant strain that lacks the invertebrate glutamate-gated chloride-selective channel (GluCl), which is the main target of the antiparasitic drug ivermectin, is resistant to Compound 1a, revealing that GluCl is the drug target. To unravel the molecular mechanism underlying the paralyzing action, we transfected mammalian cells with GluCl α and β subunits and evaluated the effect of Compound 1a on whole cell currents. Glutamate elicited macroscopic currents on cells expressing GluCl α / β heteromeric receptors. At -60 mV, macroscopic currents showed an onset of 89 ± 29 ms and amplitude-voltage relationships indicated no significant rectification. Preincubation with 0.5 mM Compound 1a (1 min) led to a ~80% decrease of the glutamate-evoked current, but the original peak current could not be fully recovered after 2 min wash. Moreover, the glutamate-activated current decreased with repeated exposure to the drug. These results indicate that Compound 1a acts as novel modulator of invertebrate GluCl receptors. In conclusion, we propose Compound 1a as a promising antiparasitic drug acting through GluCl receptors.

Effect of ions and lipids on ATPase activity of the Spf1 P5-ATPase from *Saccharomyces cerevisiae*

Petrovich GD^a, Corradi GR^a, Adamo HP^a

a - IQUIFIB-Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, UBA, Junin 956, 1113 Ciudad de Buenos Aires

P-type ATPases (P1 to P5) are integral membrane proteins that transport different ligands against its concentration gradient, through ATP hydrolysis. P5-ATPases are the least studied members of the whole family of P-ATPases and its transported substrate remains unknown. Several human P5-ATPases are implicated in neurological disorders, as the Kufor-Rakeb syndrome, a parkinsonism with dementia, hereditary spastic paraplegia, neuronal ceroid lipofuscinosis, autism and intellectual disability. With the aim of advancing the knowledge of P5-ATPases we have investigated the effect of lipids and ions on the ATPase activity of the recombinant Spf1 P5-ATPase of *Saccharomyces cerevisiae*. The highest levels of ATPase activity were achieved when asolectin from soybean was used to supplement the micelar preparation of the protein indicating that asolectin makes the best micelar environment for Spf1 ATP hydrolysis. While monovalent ions didn't affect ATPase activity Spf1, it was inhibited by mercury, aluminum, lanthanum and cadmium. Like other P-ATPases, Spf1 required magnesium to hydrolyze ATP. The dependence of Spf1 ATPase activity with Mg^{2+} was best fitted by a double hyperbola, a fact that could indicate the existence of two binding sites for magnesium. On the other hand, high concentration of other divalent cations reduced the ATPase activity. Interestingly, the Spf1 ATPase had a biphasic dependency with zinc. At low concentrations zinc increased the ATPase while it produced inhibition at higher concentrations. High inhibitory concentrations zinc in the presence of ATP changed the sensibility of Spf1 to chymotrypsin suggesting the stabilization of an inhibited conformation.

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Hypertonicity activated ion currents and ATP release in *Xenopus laevis* oocytes

Bazzi Z^a, Bracamonte MV^b, Guastaferrri FV^c, Ozu M^c, Schwarzbaum P^a, Galizia L^b

a - IQUIFIB-Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, UBA, Junin 956, 1113 Ciudad de Buenos Aires

b - Instituto de Investigaciones Médicas Alfredo Lanari, IDIM UBA-CONICET

c - Instituto de Biodiversidad y Biología Experimental y Aplicada (IBBEA) - UBA-CONICET

The *Xenopus laevis* oocyte is a well-known heterologous expression system. Several endogenous ion channels and transporters of *Xenopus* oocytes have been well characterized, but the identification of volume-sensitive endogenous channels is still a matter of debate. In native *Xenopus* oocytes hypertonic stimuli produces the activation of an endogenous ion current and simultaneous ATP release. However, the effect of cell volume decrease on the activation of these endogenous channels was not deeply investigated. Since ATP is mostly anionic at physiological pH, we wondered whether volume-sensitive ion channels mediating this endogenous current would be responsible for the observed activation of ATP efflux. To approach this question, we used heterologous expression of aquaporins (AQPs) to produce rapid cell volume changes. In this work we evaluated cell volume changes, the ionic currents and the ATP release induced by hypertonic stimuli in *Xenopus laevis* oocytes with or without heterologous expression of human aquaporin-1 (AQP1). Hypertonic-induced ion currents and ATP release were measured using two electrode voltage-clamp and luminometric techniques, respectively, while cell volume was monitored by videomicroscopy. In water injected oocytes (Ctrl) hypertonic stimuli induced cell volume decrease, activation of an endogenous ionic conductance (G_{hyper}) and a slow release of intracellular ATP. In AQP1-expressing oocytes we observed a faster volume decrease, together with a higher activation of G_{hyper} ($G_{\text{hyper}} \text{ Ctrl} = 4 \pm 0.3 \mu\text{S}$, $n = 7$ vs $G_{\text{hyper}} \text{ AQP1} = 90 \pm 27 \mu\text{S}$, $n = 6$, $p < 0.05$) and higher activation of ATP release (Relative $\text{ATP}_{\text{hyper}} \text{ Ctrl} = 34 \pm 7$, $n = 6$ vs Relative $\text{ATP}_{\text{hyper}} \text{ AQP1} = 133 \pm 23$, $n = 7$, $p < 0.05$). Results suggest the existence of an endogenous volume-sensitive ion channel at the *Xenopus laevis* oocyte, mediating ATP release.

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Ketamine effect on PC12 cell death can be mediated by voltage dependent calcium channels

Acosta L^a, Karadayian AG^b, Bustamante J^a, Lores Arnaiz S^b

a - Centro de Altos Estudios en Ciencias Humanas y de la Salud (CAECIHS), Universidad Abierta Interamericana

b - Instituto de Bioquímica y Medicina Molecular (IBIMOL; UBA-CONICET), Fisicoquímica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires

Ketamine blocks the N-methyl-D aspartate receptor (NMDAR), leading to decreased cellular calcium mobilization. The aim of this work was to study the effect of different ketamine concentrations (100-500-1000 μM) on calcium mobilization and cytosolic calcium concentrations ($[\text{Ca}^{2+}]_c$) in undifferentiated PC12 cells. Kinetic analysis of calcium mobilization was performed by Fluo-4AM after depolarization with KCl and after addition of 250 mM glutamate. Nitric oxide (NO), mitochondrial membrane potential and cardiolipin were assayed by flow cytometry using specific probes. Cell death was analyzed by PI, TUNEL, and Annexin V-FITS/PI. Results showed that the increase in $[\text{Ca}^{2+}]_c$ after addition of 70 mM KCl in control, 100 and 500 μM ketamine samples was 47% higher than after addition of 50 mM KCl, but only 8% higher for 1000 μM ketamine samples. Addition of 250 mM glutamate did not produce evident Ca^{2+} entry. Incubation with 100 μM and 500 μM ketamine induced 64% and 70% $[\text{Ca}^{2+}]_c$ decreases respectively as compared with control cells while 1000 μM ketamine induced 49% increment in $[\text{Ca}^{2+}]_c$. NO production was significantly decreased (20-35%) at all ketamine concentrations. Mitochondrial depolarization (10-12%) and slight cardiolipin depletion were observed for 100-500 μM ketamine respectively as compared with untreated cells, while exposure to 1000 μM ketamine induced 37% depolarization and 30% cardiolipin decrease. Cells incubation with 100-500 μM ketamine caused 18% and 10% of apoptosis (vs 2% for control cells) and low levels of necrosis. Levels of apoptotic cell death induced by 1000 μM ketamine were 34%, while necrosis levels were 21% as compared with control cells. We can conclude that ketamine toxicity on PC12 cells can be mediated by a decrease in calcium entry possibly by inhibiting VDCC inducing different cell death pathways. Ketamine effects in undifferentiated cells seems to be independent from antagonist action on NMDAR, due to low glutamate response.

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Measurement of Na⁺-occluded states in the Na,K-ATPase during its normal functioning

Faraj SE^a, Valsecchi WM^a, Saint-Martin M^a, Ferreira-Gomes M^a, Rossi JP^a, Montes MR^a, Rossi RC^a

a - Instituto de Química y Físicoquímica Biológicas y Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires-CONICET

According to the Albers-Post model, binding of 3 intracellular Na⁺ to the E1 state of the Na,K-ATPase triggers phosphorylation by ATP in the presence of Mg²⁺, and Na⁺ becomes occluded in the phosphorylated intermediate E1P. Na⁺ is released to the extracellular medium after the E1P→E2P conformational transition. Na⁺ occlusion has never been reported in unmodified enzyme; therefore, there is no information on the kinetics of Na⁺-transport intermediates during the normal functioning of the Na,K-ATPase. Here, we present the first results of Na⁺ occlusion during the hydrolysis of ATP.

Experiments were carried out at 25 °C in media with imidazole-HCl 25 mM, pH 7.4, using Na,K-ATPase partially purified from pig kidney. Bound Na⁺ was measured using ²²Na⁺. To stop the reactions and isolate the species with occluded Na⁺, the reaction media were injected into a chamber with a Millipore filter through which an ice-cold washing solution was flowing. The solution contained 1 mM epigallocatechin-3-gallate to stabilize the Na⁺-occluded states. Formation of the E2P intermediate was monitored using the fluorescent probe RH421.

Results from equilibrium experiments show that the level of tightly-bound ²²Na⁺: (i) increases with [Na⁺] along a rectangular hyperbola ($K_{0.5} = 3.5$ mM; max. stoichiometry aprox. 3 Na⁺/enzyme unit); (ii) in the presence of 0.5 mM Mg²⁺ the curve becomes sigmoidal ($K_{0.5} = 7.2$ mM; max. stoichiometry aprox. 3 Na⁺/enzyme unit); and (iii) decreases with [Mg²⁺] and with [Rb⁺]. The time course of tightly-bound Na⁺ after addition of 5 or 15 mM ATP and 0.5 mM Mg²⁺ showed a transient decrease, which lasted the more the higher the concentration of ATP. Experiments using RH421 suggest that these time courses reflect the formation and breakdown of phosphorylated intermediates until ATP is totally hydrolyzed. Our results are solid evidence that the tightly-bound Na⁺ remains occluded in the Na,K-ATPase and that we are measuring the occlusion of Na⁺ during ATP hydrolysis.

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Mechanism of 5-HT₃ receptor activation and modulation by allosteric drugs

Rodríguez Araujo N^a, Fabiani C^a, Bouzat C^a, **Corradi J^a**

a - Instituto de Investigaciones Bioquímicas de Bahía Blanca (INIBIBB-CONICET)

Serotonin type 3 receptors (5-HT₃) are cation-selective channels that belong to the Cys-loop receptor family. They are involved in fast excitatory transmission in central and peripheral nervous systems and are implicated in gastrointestinal and neurological functions. Five different subunits (A-E) have been identified in humans, and the A subunit is the only one capable of forming functional homomeric receptors (5-HT₃A). These receptors are activated by agonist binding to orthosteric sites located at the interfaces between two adjacent subunits at the extracellular region. Carvacrol and thymol have been classified as positive allosteric modulators that also act as allosteric agonists (ago-PAMs). To characterize their mechanism of activation and modulation we used the high-conductance form of the 5-HT₃A receptor that allows detection of single-channel openings from patch-clamp recordings. We observed that both ligands activate the receptor, eliciting openings in quick succession grouped in clusters of high open probability. Mean closed, open and cluster durations remained constant at all agonist concentrations tested. When each ago-PAM was evaluated in the presence of tryptamine (an orthosteric agonist), we observed events with mean open durations similar to those observed in the presence of tryptamine alone, but cluster duration was clearly prolonged probably due to decreased desensitization. These results suggest that the mechanism of activation is governed by the orthosteric agonist while the allosteric drug is only acting as a potentiator. Altogether, our results describe the mechanism underlying human 5-HT₃A receptor activation and modulation by two allosteric agonists and provide relevant information for the design of more efficacious and specific drugs.

Molecular Pharmacology of a Serotonin-gated Chloride Channel

Rodríguez Araujo N^a, Corradi J^a, Bouzat C^a

a - Instituto de Investigaciones Bioquímicas de Bahía Blanca (INIBIBB-CONICET)

Serotonin-gated ion channels (5-HT₃) belong to the family of Cys-loop receptors, which are pentameric proteins that mediate fast synaptic transmission. In mammals, 5-HT₃ receptors are non-selective cation channels that can be found as homomers (5-HT₃A) or heteromers when combined with 5HT3B-E subunits. The free-living nematode *Caenorhabditis elegans* is a model for the study of the nervous system and human diseases, and for antiparasitic drug discovery. *C. elegans* contains a homomeric serotonin-gated Cys-loop receptor, MOD-1, that is permeable to chloride. We here expressed MOD-1 in mammalian cells and explored the properties of activation and modulation of MOD-1 by whole cell recordings. Dose-response curves showed an EC₅₀ for 5-HT of ~1 mM, which is in the same range as that of human 5-HT₃A receptors. The analysis showed that currents do not show rectification, desensitize slowly and recover from desensitization with a time constant of about 1 s. To characterize the pharmacology of MOD-1, we tested compounds that have been shown to modulate 5-HT₃ and other Cys-loop receptors. The antiparasitic drug ivermectin (IVM), which acts as an activator or modulator of different receptors, neither activated nor potentiated MOD-1. However, pre-exposure to IVM (10-50 mM) decreased 5-HT induced currents, indicating that it acts as an inhibitor of MOD-1. The 5-HT₃ receptor potentiator, 5-hydroxyindol, did not affect MOD-1 function, whereas thymol, which is an activator and/or modulator of 5-HT₃ receptors, could only modulate MOD-1 activity. These results contribute to the understanding of the molecular pharmacology of MOD-1 as a potential drug target for anthelmintic therapy.

The blockade of low affinity neurotensin receptor (NST2) disassembles neuronal membrane association between nNOS, PSD-95 and NMDA receptor. Evidences of mitochondrial dysfunction.

Lores Arnaiz S^a, Karadayian AG^a, Gutnisky A^b, Miranda JL^b, Rodríguez de Lores Arnaiz G^b

a - Instituto de Bioquímica y Medicina Molecular (IBIMOL; UBA-CONICET), Físicoquímica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires

b - Instituto de Biología Celular y Neurociencias (IBCN; CONICET-UBA), Prof. Eduardo de Robertis, Facultad de Medicina, Universidad de Buenos Aires.

Previous results from our laboratory have shown impairment in neuronal nitric oxide synthase (nNOS) activity and expression after the blockade of low affinity neurotensin receptor (NST2) by levocabastine administration. Evidences show that post-synaptic density protein 95 (PSD-95) links nNOS with the N-methyl-D-aspartic acid (NMDA) receptor in CNS. Male Wistar rats were i.p. injected at a single dose with levocabastine (50 µg/kg) or saline and were decapitated 18 hours later. Crude mitochondrial fractions from cerebral cortex were obtained by differential centrifugation, while synaptosomal membrane fractions were isolated by differential and sucrose gradient centrifugation. Results from Western blot assays indicated that the protein expression of PSD-95, NR2B subunit of NMDA and β-actin after levocabastine administration were respectively 72, 34 and 45% lower than those in vehicle injected samples ($p < 0.05$). Mitochondrial function was significantly impaired after levocabastine treatment, presenting 27% and 29% decreases in state 4 and 3 succinate-dependent respiration respectively ($p < 0.05$). Moreover, levocabastine *in vitro* (1 µM) induced 45% and 63% decreases in state 4 and 3 succinate-dependent respiration leading to a 32% decrease in respiratory control rate ($p < 0.05$). Together with this, significant decreases in the activity of respiratory complexes were observed. In addition, ATP production rate using malate-glutamate and succinate as substrates was 94% and 57% decreased respectively ($p < 0.01$). Also, the *in vitro* addition of 1 µM levocabastine decreased ATP production rate by 92-96% with both substrates ($p < 0.001$). Findings led us to conclude that the NO synthesis impairment observed after blockade of NST2 receptor might be due to the disassembly between nNOS, NMDA NR2B subunit and PSD-95 proteins. The results were concomitant with alterations in mitochondrial function.

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The functionality of Nicotinic Acetylcholine Receptor insertes in Au(111)-supported thiolipid/DMPC bilayers

Pissinis DE^a, Díaz C^a, Prieto ED^a, Barrantes F^b, Salvarezza RC^a, **Schilardi PL^a**

a - Instituto de Investigaciones Físicoquímicas Teóricas y Aplicadas (INIFTA), CONICET - Departamento de Química, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, CC16, Suc. 4, La Plata, Buenos Aires, Argentina

b - Laboratory of Molecular Neurobiology, BIOMED, UCA-CONICET, Faculty of Medical Sciences, Av. Alicia Moreau de Justo 1600, 1107 Buenos Aires, Argentina

Biological membranes are an important interface in cells, particularly due to they hold transmembrane proteins involved in mechanisms of signal transduction, regulate the entry and exit of matter in the cell, etc. To study these processes, biomimetic systems mimicking biomembranes have been developed¹. Transmembrane protein anchoring on immobilized lipid layers on conductive substrates allows to analyze the interaction of these proteins with the membrane, and also opens the possibility of using these systems as a platform for biosensors that respond to electrochemical stimuli. In this work, we studied the insertion and function of the nicotinic acetylcholine receptor (nAChR) in Au(111)- supported bilayers. The biomimetic system was formed by incorporating nAChR into an Au(111)-supported thiolipid self-assembled monolayers (SAMs) and forming a 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) layer on the nAChR-containing SAM. We used AFM to characterize the supported bilayer and electrochemical techniques to analyze the functionality of the inserted protein. Electrochemical results show that the surface of Au (111) is coated with the thiolipid monolayer, decreasing the capacity of the double layer². This capacity is maintained after the insertion of nAChR and decreases after DMPC layer formation, but increases with the addition of the agonist Carb, due to the opening of the intrinsic ion channel of nAChR protein³. Moreover, our results suggest that the channel aperture induced by Carb is inhibited by anesthetics since the double layer capacitance is further reduced after the addition of lidocaine hydrochloride. Thus, the system appears to be a viable biomimetic model to measure ionic conductance mediated by ion-gated ion channels under different experimental conditions, with potential applications in biotechnology and pharmacology.

Contact: pls@inifta.unlp.edu.ar

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The P5-ATPase Spf1 contains a highly exposed region near the transmembrane segment M5.

Petrovich GD^a, Corradi GR^a, Adamo HP^a

a - IQUIFIB-Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, UBA, Junin 956, 1113 Ciudad de Buenos Aires

The P-ATPases are active transporters essential for cellular homeostasis. P-ATPases are known to transport ions or lipids. The P5 subgroup is not very well characterized and its transported substrate has not yet been identified. Loss of function of P5-ATPases in humans has been associated with early-onset Parkinsonism (Kufor-Rakeb syndrome) and other neurodegenerative diseases. With the aim of advancing the knowledge of the structural organization of P5-ATPases we have performed experiments of limited of the recombinant Spf1 P5-ATPase of *Saccharomyces cerevisiae* and of its fluorescent versions containing GFP at the N or at the C terminus. The products were characterized by SDS-PAGE, fluorescence, mass spectrometry and sequencing. The results show that a short exposure of Spf1 to chymotrypsin results in the split of the 135 kDa protein in a larger N-terminal fragment of about 110 kDa and a smaller peptide of 25 kDa containing the C-terminal end of the protein. This cut does not apparently affect the ability of the enzyme to hydrolyze ATP and the formation of the catalytic phosphoenzyme. N-terminal sequencing of the C-terminal fragment identified two possible cleavage sites at Ala769 (QT1A) and Ala996 (QT1B) both at a segment of the protein predicted to be exposed to the cytosol near the transmembrane segment M5. The 25 kDa size of the fragment suggest that the main cut is at QT1B. However, analysis of the amino acid sequence by PeptideCutter estimates a probability for cleavage of 54.5% for QT1B compared to 91.8% for QT1A. Moreover, secondary structure prediction and homology modeling indicates that M5 is part of a long helix starting at Ser972. Since the M5 segment is part of the transported substrate binding site in other P-ATPases it is tempting to speculate that it has a similar role in Spf1.

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Water permeability changes of *FaPIP2;1*-expressing oocytes triggered by different osmotic gradients: evidence in favor of mechanical regulation

Acuña CF^a, Galizia L^b, Guastaferrri FV^a, Bracamonte MV^b, Sutka M^a, Ozu M^a

a - Instituto de Biodiversidad y Biología Experimental y Departamento de Biodiversidad y Biología Experimental, Facultad de Ciencias Exactas y Naturales, UBA y CONICET, Buenos Aires, Argentina. Piso 4 Pabellon II Ciudad Universitaria Int. Güiraldes 2160 C1428EGA Buenos Aires ARGENTINA

b - Instituto de Investigaciones Médicas Alfredo Lanari, IDIM UBA-CONICET

Our previous works demonstrated that: 1) human AQP1 (*HsAQP1*) and *BvTIP1;2* from beet are aquaporins characterized by a non-linear relationship between the water flux (J_w) and the osmotic gradient (D_{osm}); and 2) this is due to the decrease of the osmotic permeability coefficient (P_f) mediated by membrane tension increments. On the other hand, *BvPIP2;1* (a PIP member from beet) showed a linear J_w -D_{osm} relationship, indicating that it is not mechanosensitive. Since PIPs and AQP1 share a common ancestor, we wonder if other PIPs can be mechanosensitive. Therefore, in this work we studied the transport properties of *FaPIP2;1* (from strawberry) by injecting different quantities of cRNA in xenopus oocytes. After 48 h of expression, the oocytes were subjected to different Δ osm created with mannitol (40, 90 and 140 mOmol.Kg_w⁻¹). Oocytes volume changes were registered by videomicroscopy and J_w and P_f were determined from the initial slope of volume kinetics. Controls were performed with water-injected or 100 μ M amphotericin-incubated oocytes. While controls show linear J_w -D_{osm} relationships, *FaPIP2;1*-expressing oocytes show deviations from linearity, similar to previous reports with *HsAQP1* and *BvTIP2;1*. Oocytes injected with 5 ng of *FaPIP2;1*-cRNA show a significant decrease ($p < 0.05$) of P_f (cm.s⁻¹) with high osmotic gradients: 0.023 ± 0.008 (40 mosmol.Kg_w⁻¹, N=3, n=6) vs 0.008 ± 0.001 (140 mosmol.Kg_w⁻¹, N=5, n=10). Similar results were observed with 10 and 14 ng. In other experiments, 5ng of *FaPIP2;1*-cRNA/oocyte were tested without vitelline envelope (VE), observing the same result. In addition, P_f determinations (cm.s⁻¹) with the same gradient (160 mosmol.Kg_w⁻¹) in *FaPIP2;1* injected-oocytes (5 ng/oocyte) are not dependent on the absence (0.019 ± 0.005 , N=1, n=6) or presence (0.017 ± 0.007 , N=1, n=3) of the VE. Our results suggest that: 1) *FaPIP2;1* responds like mechanosensitive aquaporins do; and 2) the VE could not be involved in the mechanical regulation mechanism.

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P5-ATPase ATP13A2-overexpression in SH-SY5Y cells modifies the lipid homeostasis

Marcos AL^a, Solis Pavesio L^a, Corradi GR^a, Mazzitelli LR^a, Adamo HP^a, de Tezanos Pinto F^a

a - Instituto de Química y Físicoquímica Biológicas y Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires-CONICET

The P-type ion pumps are membrane transporters energized by ATP-hydrolysis. They were classified into five subfamilies termed P1-P5; the substrate specificity of P5 subfamily is still unknown. Five genes named *ATP13A1-ATP13A5* that belong to the P5-ATPases are present in humans. Mutations of the *ATP13A2* gene, also known as *PARK9*, were initially associated with a form of Parkinson's Disease (PD), a form of NCL (CNL12) and of hereditary spastic paraplegia (SPG78). *ATP13A2* is localized in lysosomes and late endosomes (LEs). Dysfunction of this protein diminishes the lysosomal degradation, the autophagic flux and the exosome externalization. We have previously shown that *ATP13A2* expression caused a reduction of the iron-induced lysosome membrane permeabilization, which suggests that *ATP13A2* overexpression improves the lysosome and LE membrane integrity. In this work, we stably expressed the *ATP13A2* in SH-SY5Y human neuroblastoma cell line. By fluorescence microscopy, we found that the expression of *ATP13A2* increases the accumulation of the fluorescent analog phosphatidylethanolamine (NBD-PE) while reduces the accumulation of ceramide (NBD-ceramide). In immunofluorescence assays, we found that the expression of *ATP13A2* reduces the content of bis(monoacylglycerol)phosphate (BMP). Besides, the triglyceride and cholesterol content is reduced in *ATP13A2*-expressing cells, while the synthesis of polar lipids is increased. Altogether these results show that *ATP13A2* overexpression modifies the lipid homeostasis in SH-SY5Y cells. As BMP is required for the lipid degradation process and the exosome biogenesis inside the acidic compartments, these results suggest that *ATP13A2* may be modifying the lipid digestion capacity and/or the redistribution of lipids in these subcellular organelles.

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Development of multi-enzymatic complexes for the improvement of cellulolytic activity

Gorojovsky N^a, Iglesias Rando MR^a, Garrone N^a, Santos J^b, Dodes Traian MM^a, Zylberman V^c, Goldbaum FA^d, Craig PO^a

a - Departamento de Química Biológica, FCEyN, UBA - IQUBICEN, CONICET

b - IQUIFIB-Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, UBA. Junin 956, 1113 Ciudad de Buenos Aires

c - Inmunova

d - Fundación Instituto Leloir

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. However, the industrial production of natural cellulosomes has serious limitations because of 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 and the development of artificial cellulosomes. For the assembly of these multienzymatic particles we use a non-covalent coupling strategy through high affinity heterodimeric peptides 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, including endoglucanases, exoglucanases, xylanases, beta glucosidases and cellulose binding domains from different organisms, fused to the coupling peptide. The amount and solubility of these fusion proteins were compared to the original isolated domains. The coupling peptide doesn't perturb the solubility of the target proteins. At least one member of each functional category is solubly expressed in significant amounts and has been able to be purified by affinity chromatography. We present advances in their functional characterization.

We have successfully assembled several of the target proteins to the oligomeric scaffold encouraging further development of the artificial cellulosomes. It is expected that these complexes will help to increase the enzymatic lignocellulose degradation, reducing the cost of bioethanol production.

Biocompatible supramolecular platforms to modulate cell adhesion

Pasquale MA^a, Muzzio NE^{b,c}, Marmisolle WA^a, von Bilderling C^{c,a}, Cortez LM^a, Pietrasanta LI^{d,c}, Azzaroni O^a

a - Instituto de Investigaciones Físicoquímicas Teóricas y Aplicadas (INIFTA), (UNLP, CONICET), Sucursal 4, Casilla de Correo 16, 1900 La Plata, Argentina

b - Centro de Investigación de Proteínas Vegetales (CIPROVE), Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas, Universidad Nacional de La Plata (UNLP), La Plata, Argentina

c - Instituto de Física de Buenos Aires (IFIBA, UBA-CONICET), and Centro de Microscopías Avanzadas, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, C1428EHA Buenos Aires, Argentina

d - Departamento de Física, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, C1428EHA Buenos Aires, Argentina

Cell adhesion modulation via biologically inspired materials plays a key role in the development of realistic platforms to envisage mechanistic descriptions of many physiological and pathological processes as well as the design of biomedical devices.

This contribution shows the control of cell adhesion and proliferation of three different cell lines employing a novel biologically inspired supramolecular coating generated via dip coating of the working substrates in an aqueous solution of polyallylamine in the presence of phosphate anions in one-step procedure.

Results reveal that selective cell adhesion can be controlled by varying the deposition time of the coating. Cell proliferation experiments showed a cell type-dependent quasi-exponential growth demonstrating the nontoxic properties of the supramolecular platform. From a certain deposition time, the supramolecular films displayed antiadhesive activity towards cells, irrespective of the cell type. However, these antiadherent substrates recover good adhesive properties after thermal annealing. These results were interpreted based on the changes in the coating hydrophilicity, topography and stiffness. This material may have uses either to improve cell adhesion or as an antifouling surface. The former would be achieved employing thin coatings or annealed ones and antifouling properties would be obtained for thicker unannealed coatings. The proposed approach is simple and flexible, and offers opportunities for the development of tunable, biocompatible architectures to control cell phenotype of interest for biomedical applications.

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Bovine serum albumin nanohydrogels as a potential carrier for drug delivery

Velez EMdM^a, Maldonado Galdeano C^b, Chehín R^a, Cháves AS^a

a - IMMCA (Instituto de Investigación en Medicina Molecular y Celular Aplicada)

b - CERELA (Centro de Referencia para Lactobacilos)

One of the most important impediments that therapeutic drugs have is their arrival at the biological target (physical barriers, dilution, environmental stability, etc). In this sense, hydrogels have emerged as a potential candidate to compete with many of the existing materials associated with drug delivery. Hydrogels are three-dimensional networks with high water absorption, capable of being loaded with different compounds and then release them in a specific condition. The aim of the present work is to characterize the conditions in which albumin could aggregate to produce hydrophilic nanomaterials with high loading capacity and good releasing profile in biological conditions. Different aggregation conditions were explored and the hydrogels obtained were characterized by using fluorescence and infrared spectroscopy as well as swelling ratio (SR) with H₂O or DMEM culture media. Those with best SR were probed for loading/releasing capacity with different dyes and the most efficient were tested *in vivo* to study biocompatibility. To do this, we used a standard murine model; hydrogels were injected in the peritoneal cavity or orally administered. The inflammatory response was tested according Maldonado Galdeano 2011⁽¹⁾. The low immune response indicate that the selected preparation could be able to be functionalized with specific antibodies to reach different targets and this novel nanomaterial could represent a potential and promising system for drug delivery.

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Crosslinked domains of apoA-I but not of Δ K107, are involved in rHDL's shape

Díaz Ludovico I^a, Garda HA^a, Gonzalez MC^a

a - INIBIOLP, CCT-La Plata, CONICET, Facultad de Ciencias Médicas, UNLP, 60 y 120, La Plata

Human apolipoprotein A-I (ApoA-I) is the major protein component of high density lipoproteins (HDL) playing a pivotal role in reverse cholesterol transport. A natural mutant of ApoA-I having a deletion of the single residue Lys107 (K) induces severe atherosclerosis. The structural and functional effects due mutation are studied to elucidate the pro-atherogenicity of this protein.

In this study we investigate the structural effects of K107 deletion in a comparative approach with wild type (W) protein. Attending to structural alterations caused by deletion we have crosslinked with BS3 the lysine residues of both proteins in the native monomeric state (KBS3 and WBS3).

Tryptophan fluorescence is red shifted in the deletion mutant (K or KBS3) compared with the wild type protein (W or WBS3) but it is not affected by crosslinking.

The micellization of multilamellar DMPC liposomes (145:1 molar ratio) was evaluated with crosslinked (WBS3 and KBS3) and un-crosslinked proteins. The rate of the micellization process was decreased by the crosslinking although the efficiency of the process at final point was not affected.

The size of HDL complexes formed by DMPC micellization were determined by polyacrylamide native electrophoresis and shape was visualized by TEM. Cross-linked proteins do not form the small sized populations of lipoprotein complexes observed with uncrosslinked proteins. TEM observation indicates that generation of discoidal complexes is decreased by crosslinking of the wild type protein but is less affected in the case of the deletion mutant. Thus, we can conclude that the ability to generate discoidal HDL complexes is reduced exclusively by crosslinking of lysine 107.

Hybrid albumin/gold nanoparticles toxicity in Zebrafish larvae

Feas DA^a, Achilli E^b, Flores C^b, Alonso S^a, Grasselli M^b, Chiaramoni N^a, Prieto MJ^a

a - Laboratorio de Biomembranas (LBM), GBEyB, UNQ-IMBICE-CONICET

b - Laboratorio de Materiales Biotecnológicos (LaMaBio), GBEyB, UNQ-IMBICE-CONICET

The use of nanotechnology in medicine is based on the preparation of nanostructures, such as nanoparticles (NPs). NPs can be targeted to specific tumor tissues and enhance the intracellular activation properties.

In this context, we prepared core/shell gold/Albumin NPs (Alb/Au-NPs) by crosslinking with ionizing radiation. Preparation of NPs containing an inorganic core, such as gold, can be very useful for NP tracking and detection. Also, albumin is a high biocompatible, biodegradable and non-antigenic protein, as well as malleable.

Recently we have advanced in the study of free radicals formed during the irradiation of NPs in the presence of ethanol, and the byproducts of this irradiation. The ionizing irradiation (0.5 to 5 kGy, gamma rays) of binary aqueous ethanol mixtures leads to three main final products: molecular hydrogen, 2,3-butandiol, and acetaldehyde as a consequence of dimerization or dismutation reactions. The presence of butandiol was confirmed by FT-IR spectroscopy, whereas the presence of acetaldehyde was confirmed by a colorimetric method.

Based on this, the main goal of this work was to study the toxicity of Au-NPs combined with bovine serum albumin (BSA) and human serum albumin (HSA) in zebrafish larvae.

To carry out this work, irradiated, non-irradiated and desalted irradiated Alb/Au-NPs were tested in zebrafish larvae. The spontaneous movement, viability, heart rate and morphological changes of 120 hpf zebrafish larvae were studied as parameters of toxicity. Non-irradiated and desalted irradiated Alb/Au-NPs exhibited no significant variations in the studied parameters. On the contrary, larvae exposed to irradiated Alb/Au-NPs showed a significant decrease in the spontaneous movement until 24 hours post incubation (hpi) and mortality at 48 hpi. For those above, the final products obtained after the ionizing irradiation, which are isolated after desalination, might be responsible for larvae toxicity.

Acknowledgments

UNQ, IMBICE, CONICET.

PAMAM Dendrimers Enhance the Solubility of Curcumin in Aqueous Media through Complexation in their Internal Pockets

Igartúa DE^a, Martínez C^a, Alonso S^a, Prieto MJ^a

a - Laboratorio de Biomembranas (LBM), GBEyB, UNQ-IMBICE-CONICET

Curcumin (CUR) is a natural polyphenol derived from the turmeric *Curcuma longa*, which is used as a spice, food coloring, and medicine in oriental practices. CUR has multiple mechanisms of action that could modulate the Alzheimer's Disease progression: (a) inhibits acetylcholinesterase enzyme, (b) inhibits the formation of aggregates of A β and promotes the disintegration of those already formed, (c) inhibits TAU phosphorylation, and (d) acts as an antioxidant and anti-inflammatory, reducing levels of oxidative stress. However, clinical studies did not have positive results, which could be due to CUR insolubility in aqueous solutions, short half-life, and low bioavailability. For the aforementioned, the CUR is a candidate to be used in drug delivery systems. PAMAM dendrimers generation 4.0 and 4.5 (DG4.0 and DG4.5) are polymers that would increase the solubility of hydrophobic drugs. The present work aimed to obtain and characterize complexes between CUR and PAMAM dendrimers.

First, the effect of dendrimer:CUR molar ratio on the solubility of CUR was studied. The ratio 1:10 D:CUR was the optimal complexation condition, which resulted in complexes with seven mol of CUR for each mole of DG4.0, or four mol of CUR for each mole of DG4.5. These results reflected an increase in solubility from 1.6 μ M (free CUR) to 170 μ M for DG4.0-CUR and 100 μ M for DG4.5-CUR.

Then, the complexes were characterized by studying the time-stability and after the lyophilization process. In addition, the dendrimer:CUR interaction was studied using FTIR. The analysis of these assays showed that the interaction occurs in the hydrophobic pockets of the dendrimers.

So far, a CUR delivery system based on dendrimers capable of complexing and significantly increasing the solubility of the CUR in aqueous media has been developed. It is proposed, as a short-term perspective, to study the activity of these complexes in cell culture (*in vitro*) and in zebrafish (*in vivo*).

Acknowledgments

UNQ, IMBICE, CONICET.

Synthesis and Characterization of Nanoparticles and Nanofibers from Whey Protein Concentrate.

Burgos I^a, Muratori P^a, Perillo MA^a

a - IIBYT (CONICET-UNC), Departamento de Química, Cátedra de Química Biológica, Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba

Waste from the dairy industry produce a significant environmental impact in the region. We propose the reuse of one of the most abundant wastes, whey serum, rich in proteins, for the synthesis of biomaterials with biotechnological interest. Nanoparticles (Nps) synthesized from whey proteins concentrate (WPC) are of interest to be used as nano-transporters of molecules of low solubility or stability and are characterized as biocompatible and biodegradable. Moreover, employing the same whey proteins self-aggregated into fibrillar nano-structures (Nfs), we proposed to synthesize protein films with various biotechnological applications. Here we have applied different protocols (with and without SDS, Urea, β -Mercapto Ethanol as a reducing agent, different incubation times (IT) at 85°C, desalting), and obtained Nps with WPC desalted in the absence of SDS and 1 hour IT. The NPs observed in TEM images were round and the diameters ranged between 20 and 150 nm, and the median was 44 nm. Nfs were obtained with desalted WPC in the presence of 1% SDS and 15 minutes IT. TEM images showed that the Nfs were mainly a supercoiled structure of 50 nm of thickness and 0.8 mm length approximately. Further characterization of the nanostructures was performed by analytical centrifugation, dynamic light scattering and N₂ BET isotherms. This information will be usefull for the setup of drug adsorption/desorption experiments in a next research step.

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Azido ruthenium, a Ca²⁺-like photoactivatable reagent used in biophysical chemistry teaching

Ontiveros M^a, Saffioti NA^a, Rinaldi D^a, Delfino JM^a, Rossi JP^a, Mangialavori I^a, Ferreira-Gomes M^a

a - Instituto de Química y Físicoquímica Biológicas y Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires-CONICET

Photoaffinity labeling enables to covalently bind ligands to proteins to determine their relative spatial arrangement. Analogues of natural ligands illuminate structural features of binding sites. A photoactivatable probe implies the incorporation of two important functionalities: (i) a unit that imparts specificity, responsible for the reversible binding to the target protein, and (ii) a photo reactive functional group, allowing photo inducible permanent binding. We describe here a laboratory exercise to straightforwardly demonstrate the light dependent binding of the photo probe azido ruthenium (AzRu) for identifying the Ca²⁺ binding sites in a protein. Ru²⁺ mimics Ca²⁺, the specific unit, and azide is the photoreactive moiety. Hence, AzRu binds specifically and covalently to Ca²⁺ binding proteins after exposure to ultraviolet radiation at 290 nm.

AzRu was assayed with the sarco/endoplasmic reticulum Ca²⁺-ATPase (SERCA) and the plasma membrane Ca²⁺-ATPase (PMCA). Both pumps use the hydrolysis of ATP as the energy source to drive Ca²⁺ from the cytoplasm to the extracellular medium or the SR/ER lumen, respectively. The effect of AzRu on Ca²⁺-ATPase activity was evaluated, using a low-cost colorimetric assay. Each enzyme was incubated for 15 min with AzRu at different concentrations with or without UV irradiation. Afterwards, both non-irradiated and UV-irradiated samples were appropriately diluted for the enzyme assay. Both pumps show inhibition by AzRu only when UV-irradiation takes place. In this way, using an easy, low-cost method students are able to carry out the key experiment demonstrating the causative link between photoactivation and irreversible binding of the probe to the enzyme.

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How to distinguish ligand binding mechanisms: an example of conformational selection disguised as an induced fit

Faraj SE^a, Rossi RC^a, Montes MR^a

a - Instituto de Química y Fisicoquímica Biológicas y Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires-CONICET

Ligand-binding and the effect of small molecules on enzyme activity are topics of increasing interest in life sciences curricula since they are critical for drug rational design and the development of new therapeutic agents. In a regular undergraduate course of chemical biology, when addressing enzyme kinetics, the two limiting cases of induced-fit and conformational selection mechanisms are usually presented to students. In the first case, ligand binding occurs before the conformational change takes place, whereas in the latter the conformational change happens before ligand binding. For the sake of simplicity, most of the work done on this subject assumes rapid-equilibrium for ligand binding without further evidence.

We developed a laboratory exercise that demonstrates that assuming rapid-equilibrium may result in a wrong characterization of a ligand-binding mechanism as an induced fit. Students measure eosin fluorescence change induced by the E1 → E2 conformational transition of the Na,K-ATPase upon addition of Pi or BeF₃⁻. Students observe that Pi and BeF₃⁻ produce opposite behaving dependences of the observed rate constants (k_{obs}) with ligand concentration. Assuming rapid equilibrium for the binding of both ligands, these results suggest a conformational selection mechanism for Pi and an induced-fit mechanism for the binding of BeF₃⁻. However, if no rapid equilibrium assumptions are invoked, both processes may be explained by a conformational selection mechanism.

This experiment serves as the basis for discussing the consequence of not being extremely cautious when invoking approximations about not-very-well-known systems and the importance of deeply understanding chemical models used to classify processes.

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Membrane proteins and their lipid environment: an example of a pedagogical strategy to promote interdisciplinary teaching and learning in biophysics

Pignataro MF^a, de Sautu M^a, Rossi JP^a, Delfino JM^a, Ferreira-Gomes M^a, Mangialavori I^a

a - Instituto de Química y Físicoquímica Biológicas y Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires-CONICET

*Both authors contributed equally to this work.

One of the most important challenges in the teaching and learning processes is to be able to relate new contents with previous ones so that students can reaffirm and reshape the acquired knowledge. To work out this strategy, teachers and students often resort to similar prior knowledge, offering the new content as an extension or modification of what has already been incorporated (a so-called "backwards and forwards" B&F strategy). In this same line, what could be considered a "horizontal" H strategy refers to the connection of the contents among different areas of knowledge to favor a broader learning. A clear example is the teaching of biophysics of biological membranes and enzyme kinetics at undergraduate and graduate levels. The contents of both topics use, reformulate and expand the knowledge acquired previously (B&F strategy), but very rarely employ an H strategy, an instance when students can use new contents to reformulate knowledge involving other areas.

Therefore, considering the existing link among sub-disciplines in biophysics and the fundamental need in professional training to find relationships aiming at creating critical and comprehensive knowledge, we propose a laboratory exercise where graduate students evaluate the effect of different lipid environments on the activity of the plasma membrane calcium pump (PMCA). The main goals are: (1) the development of an experimental design fit to reconstituted PMCA in different amphiphilic compositions and measure the enzyme activity in these environments and, (2) the analysis and discussion of results within the framework of the chosen experimental design.

This is an extension of a topic that we have been imparting for 10 years in the course "Methods for the study of protein conformation and interactions: MECPI" (School of Pharmacy and Biochemistry-UBA) with optimal results tested through several surveys to the students at the end of the course.

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Teaching the use of analytical size exclusion chromatography and light scattering for size determination of challenging proteins

Valsecchi WM^a, Santos J^a, Delfino JM^a, Faraj SE^a

a - Departamento de Química Biológica e IQUIFIB (UBA-CONICET), Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires

Analytical size exclusion chromatography (SEC) is a simple yet powerful and widespread method for mass and size estimation of proteins, commonly taught in most biological chemistry undergraduate and graduate courses. However, in an advanced approach to this topic, students are immediately confronted with the fact that SEC results may not accurately describe the molecule under study given that this technique relies on the calibration with reference molecules and assumes certain approximations and ideal conditions—especially protein conformation and absence of interactions with the matrix. Static and dynamic light scattering (SLS and DLS) are two alternative methods that allow, respectively, the absolute determination of the molecular weight and the diffusional coefficient, basically on the basis of the interaction of the molecule with light.

In this practical, students make use of SEC, SLS and DLS to determine the molecular weights and hydrodynamic radii of a set of three proteins: bovine serum albumin (BSA), Hypoxanthine phosphoribosyltransferase (HPRT) from *Trypanosoma cruzi* and human Frataxin (FXN), and analyse the results to make judgments based on the criteria generated during the lecture. These proteins provide a challenging scenario: BSA presents multiple aggregation states, HPRT is found to be a monomer by SEC, but a tetramer by SLS, and FXN is a negatively-charged protein that experience some electrostatic repulsion with the gel matrix.

This activity promotes a further understanding of SEC, SLS and DLS, and improves students' skills of constructing meaning from data. The assessment of the implementation showed that students managed to process SEC and SLS data, and gave an explanation to their observations, gaining a deeper awareness of the pros and cons of each technique. As a result, by the end of the activity students were able to correctly select the most appropriate means for the characterization of a sample of a given size, concentration and complexity.

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Surface and semiconductor properties of Langmuir monolayers made of bio-inspired tyrosine rich amphiphilic peptides

Bolaño Alvarez A^a, Pino M^a, Petersen S^b, Fidelio GD^a

a - CIQUIBIC, Departamento de Química Biológica, Facultad de Ciencias Químicas, CONICET, Universidad Nacional de Córdoba, Córdoba, Argentina.

b - Department of Health Science and Technology, Faculty of Medicine, Aalborg University.

Langmuir monolayer at air-water interface allows two-dimensional nano-scale organization of amphiphilic molecules at very high molecular density. We present the surface properties of two peptides with the same amphiphilicity but with reversal isomerism: PF3 and PF4 with the sequence Ac-KKGALLLLGYYY-NH₂ and Ac-YYYGLLLLAGKK-NH₂, respectively. Both have a central hydrophobic region (Leu)₅ flanked by a hydrophilic domain (Lys)₂ and a (Tyr)₃ rich domain. PF3 has the hydrophilic domain at the N-terminal and PF4 at the C-terminal. Both peptide isomers form insoluble monolayers with reproducible Pi-A isotherms with similar molecular areas compatible with a perpendicular organization at the interface, high lateral stability and differing in their surface potential due to its intrinsic isomerism. Both peptides are UV light sensitive. When a close packed film formed by either PF3 or PF4 is illuminated with a 265 nm UV light source, the Tyr rich domain laterally forms intermolecular covalent formation compatible with di-Tyr formation. This di-Tyr like formation at the interface was detected by fluorescence spectroscopy with the typical emission between 410-420 nm. We have also developed an electronic device to measure lateral conductivity in Langmuir monolayers. PF3 and PF4 have a particular profile of conductivity upon illumination when compared with non Tyr containing peptide. We observed a greater conductivity for PF4 compared with PF3 attributable to the different dipolar organization for PF4 at the interfacial region. By using Brewster Angle Microscopy, we have observed heterogeneity on the film upon illumination with appreciable irregularity in the values of the reflectivity for both peptides attributable to cross-linked di-(poly)-Tyr domain. However, PF3 seems to be more heterogeneous than PF4. We demonstrate that Tyr-rich peptide films may behave as bio-semiconductors changing conductivity depending on illumination and disposition of the peptide amide backbone.

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

Cababie LA^a, González-Flecha FL^a, Incicco JJ^a, Mikkelsen E^a, Gebhard LG^b, Gamarnik AV^b

a - Instituto de Química y Físicoquímica Biológicas y Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires-CONICET

b - Fundación Instituto Leloir-CONICET

Dengue virus (DENV) NS3 protein is a helicase that catalyzes the hydrolysis of ATP and couples the free energy of this reaction to the translocation on single strands and to unwind double stranded RNA. Using a fluorescent 10 base-RNA oligonucleotide (F-p-R₁₀), we performed spectroscopic titration experiments between NS3 and RNA in the presence of different concentrations of monovalent cations at different temperatures. From the temperature dependence of the observed equilibrium association constant, we obtained the enthalpic and entropic contributions to the Gibbs free energy change due to the salt effect on the equilibrium between NS3 and ssRNA. Our results indicate that the association of NS3 to F-p-R₁₀ is enthalpically driven under all experimental conditions tested and the effect of salt concentration on $\Delta_r G_{\text{obs}}^{\circ}$, resides almost entirely in its entropic term $T \cdot \Delta_r S_{\text{obs}}^{\circ}$, which decreased linearly with $\log[K^+]$.

On the other hand, we studied the effect of various ligands participating in the catalytic cycle of ATP hydrolysis on the NS3-RNA interaction. Isothermal titration calorimetry was used to determine the characteristic thermodynamic parameters of the binding between NS3h and RNA in presence of ADP, and phosphate. Our results indicate that the observed association constant for the binding between NS3h and p-R₁₀ (the counterpart unlabeled of F-p-R₁₀) is lower in the presence than in the absence of ADP; decreasing ~3 orders of magnitude. Additionally, in the presence of phosphate the observed association constant also decreases although to a lesser extent than when ADP is present.

Towards novel positron emission tomography probes for the detection of α -synucleinopathies

Machin MB^a, Sandobal J^b, Messina G^b, Casale G^b, Ferrario J^c, Bastianello M^d, Varela O^e, Avila CL^a, Chehín R^a

a - IMMCA (Instituto de Investigación en Medicina Molecular y Celular Aplicada)

b - Laboratorio Bacon SA

c - Instituto de Investigaciones Farmacológicas (ININFA) / CONICET - UBA

d - Hospital Universitario CEMIC

e - Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales (FCEN)

The results of several studies have shown that the evolution and prognosis of Parkinson's disease (PD) correlates with α -synuclein aggregation in brain. This information indicates that to monitor progression of the patient with PD by positron emission tomography (PET), a specific *in vivo* neuroimaging marker would be needed to label the α -synuclein fibers. However, nowadays, no PET probes are available for selective detection of pathological α -synuclein in this disorder.

The aim of this study was to evaluate novel chemical entities that could have high affinity and selectivity for AS aggregates and which could serve as compounds for PET radiotracers development. We have previously reported that doxycycline can bind α -synuclein aggregates but not monomeric α -synuclein. For this reason, we propose the doxycycline and novel synthetic analogues as candidates for α -synuclein imaging PET radiotracers.

The binding affinity of these compounds to α -synuclein fibrils was determined by competition binding assays using thioflavin T dye. K_i values were calculated from EC_{50} values using the equation $K_i = EC_{50} / (1 + [radioligand] / K_d)$. Equations were fitted to the experimental data by nonlinear regression. The data described here could provide valuable information for the design of new doxycycline probes which bind preferentially to α -synuclein fibrils. *In vitro* binding assays demonstrated that these compounds have affinity for insoluble AS filaments. These results suggest that doxycycline and their analogues are promising candidates as PET radiotracers for *in vivo* detection in PD patients.

Noninvasive monitoring of α -synuclein protein aggregates in the living brain will provide information regarding α -synuclein pathophysiology. This information will be potentially useful in the study of the early diagnosis and prognosis of patients with PD.

Central role of electrostatics in mediating the interactions of Bax with mitochondrial outer membrane at the beginning of apoptosis.

Jaure O^a, Zamarreño F^a, Obiol D^a, Amundarain MJ^a, Viso J^a, Costabel M^a
a - Grupo de Biofísica. Departamento de Física, Universidad Nacional del Sur (UNS), IFISUR (UNSCONICET), Bahía Blanca, Argentina.

The binding of proteins to the membrane surface plays a critical role in a wide variety of biological processes. It has been observed that the membrane association of many proteins is mediated by non-specific electrostatic interactions between a group of basic residues in the protein and acid phospholipids in the membrane, thus determining its activity and regulation. This is the case of the Bax protein, a pro-apoptotic member of the Bcl-2 family of proteins that remains inactive until it receives a signal of cell death. The activation process consists of initial conformational changes, translocation to the mitochondrial outer membrane (MOM), insertion, oligomerization, pore formation and consequent release of apoptogenic factors.

Free Electrostatic Energy of Binding, was computed using Finite Difference Poisson Boltzmann Equation (FDPB) method as implemented in software APBS (Adaptive Poisson Boltzmann Solver). This type of calculations provided a starting point for further computational analysis through molecular dynamics simulations (MD). To this end, we used GROMACS simulation package.

The way in which the charge is distributed over the surface of Bax may have profound implications for the translocation to MOM at the beginning of the apoptotic process. For this reason we carried out an exhaustive analysis of the equipotential surfaces of Bax facing membranes with an increasing content of anionic lipids. In addition, we observed that the region responsible for the electrostatic association between Bax and membrane is relatively flat. This physical characteristic makes it possible to differentiate between two possible states that Bax might adopt when approaching the membrane: the peripheral union of Bax to the membrane in an inactive state, and Bax oriented for the correct insertion in MOM. In the present work, we demonstrated the influence of non-specific electrostatic interactions in the first approach between Bax-membrane.

CMT-3 inhibits α -synuclein aggregation and remodels its mature fibrils: a possible ready to use neuroprotector

González Lizarraga MF^a, Sequeira S^a, Avila CL^a, Ploper D^a, Socías B^a, Barbosa L^b, Itri R^b, Raisman-Vozari R^c, Pietrasanta LI^d, Chehín R^a

a - IMMCA (Instituto de Investigación en Medicina Molecular y Celular Aplicada)

b - IFUSP, São Paulo, Brazil

c - ICM - Institut du Cerveau et de la Moelle Epinière - Hôpital Pitié Salpêtrière

d - IFIBA - INSTITUTO DE FISICA DE BUENOS AIRES

Parkinson's disease (PD) is an aging-related neurodegenerative disorder characterized by the aggregation of α -synuclein in dopaminergic neurons. Considering projections of the world's future population, the number of people with PD will reach around 14 million in 2040 and threatens to breakdown healthcare systems worldwide. Unfortunately, drug development programs need 10 to 17 years from bench to bed. Approaches such as drug repurposing and repositioning save time by using pre-existing and approved drugs for new indications. In this way, we demonstrated that doxycycline is able to reshape alpha-synuclein oligomeric species reducing their toxicity, seeding capacity and propensity to form fibrillar species. However, the antibiotic activity of doxycycline represents a hurdle for its repositioning in long-term treatments. Then, we searched for molecules that preserve the antiaggregant, anti-neuroinflammatory and antioxidant properties of doxycycline but with reduced or null antibiotic effect to be studied as candidates for repositioning.

According to our results, CMT3, a chemically modified tetracycline, has exceptional characteristics to interact and reshape/disrupt cross-beta structure. By using a combination of biophysical techniques, we demonstrated that this molecule inhibits alpha-synuclein aggregation and remodels mature fibrils. Moreover, it has striking physicochemical properties to cross the brain blood barrier and is more efficient than doxycycline inhibiting neuroinflammation. This property, together with its antioxidant, antiaggregant, and anti-inflammatory effects, renders CMT-3 as an ideal drug to be repurposed and enter clinical trials for PD and other synucleopathies.

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

Rosu S^a, Toledo L^b, Urbano B^b, Sanchez Donoso S^b, Calabrese G^c, Tricerri MA^a

a - INIBIOLP (Fac. de Cs. Médicas, UNLP-CONICET)

b - Departamento de Polímeros. Facultad de Ciencias Químicas. Universidad de Concepción, Concepción, Chile.

c - Facultad de Farmacia y Bioquímica- Cátedra de Biología Celular y Molecular

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

Human apolipoprotein A-I (apoA-I Wt) under physiological conditions does not bind GAGs, but our results interestingly show that ApoA-I Arg173Pro (a natural mutant involved in cardiac amyloidosis) forms heparin/protein complexes at pH 7.4 with higher efficiency than the Wt¹. These results indicate that electrostatic interactions could play a key role in the interaction of apo A-I with the extracellular matrix (ECM). In order to further study the specific role of the matrix's charge on the interaction of apoA-I with GAGs, we synthesized polymers having different ratios of sulfated or hydroxylated monomers (sodium 4-styrene sulfonate (SSNa) and 2-hydroxyethyl methacrylate (HEMA)) and studied the binding of apoA-I Wt or Arg173Pro, both fluorescently labeled.

Our results indicate that both proteins are highly retained as long as the negative charge increases, and in addition it was shown that the mutant is more retained than the Wt, indicating that the retention of specific proteins in the ECM could be part of the pathogenicity.

We concluded that charge and chemical composition of the ECM could mediate apoA-I binding to GAGs and as a consequence alter the delicate equilibrium of protein and function.

¹ Rosu, S.A., Rimoldi, O.J., Prieto, E.D. et al. PLoS One 2015; 10: e0124946.

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The involvement of CHICUP dityrosine cross-linking in tau pathologic assembly: impliceance in Alzheimer's disease.

Sequeira S^a, Chehín R^a, Avila CL^a

a - IMMCA (Instituto de Investigación en Medicina Molecular y Celular Aplicada)

Alzheimer's disease (AD) is an age-related neurodegenerative disorder associated with amyloid aggregation of tau protein in neurofibrillary tangles (NFTs) in human brain. The causes of tau aggregation have not yet been clarified, but the oxidative stress and metal rich environments have been implicated in triggering the toxic assembly. Besides the extensive damage on protein, lipid and nucleic acids reported under oxidative stress, it has also been implicated in dityrosine cross-linking formation in Amyloid- β (A β) through a metal catalyzed oxidation (MCO) process. Moreover, recent studies revealed the presence of these dityrosine crosslinks in *ex-vivo* Lewy's body from brain tissue in Parkinson disease (PD) patients. Similar α -synuclein aggregates were obtained *in vitro* via MCO in the presence of Cu²⁺ and hydrogen peroxide. This reaction is specifically called CHICUP (Copper and Hydrogen Peroxide Induced Cross-Linking of Unmodified Proteins) and its physiological relevance was proposed due to its brain-occurring chemistry.

Despite the old relationship between impaired Cu²⁺ homeostasis and AD, its role in the ethiopathology remains unraveled. Surprisingly, there are not extensive studies on the MCO effect on tau except for the binding of Cu²⁺ to a 198 aminoacid fragment of the protein inducing a limited amount of aggregation, measured by dynamic light scattering (DLS). In this context, the main purpose of this study was to go in depth in how Cu²⁺ could be involved in tau aggregation and whether oxidative stress promotes the environment for the MCO. For this purpose, we have studied the effect of Cu²⁺ in the presence of hydrogen peroxide on tau by monitoring the formation of dityrosine cross-links through biophysical techniques. The results obtained herein allows us to get a better understanding on how Cu²⁺ and hydrogen peroxide, trigger dityrosine cross-linked tau aggregates which may behave as seeds in tau NFT formation and AD development.

HeLa cells spheroids as drug diffusion and light dosimetry model in photodynamic therapy

Etcheverry ME^a, Pasquale MA^a, Ponzinibbio C^b, Garavaglia M^c

a - Instituto de Investigaciones Físicoquímicas Teóricas y Aplicadas (INIFTA) (CCT CONICET La Plata, UNLP y CIC)

b - Facultad de Ciencias Médicas, Cátedra de Patología B, Universidad Nacional de La Plata

c - Centro de Investigaciones Ópticas (CCT-CONICET La Plata, UNLP and CIC-BA), Gonnet, La Plata, Argentina)

The outcome of the photodynamic therapy (PDT) depends on several factors such as genotype of cells, the nature of cell death, the environmental conditions, and PDT dosimetry, i.e., light intensity and photosensitizer concentration and its distribution at the affected tissue.

In this contribution, we have carried out experiments on three dimensional HeLa cell spheroids (approximately 500 μm in size) to study the effect of dosimetry of the treatment. Meta-tetrahydroxy-phenyl chlorine (m-THCP) was employed as photosensitizer and the illumination was performed employing LED sources of 395 nm and 660 nm with an exposure time was in the range 4 - 12 minutes, and the drug concentration from 0.1 to 2.0 $\mu\text{g}/\text{ml}$. For asymmetric cell aggregates, light was directed from different angles. Light penetration was measured placing the spheroids between two coverslips and the transmitted light detected by a spectrometer. The outcome of the treatment was analyzed by confocal fluorescence microscopy after cell staining with vital staining. Intensity of acridine orange was evaluation from images to evaluate cell death. To assess the spheroid dynamics after illumination, cell death at different planes of the spheroid comparing, was compared at different times post treatment (3h, 12 h and 24 h) employing groups of spheroids illuminated with either 630 nm or 395 nm light, maintaining the fluence rate constant at 12,3 $\mu\text{W}/\text{cm}^2$.

Results indicate that the amount of death decreases in going from the surface of the spheroid closer to the light source to the lower planes. The fraction of dead cells follows a quasi-exponential relationship that correlates with light penetration and photosensitizer diffusion. Similar results have been reported in literature. This preliminary study indicates that the 395 nm LED light was more efficient than the 660 nm in agreement with 2D HeLa cells assays. Cell spheroids appear to be useful to obtain information about the dosimetry during PDT treatment.

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Single image deconvolution with super-resolution using the SUPPOSE algorithm

Toscani M^a, Martínez S^b, Martínez O^a

a - Laboratorio de Fotónica, Departamento de Física FIUBA - CONICET

b - IMAS, Departamento de Matemática FCEyN UBA - CONICET

We present the results of super-resolution deconvolution of fluorescent intracellular images using the SUPPOSE algorithm. The image is acquired using a standard fluorescence microscope and a CMOs low noise high dynamic range camera. The algorithm relies in assuming that the image source can be described by an incoherent superposition of point sources and a precise measurement of the microscope point spread function (PSF). The deconvolution problem is converted into finding the number of sources and the position of the sources. The retrieval is performed using a genetic algorithm that finds an adequate fit to the measured signal. The method is particularly fit for sparse data, such as encountered in typical intracellular experiments, where only a minor fraction of the explored volume has fluorescent markers. The technique retrieves by construction only positive values for the spatial density, avoiding the need for nonlinear constraints found in prior deconvolution techniques such as Tikhonov-Miller and Richardson-Lucy. The method automatically subtracts the background from the image. Based on the measured point spread function with the information of the quality of the fit and the information on the noise figure of the camera as a function of the read signal, the method provides a predictor of the uncertainty in the reconstruction both in lateral resolution and amplitude. A fivefold increase in resolution is shown both by inverting a synthesized artificial image and using known beads clusters. The algorithm was applied to reconstructing images from bovine pulmonary artery endothelial cells with fluorescent labels for the F-actin, microtubules and mitochondria. The algorithm is used for the reconstruction requires the precise measurements of the PSF and the noise figure of the camera. It can be applied to reconstruct the image with super-resolution down to $\lambda/10$ and also to increase the resolution using a low magnification for wide field objective.

Spectroscopic and physicochemical characterization of PAMAM dendrimers generation 4.0 and 4.5 as pH-function

Ybarra DE^a, Igartúa DE^a, Tuttolomondo ME^b, Alonso S^a, Alvira FC^a

a - Laboratorio de Biomembranas (LBM), GBEyB, UNQ-IMBICE-CONICET

b - INQUINOA (CONICET-UNT) Instituto de Química del Noroeste Argentino, Ayacucho 471, San Miguel de Tucumán, CP 4000, Tucumán, Argentina

The aim of this work is the physicochemical characterization of polyamidoamine (PAMAM) dendrimers (D) of generation (G) 4.0 and 4.5, as a function of the pH. PAMAM D are polymers with a branched structure chemically synthesized from a core of ethylenediamine with progressive addition of polyamidoamine groups. These nanoparticles can have complete G (as DG4.0), with a primary amine as terminal groups; or intermediate G (as DG4.5), with carboxylic acid as terminal groups. Both D have interior pockets with amide and tertiary amines. The internal and terminal ionizable groups of the D would change their charge in function of the physicochemical environment. The fluorescence emitted by PAMAM D is currently a matter of research, since they do not present traditional fluorophores. It is known that they have two fluorophores. Only one of these fluorophores is identified, while the second is the subject of controversy nowadays.

Raman, UV-Vis and fluorescence spectroscopies were used to study the changes that pH causes in the PAMAM D. Proton binding constants (pK_a) were determined for DG4.0 and DG4.5 by potentiometric titration. Raman spectra showed signals of tertiary amines and carbonyl of the carboxylic acid and amide bond. On the other hand, transitions corresponding to amides and tertiary amines are found on UV-Vis spectrum. Tertiary amines absorb with different intensity according to the pH condition. The detected fluorescence emission reinforces the idea that the main fluorophore in PAMAM D are the inner tertiary amine. However, we found an unusual behavior of the DG4.0 fluorescence with the pH, while the behavior of fluorescence of DG4.5 are shown for the first time. These results could be helpful to understand the structural behavior of PAMAM D according to its environment and to predict its possible interactions with organic molecules of biomedical interest.

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Unraveling" the cytoskeleton: structure and biological role in stem cells using fluorescence correlation techniques

Romero JJ^a, Asplanato L^b, Guberman A^a, Bruno L^b, Levi V^a

a - Departamento de Química Biológica, FCEyN, UBA - IQUBICEN, CONICET

b - Departamento de Física, FCEyN, UBA - IFIBA, CONICET

Cytoskeleton is a complex network of interconnected polymer filaments, fundamental for cellular mechanics. It is suggested that its active forces can regulate the organization and dynamics of chromatin, and indirectly gene expression. Our goal is to understand how these networks respond and transmit mechanical signals to the cell nucleus during the differentiation process. So, we studied the organization and role of the cytoskeleton in mouse embryonic stem cells (mESCs), both in undifferentiated state and during differentiation, using a non specific differentiation protocol.

We cotransfected W4 mESC with actin, α -tubulin or vimentin expression vectors fused to GFP, together with H2B-mCherry to label the chromatin. We obtained confocal images and 3D reconstructions to understand the organization of the different networks, and combining experiments of

fluorescence recovery after photobleaching and fluorescence correlation spectroscopy (FCS) we studied its dynamics. In contrast to what is usually observed for adherent cells and differentiated to defined lineages, which present clearly structured networks, in undifferentiated mESCs microtubules do not form a defined network, affecting the viscoelastic properties of the cytoplasm.

At the same time, we selectively depolymerize different filaments of the network, in order to study the effect on cellular architecture and particularly nuclear positioning. We observe that during the differentiation process, networks begin to mature and define, giving rise to changes in cytoskeleton structure, function of each family of filaments and in the viscoelasticity of the cytoplasm.

On the other hand, we complemented these results with FCS experiments using EGFP tandems as diffusional tracers in the cytoplasm. We analyze the obtained diffusional time constants and anomalous index, to corroborate the structure of the network and characterize the rheological properties of the cytoplasm in both differentiated and undifferentiated state.

***In vivo* systems to study class II bacteriocins toxicity and immunity**

Ríos Colombo NS^a, Chalón MC^a, Galván AE^a, Navarro SA^a, Lanza L^a, Fernandez de Ullivarri M^a, Barraza DE^a, Acuña L^b, Minahk CJ^a, Bellomio A^a

a - INSIBIO (CONICET-UNT) Instituto Superior de Investigaciones Biológicas, Chacabuco 461 - San Miguel de Tucumán, CP 4000, Tucuman, Argentina

b - IPE Instituto de Patología Experimental

Class II bacteriocins are membrane-active peptides that act over a narrow spectrum of bacterial targets and have a great potential application as antibiotics in medical sciences. They act on the cytoplasmic membrane dissipating the transmembrane potential by forming pores. There is solid evidence that membrane receptor proteins are necessary for their function, however the precise role of this receptor and the nature of the pore remain elusive. The most accepted model suggest that bacteriocins bind the receptor to change its conformation, creating a channel that remains open. Nonetheless, several studies support a second model in which the bacteriocin is able to disrupt the membrane itself and the receptor might act just as an anchor allowing the subsequent bacteriocin insertion to form the pore. In order to reveal whether or not the pore structure involves the specific receptor, we designed chimeric peptides fusing the membrane protein EtpM with different class II bacteriocins. We chose *E. coli* as a receptor-free expression host. The fusion EtpM-bacteriocin anchors each bacteriocin to the membrane and kills the expressing host cell, even in the absence of the specific receptor. These results are in line with the second model in which the pore is formed through a receptor-independent interaction with the lipid bilayer. The effect of these interactions was also analyzed, through a fluorophore that changes its fluorescence intensity according to transmembrane potential.

On the other hand, an immunity protein protects the producer strain against its own bacteriocin. For antimicrobials under investigation for clinical applications, the potential emergence of resistant pathogens and the study of immune mechanisms are a primary concern. Though no direct *in vitro* interaction bacteriocin-immunity has been reported before, by using an *in vivo* system, we present evidence that this binding might occur, not in aqueous solution but in a membrane inserted conformation.

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Raft-like model membranes for biomolecular interaction studies

Daza Millone MA^a, Vázquez R^b, Maté S^b, Vela ME^a

a - Instituto de Investigaciones Fisicoquímicas Teóricas y Aplicadas (INIFTA), CCT- La Plata, CONICET. Universidad Nacional de La Plata. Sucursal 4 Casilla de Correo 16, La Plata (1900) Argentina.

b - Instituto de Investigaciones Bioquímicas de La Plata (INIBIOLP), CCT- La Plata, CONICET. Facultad de Ciencias Médicas. Universidad Nacional de La Plata. 60 y 120, La Plata (1900) Argentina.

Biomimetic membrane models are promising platforms to complement in vitro cell-screening assays in the analysis of biochemical and biophysical interactions. The design and study of these platforms is not only important regarding cell biology research but also from advanced applications in pharmaceutical industry. In particular, the presence of segregated domains or “lipid raft-like domains”, in the membrane interface plays a critical role in studies of biomolecular interactions¹. Among the new techniques employed for these studies, Surface Plasmon Resonance (SPR) provides a label-free analytical approach that allows high-throughput screening of the structural and compositional factors that mediate the binding of bioactive molecules to the membrane.

Here, we present results concerning the preparation of a supported lipid bilayer (SLB) on a SPR chip from vesicles of a ternary lipid mixture (DOPC/SM 16:0/Cho, 2:1:1 molar ratio). This mixture was previously characterized on mica surfaces² and exhibits phase coexistence, i.e. a liquid-ordered (L_o) phase enriched in sphingomyelin (SM) and cholesterol (Cho) which is segregated from the liquid-disordered (L_d) phase composed mainly of DOPC. We demonstrate that these domains have similar nanomechanical properties on DTT-Au surfaces by means of Force spectroscopy (FS). Finally, we evaluate the biomimetic properties of the SLB employing a Cho-extracting drug. The results show that the percentage of Cho release bears close resemblance to the one reported for erythrocytes and the kinetics of the depletion followed a biexponential model, consistent with two pools of Cho³, i.e. the presence of Cho in segregated domains.

Contact: dazamillone@inifta.unlp.edu.ar

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Structural protein-protein interaction study of complexes involved in the regulation of *A. thaliana* growth by Double Electron-Electron Resonance

Miotto MC^a, Crespo R^a, Rasia RM^{b,a}, Tabares LC^c

a - Institute of Molecular and Cellular Biology of Rosario, Ocampo y Esmeralda, Rosario, Argentina.

b - Department of Biological Chemistry, School of Biochemistry and Pharmacy, University of Rosario, Suipacha 531, Rosario, Argentina

c - Institute for Integrative Biology of the Cell (I2BC), Department of Biochemistry, Biophysics and Structural Biology, Université Paris-Saclay, CEA, CNRS UMR 9198, Gif-sur-Yvette, F-91198, France

Nanometric distances determination by Double Electron-Electron Resonance (DEER or PELDOR) has become an important tool in structural biology. DEER measures the distance between two paramagnetic centers by determining the strength of their magnetic interaction. These centers are in general introduced by "side-directed spin labeling" in which an EPR active specie, most commonly a nitroxide or a Gd(III) chelator, is selectively attached to the molecule of interest via a cysteine residue. In this way the pairwise distances between such spin-labels can be used as distance constrain to build a structural model.

Plant development is a finely regulated process. The Growth-Regulating Factors (GRF)/GRF-Interacting Factors (GIF) system is formed by two families of activators and transcriptional coactivators. A range of studies have demonstrated the function of the combined action of these proteins on plant development, but there is no biophysical evidence of such protein-protein interaction so far. GRFs/GIFs interaction is mediated by their QLQ and SNH domains. Molecular modeling shows that SNH form a helix-loop-helix structure while QLQ form a single helix. We have used DEER to characterize the structure of the complexes of GIF1 with its partners GRF1 and BRM. To this end the proteins were spin-labeled at selected positions and DEER experiments were performed for the single proteins as well as on the complexes. DEER results showed that both GRF1 and BRM exist in a monomer/homodimer equilibrium in solution. DEER experiments on the GIF1/GRF1 complex labeled at different positions showed that these proteins bind in a 1:1 complex forming a three-helix bundle with the C-terminal of GRF I pointing towards the GIF I loop. Studies on the GIF I/BRM complex suggest that GIF1/BRM is a 1:2 complex. This study represents the first structural information available on the interaction of GIF1 with its partners and highlight the usefulness of DEER as a structural tool.

Contact: leandro.tabares@cea.fr

Dynamics of Oct4, Nanog and HP1 in embryonic stem cells

Oses C^a, Cosentino S^a, Alvarez Y^a, Guberman A^a, Levi V^a

a - Departamento de Química Biológica, FCEyN, UBA - IQUBICEN, CONICET

Transcription factors (TFs) dynamic interactions with DNA targets depend, among multiple parameters, on chromatin landscape. Despite TFs-chromatin interactions play a relevant role in the modulation of gene expression we still do not know how the complex temporal and spatial network of interactions defines transcriptional response in stem cells (SCs).

Embryonic SCs (ESC) studies are relevant for understanding embryo development and for their potential clinical applications. These cells have two important properties: an unlimited possibility of self-renewal and pluripotency, which depends on specific TFs such as Oct4, Sox2 and Nanog that repress genes involved in differentiation and induce genes that preserve an undifferentiated state.

In this work, we used fluorescence correlation spectroscopy (FCS) analysis to quantitatively explore the dynamical organization of TFs in the nucleus of ESC wild type (wt) and an ESC line knockout for a chromatin remodeler, the histone acetyltransferase Kat6B (Kat6B^{-/-}). Since histone acetylation usually makes a more permissive chromatin, we expected a different dynamical distribution of TFs and heterochromatin associated proteins between Kat6B^{-/-} and wt ESCs.

We transfected ESC wt and Kat6B^{-/-} cells with vectors encoding Oct4, Nanog or HP1 fused to enhanced green fluorescent protein (GFP), and measured fluorescence fluctuations as a function of time using confocal microscopy. We analyzed the FCS data with a model that considers fast and slow interactions with DNA targets. Results showed statistical significant differences between both cell lines for both TFs and HP1, which are consistent with a less permissive chromatin in Kat6B^{-/-} ESC line.

These results may provide important clues for understanding the transcriptional response. Furthermore, they show that FCS is a useful tool to evaluate how biological processes modulate TFs partition in the nucleus and could help us to understand early embryo development in future studies.

Quantitative imaging of nuclear proteins in embryonic stem cells

Vernerì P^a, Vazquez Echegaray C^a, Stortz M^a, Guberman A^a, Levi V^a

a - Departamento de Química Biológica, FCEyN, UBA - IQUBICEN, CONICET

Pluripotency of embryonic stem cells (ESC) depends on transcription factors (TFs) such as Oct4, Sox2 and Nanog, which induce genes necessary to preserve an undifferentiated state and repress genes related to differentiation.

Gene expression not only depends on the expression levels of TFs but also on dynamic changes in intracellular distribution that could affect TF-chromatin interactions.

The dynamical interactions of TFs and DNA targets play a fundamental role in different stages involved in gene expression, highly relevant in the control of cell differentiation and development.

Exploring the transcription machinery's dynamics in living cells is essential to understand how the dynamical organization of nucleus and specifically, the dynamics of chromatin-associated proteins and TFs influence the transcriptional output.

We used fluorescence correlation spectroscopy (FCS) to quantitatively explore the dynamical organization of TFs in the nucleus of ESC. We used stable ESC lines encoding Oct4 or Sox2 C-terminally fused to a yellow fluorescent protein. We focused our studies on the dynamics of Oct4 and Sox2 in undifferentiated and differentiated ESC. Besides, we transfected the cells with vectors encoding chromatin-associated proteins fused to mCherry and analyzed how chromatin remodel during differentiation.

Our results show that in the undifferentiated state, Oct4 is distributed homogeneously in the nucleus and analyzed FCS data fit with a model that considers fast and slow interactions with chromatin targets. In addition, when cells are submitted to a differentiation protocol, the process is accompanied by a repartitioning of Oct4 and Sox2 in distinguishable foci. We also found that this change in TFs dynamics is accompanied with changes in TFs-chromatin interaction times and chromatin remodeling. These studies may provide important clues for understanding how the fine modulation of TF-DNA interactions in the cell nucleus regulates the transcriptional response.

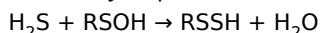
A quantum classical study of the reactivity hydrogen sulfide towards methylsulfenic acid

Semelak J^a, Zeida A^b, Foglia N^a, Trujillo M^b, Estrín DA^a

a - Facultad de Ciencias Exactas y Naturales, UBA

b - CEINBIO, FMed-UdelaR, Montevideo, Uruguay

Hydrogen sulfide (H₂S) has been identified as an endogenous signalling molecule and many physiological and pathophysiological processes have been linked to its activity. H₂S is not able to react with reduced thiols (RSH). However, H₂S is able to react with oxidized thiol derivatives. The reaction of H₂S with sulfenic acids (RSOH) shields *hydropersulfides* (RSSH):



Hydropersulfides are thiols (RSH) derivatives species that may play an important role in cellular redox regulation and possible other physiological functions related with signaling and catalysis.

Despite the growing attention of the bio-medical community in this novel species, the information reported in bibliography is still sparse and many questions about their reactivity, formation mechanisms and its relevance in biological systems have not been answered yet.

In this work, we present an exhaustive study of the reaction of H₂S toward al sulfenic acid model employing hybrid quantum mechanics-molecular mechanics (QM/MM) molecular dynamics (MD) simulations, using an umbrella sampling approach aiming to understand the whole processes in aqueous solution, in a realistic solvent representation at room temperature. This simulation scheme allowed us to obtain thermodynamical information such as the free energy profile in addition to microscopic insight about electronic structure changes throughout the reaction. In addition, we study the importance of including a water molecule as an explicit participant of the reaction exploring the Minimum Energy Pathway of the reaction in vacuo by means of Nudged Elastic Bands simulations.

The results reported herein suggest that the reaction occurs by a nucleophilic attack of HS⁻ to the S atom of the sulfenic acid and that the solvent role might not only be related with solvation of the species but protonation states rearrangement along the process.

Keywords: Hydrogen sulfide, Hydropersulfides, Reactive Oxygen Species, Oxidative Stress, Mechanism, QM/MM.

Collective flight: modelling collective turns in starling flocks

Grigera TS^a

a - Instituto de Física de Líquidos y Sistemas Biológicos (IFLYSIB), CONICET-UNLP; Departamento de Física - Universidad Nacional de La Plata

Many animal species form groups (swarms, flocks, schools) which move in a coordinated fashion without any individual taking the role of leader. The phenomenon of global order arising from simple local interactions rules is similar to that witnessed in equilibrium phase transitions with spontaneous symmetry breaking; accordingly flocking has been studied with statistical physics methods. In this spirit we discuss observations of starling flocks during a collective change of flight direction, which show that the information to change direction propagates with constant speed and negligible attenuation. This wavelike propagation of information contrasts with prevailing models of flocking behaviour, which predict a slower, dissipative (diffusion-like) propagation. We show how to build a relatively simple model that accounts for this observation. The model provides a quantitative expression for the speed of propagation of information, according to which transport is swifter the stronger the flock's orientational order. This prediction is verified by experimental data.

Construction of a structural model of bovine caltrin protein interacting with artificial membranes

Grasso EJ^a, Coronel CE^a

a - IIBYT (CONICET-UNC), Departamento de Química, Cátedra de Química Biológica, Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba

Bovine caltrin (calcium transport inhibitor) is a small and basic protein of the seminal plasma which binds to epididymal spermatozoa during ejaculation, and inhibits the sperm extracellular Ca^{2+} uptake. This inhibitory activity seems to play an important regulatory function, preventing the premature acrosomal exocytosis and the hyperactivated motility during the sperm journey along the female reproductive tract. The binding of bovine caltrin to specific regions of the spermatozoa, where Ca^{2+} influx may take place to trigger these two Ca-dependent physiological processes, suggests the existence of receptor molecules or precise protein-phospholipids arrangements in the sperm membrane. At present, the molecular mechanisms of recognition and interaction between caltrin and sperm membranes have not been elucidated. In this work we have built a theoretical model of the interaction between bovine caltrin and model membranes by using several bioinformatics tools. A hydrophobic 13-residues (PKLLETFLSKWIG) peptide with favorable thermodynamic properties to penetrate into biological membranes was identified in the protein primary structure. It may be responsible of caltrin-sperm binding to modulate the intracellular signaling of these two Ca-dependent physiological events.

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Demonstration of Substrate Specificity of Bile Salt hydrolase from *Lactobacillus reuteri* CRL 1098 Using Molecular Docking Analysis

Bustos AY^a, Taranto MP^b, **Ledesma A^a**

a - CIBAAL Centro de Investigación en Biofísica Aplicada y Alimentos CONICET UNSE

b - Centro de Referencia para Lactobacilos (CERELA-CONICET)

The microbial enzyme Bile Salt Hydrolase (BSH) releases free BA plus amino acid (taurine or glycine) from the Conjugated Bile Acids (BA). Currently, BSH inhibitors may become novel and attractive growth promoters. Detailed knowledge of BSH substrate preferences provide a solid foundation for rationally BSH inhibitor design.

The aim of this work was to characterize the substrate specificity of BSH from *Lactobacillus reuteri* CRL 1098 using molecular docking analysis and DFT calculations. The deconjugation of both taurocholate and glycocholate optimized from Gaussian Program, was performed using AutoDock 4.2 tool with a semiempirical free-energy force.

Our results showed that CRL 1098-BSH exhibited greater hydrolysis toward glycoconjugated BA compared to tauroconjugated ones. In fact, the molecular docking illustrated that both conjugated are embedded into binding pockets with the binding energy (BE) of -7.31 and -5.72 kcal/mol, respectively, indicating an energetically favorable binding for glycocholate as compared to taurocholate.

In addition, docking results indicate a polar or charged nature of the residues in the binding site in accordance with the five catalytically important amino acids residues (Cys, Asp, Asn, Asn, Arg) are highly conserved in CRL 1098 strain. Hydrogen bonding interaction can be observed between these residues within both taurocholate and glycocholate. The formation of less hydrogen bonds was observed in the interactions of taurocholate with BSH as compared to glycocholate, reflecting a lesser binding affinity between the ligand and receptor. All these correlated well with experimental data of glycocholate being more favorable to bind with BSH as compared to taurocholate.

In this work we provide the molecular basis for substrate recognition of BSH from the probiotic CRL 1098 strain. These data could be useful for development of safe and cost effective BSH inhibitors as a replacement of current antibiotic growth promoters.

Drug development for inhibition of glutamine synthase from *Mycobacterium tuberculosis*

Storni B^a, Issoglio F^a, Luque FJ^b, Estrín DA^a

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

b - Departamento de Nutrición, Ciencias de la Alimentación y Gastronomía. Facultad de Farmacia. Universidad de Barcelona

Glutamine synthase enzyme (GS) catalyzes the production of glutamine from glutamate and ammonium, using ATP and Mg²⁺ as cofactor. In mammals is related with the maintenance and correct functioning of the nervous system. In bacteria (i.e. *Mycobacterium tuberculosis* -*Mtb*-, the causing agent of "Tuberculosis" infection), fulfills a fundamental role in the biosynthesis of the cell wall, being essential for its proper growth.

Being this disease one of the most common among worldwide population (one third suffers it, according to the W.H.O.), GS is a study target for new developing drugs which may inhibit it, working as antibiotics against *Mtb*. Nevertheless, even though it is possible to carry on successful therapies, long-term treatments result in more resistant strains, even under the effect of multiple drugs.

With the ambition to find a functional drug against *Mtb*, a virtual screening (VS) method is used, which allows to find potentially appropriate substrates that may inhibit by competition the enzyme. A score is obtained from these results, suggesting how strong the enzyme-ligand union is (the lower the score, the stronger the union). On the other hand, a potentially capable inhibitor of GS in *Mtb*, must have a high score when the VS is performed over human GS, that is, a bad interaction.

Finally, from analysis of the results obtained with the VS, molecular dynamics simulations were carried out to study specific interactions between likely inhibitors and GS, for both human and bacterial GS.

Out of these molecular modeling tools derive preliminary conclusions, from which, later on, *in vivo* and *in vitro* experiments are designed to test the potential inhibitor's efficiency.

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Energetic frustration of proteins evolutionary adapted to extreme temperature conditions

Matias S^a, Iglesias Rando MR^a, Garrone N^a, Craig PO^a

a - Departamento de Química Biológica, FCEyN, UBA - IQUBICEN, CONICET

The energy landscape of natively folded proteins is minimally frustrated. This property obeys the necessity of proteins to fold and fulfill their functional role in biological times. The energy difference between native and non-native conformations, the "energy gap", determines the foldability of proteins. This energy gap is the product of evolutionary processes that selected sequences that best fit the structure, function and dynamics of each protein. The concept of minimal frustration assumes that the strength and consistency of native interactions between residues is much higher than non-native interactions. The probability of proteins to escape from non-native energy traps in the folding process depends on the stability of the local trap and the temperature of the environment. Therefore, it is possible that proteins adapted to fold and function in organisms that live at different temperature conditions, would tolerate different levels of frustration in their energy landscape. In this work we explore the global and local frustration of proteins from extremophile organisms. We evaluate parameters related to the foldability and conformational flexibility of these proteins. For this aim, we use protein homologs from psychrophilic, mesophilic and thermophilic organisms with structure solved at high resolution. We calculate the energetic frustration of these proteins with the 'frustratometer2.0' program that uses the coarse grained force field potential of the AWSEM program, designed for protein structure prediction. The results illustrate the challenges of this analysis and suggest possible explanations to the expected necessity of improving foldability and increasing flexibility of the native state at decreasing temperatures.

Exhaustive computational analysis of *Ascaris suum*'s NPA-ligand interaction. Searching for a gate in

Zamarreño F^a, Viso J^a, Amundarain MJ^a, Costabel M^a

a - Grupo de Biofísica. Departamento de Física, Universidad Nacional del Sur (UNS), IFISUR (UNSCONICET), Bahía Blanca, Argentina.

Nematode polyprotein allergens (NPAs) are atypical lipid-binding proteins. Due to the fact that these proteins have no counterparts in vertebrates and because helminthes parasites have a restricted lipid metabolism, and must acquire simple and complex lipids from their hosts, NPAs represent potential targets for drug design.

ABA-1A is the most common repeating unit of the NPA of *Ascaris suum*. In this work we aim at understanding the molecular basis of ABA-1-A - lipid interaction to evaluate them as potential anthelmintics drug targets.

Using an in-house developed software and APBS, the solutions of the Poisson-Boltzmann equation were used to calculate the electrostatic interaction energy between ABA-1-A and oleate for 3000 possible configurations. In order to select the most suitable ABA-1-A - oleate configurations, the difference between the electrostatic energy of a system compound by ABA-1-A and oleate, and the electrostatic energy calculated for both, ABA-1-A and oleate, individually were calculated for all relative positions. Lowest values were considered as best interactions.

A small group of minimum electrostatic energy for ABA-1-A - oleate relative position was selected, in order to use them as initial configurations for Molecular Dynamics (MD) study of ABA-1-A - oleate interaction.

Results from MD suggest that ABA1-A seems to have more than one putative gate for oleate entrance to the protein hydrophobic core, like other Lipid Binding Proteins, such as Rat LFABP. Additionally, we were able to identify residues with high interaction with oleate.

Interaction between different free fatty acids and the nicotinic acetylcholine receptor inserted in a lipid bilayer. A Molecular Dinamic approach.

Obiol D^a, Amundarain MJ^a, Jaure O^a, Zamarreño F^a, Viso J^a, Antollini S^b, Costabel M^a

a - Grupo de Biofísica. Departamento de Física, Universidad Nacional del Sur (UNS), IFISUR (UNS-CONICET), Bahía Blanca, Argentina.

b - Instituto de Investigaciones Bioquímicas de Bahía Blanca (INIBIBB). Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur (UNS- CONICET), Bahía Blanca, Argentina

The nicotinic acetylcholine receptor (nAChR) is blocked in a non-competitive way by free fatty acids (FFAs) and the site of action might occur at the lipid-AChR interface. The isomerism and position of the double bond are considered crucial for this blocking mechanism. The objective of this work was to perform molecular dynamics (MD) for a system consisting of a nAChR model inserted into a lipid bilayer composed of cholesterol, palmitoyl oleoyl phosphatidylamine and palmitoyl oleoyl phosphatidylcholine to evaluate the influence of different FFAs on the nAChR conformational changes. The parameters that evaluate the quality of the model like molpdf, DOPE, GA341, QMEAN and ZSCORE were maintained or improved with respect to the AChR from *Torpedo marmorata* (2BG9) published in the Protein Data Bank. To this minimized and balanced system we made three replacements, in annular and non-annular sites of the receptor, with one of the following FFAs: cis-18:1 ω -6, cis-18:1 ω -9, cis-18:1 ω -11, cis-18:1 ω -13 or trans-18:1 ω -9. We obtained a total of 10 systems, plus the control system formed only by the model of the AChR included in the lipid bilayer. The results show the stability of the system along the entire MD trajectory by the root-mean square deviation (RMSD), root-mean square fluctuation (RMSF), the radius of rotation of the protein, the electrostatic potential along the lipid bilayer, the thickness of the lipid bilayer and the area per lipid.

These preliminary results show a good correlation with previously published studies of our group, concurring with oleic acid (cis-18:1 ω -9) located near the TM4 segments and elaidic acid (trans-18:1 ω -9) positioned near the TM4 segments and confirming that the double bond and the isomerism of FFAs effectively influence AChR conformation.

Modelling of soil nitrous oxide emissions as influenced by two gas sampling protocols, under the biophysical environment of the Southeastern Pampas (Argentina). Preliminary results.

Cambareri GS^a, Lewczuk N^a, Venterea R^b, Posse G^c, Cambareri M^d, Echarte L^a

a - INTA Balcarce

b - US Department of Agriculture / University of Minnesota

c - Instituto de Clima y Agua (INTA Castelar)

d - Facultad de Ciencias Agrarias -Universidad Nacional de Mar del Plata

The main goal of this study was to simulate N₂O emissions with a mechanistic model during one year of samplings, using observed data from two non steady state (NSS) chambers designs. The study was conducted at INTA Balcarce Research Station (37°45'S, 58° 18'W), where gas sampling with NSS chambers was performed on a Mollisol soil (5.2 ± 0.2 % OM, 0.23 ± 0.013 % total N, and soil bulk density = 1.28 g cm⁻³). Two base designs were evaluated: (i) standard non perforated base (BN) and (ii) perforated bases, from April 2017 to December 2017, with 12 sampling dates during the sequence fallow / corn. Steel bases were buried at 8-cm depth and installed during April under fallowed soil, removed during August (17/08) and re-installed soon after corn planting (16/11). Data of N₂O concentration were determined at the Department of Soil, Water and Climate (University of Minnesota) through gas chromatography, then N₂O emissions were calculated. The model used to simulate N₂O emissions was DNDC (Denitrification / Decomposition model), evaluating root squared medium error (RMSE), RMSE normalized by range (NRMSE) and the index of agreement (*d'*) as indexes for the model performance. The simulation resulted in 6 emissions peaks, out of which only 2 were captured by the samplings. BN emitted 41.3 g N-N₂O ha⁻¹ day⁻¹ at emission peak (4/12), while the simulation resulted in 40.2 g N-N₂O ha⁻¹ day⁻¹ occurring 4 days after the observed peak. For BP, N₂O emission peaked at 15/12 (97.3 g N-N₂O ha⁻¹), while simulation run resulted in an emission peak (139.5 g N-N₂O ha⁻¹) 2 days after the observed peak. The value of RMSE was lower for BP than for BN (39.7 vs. 73.1 g N-N₂O ha⁻¹), with a similar trend for NRMSE (BP= 40.6 % vs. BN=80 %). Regarding to *d'* (unitless), DNDC had a better fit with BP than with BN (0.48 vs. 0.05, respectively). Our results indicated that the base design should be taken into account to evaluate DNDC performance for simulating N₂O emissions in future studies.

Acknowledgments

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Molecular dynamics study of quercetin and rutin in solution

Campo MG^a, Corral GM^a

a - Facultad de Ciencias Exactas y Naturales. UNLPam.

Flavonoids are the most abundant phenolic compounds in plants. Their physiological potentials have attracted much attention in relation to their role in the cellular and extracellular antioxidant defense against oxygen radicals. Quercetin is one of the most abundant bioflavonoids present in most edible fruits and vegetables. It consists of two aromatic rings A and B linked by an oxygen-containing heterocyclic ring C. It has been used for the treatment of inflammation, arteriosclerosis, bleeding, allergy and swellings. Classic molecular dynamics technique is used to study the behavior and hydration structure of quercetin and rutin in solution. Rutin is the glycoside combining the flavonol quercetin and the disaccharide rutinose. The models of flavonoid molecules are developed based on the Amber99 force field, except for the atomic charges, which are calculated with the ESP module of the NWChem software using the 6-31G base. The hydration structure studied by analyzing the radial distribution functions, the hydrogen bonds distributions between the molecule and the solvent, the structural order parameter of the water, while the dynamic aspects are analyzed by studying the diffusion coefficient of the molecules in the solution. The most significant characteristics of the flavonoids hydration are obtained, which allow to obtain conclusions about their interaction with other molecules possibly present in the solution.

Study of the molecular mechanisms associated to the dynamic and structural properties in the protein aggregation of TRP-cage

Espinosa YR^a, Faundez C^b, Romero U^b, **Ferrara CG**^{b,a}

a - Institute of Physics of Liquids and Biological Systems (IFLYSIB), CONICET and National University of La Plata, Argentina

b - Institute of Engineering and Agronomy, National University Arturo Jauretche, Florencio Varela, Argentina

The study of the protein aggregation process is of vital importance to understand many of the biological events that occur in the cell.

There are a series of conformational disorders in the folded protein that responsible for the appearance of pathologies such as type 2 diabetes or neurodegenerative-type disorders such as Alzheimer's, Parkinson's or Huntington's disease, even being fatal as is the case of spongiform encephalopathies transmissible, for example, Creutzfeldt-Jakob disease.

The molecular mechanisms that trigger these diseases are the result of the protein conformational disorders (DCP), a disorder that has received great attention in recent years. If we only take Parkinson's disease as a reference, we would see that its prevalence is close to 2% in populations older than 65 years, which transforms this disease and the rest of the PCDs into a cause of growing concern for the general population.

In the present work, we show the first results obtained from a dynamic and structural analyzes of the protein aggregation process of TRP-cage, where the all analyzes were performed using Molecular Dynamics simulations (DM).

The systems studied formed aggregates composed of 2 to 4 proteins, under physiological conditions (saline concentration) and thermodynamic (temperature and pressure) stable, attempting to adequately represent the biological conditions of the process and how thermodynamic variations modify the kinetics of aggregation and the structure of the aggregate.

The results show the analysis of the solvent exposed surfaces (SASA) of the proteins, of the proteins in the state of aggregation, of hydrophobic and hydrophilic groups, different amino acids, interdistance between the hydrophobic cores of the proteins, energies of interaction between groups, radial distribution functions and other parameters that characterize the dynamics and structure of protein aggregation.

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The role of interactions on the collective behavior of particles confined in a corridor

Moreno JC^a, Rubio Puzzo ML^b

a - Laboratorio de Instrumentación, Automatización y Control (LIAC) - Departamento de Ciencia y Tecnología - Universidad Nacional de Quilmes; CONICET; Ingeniería en Informática - Departamento de Tecnología y Administración - Universidad Nacional de Avellaneda

b - Instituto de Física de Líquidos y Sistemas Biológicos (IFLYSIB), CONICET-UNLP; Departamento de Física - Universidad Nacional de La Plata

Collective behavior is the result of the interaction of a large number of self-propelled particles, which spontaneously and under certain specific conditions move orderly. Many groups of living beings (from cells and bacteria to fish, birds, mammals and even humans) has this specific kind of motion under particular conditions. The configuration of the particles positions and its time evolution can be analyzed with the tools of the statistical physics, searching the existence, determination and characterization of ordered phases in non-equilibrium systems. In this context, simple but non-trivial models have been proposed with the purpose to contribute to the understanding of this complex behavior.

The Vicsek Model (VM) was the pioneer model of computational physicist to study the collective motion. In this simple model, particles with constant speed v_0 assumes the average direction of motion of their closest neighbors distorted by the existence of a noise of amplitude h . However, the introduction of complex interactions are needed in order to understand more realistic problems, such as socio-psychological forces, comprehension and friction. In this way, the Social Force Model (SFM) takes into account these interactions because it was particularly designed to reproduce the motion of pedestrians.

Our work focus the attention on the role of the interactions between particles confined in a corridor. By means of Monte Carlo simulations, we study the effects of these interactions on the configurations that the system reaches in the steady state, and the order-disorder transition.

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Unravelling the molecular basis for allosteric effectors specificity in ancestral hemoglobins by molecular dynamics techniques.

Pettinati M^a, Larregui F^a, Issoglio F^a, Estrín DA^a, Alonso de Armiño DJ^a

a - Universidad de Buenos Aires-CONICET, DQIAQF-INQUIMAE, Facultad de Ciencias Exactas y Naturales

Oxygen binding in the heme active site in hemoglobins (Hb) of most vertebrates, is negatively regulated by the binding of organic phosphates at a structurally remote site in the same protein (allosteric regulation). However the exact identity of this allosteric effector varies greatly amongst different species and in few is this more true than in crocodillians, where Hb-oxygen affinity is allosterically regulated by bicarbonate ions, instead. In order to understand the structure-function relations of the evolutionary steps that lead to such singular adaptation it is imperative to study the last common ancestor of both crocodillians and birds still capable of binding ATP. As part of an ongoing project aimed onto the understanding of the evolution of hemoglobins, such common ancestor, an archeosaur Hb (archHb) protein, has been very recently obtained by protein engineering in Dr. Jay Storz laboratory using an experimental approach known as "Ancestral Protein Resurrection", and its crystal structure elucidated. We hereby present a preliminary comparative study of this ancestral archeosaur Hb protein and human Hb, with the objective of determining the molecular determinants of the allosteric effector specificity using molecular dynamics simulation techniques.

Using Virtual Screening for the discovery of new GABAergic insecticides: assessment of Rdl homology models and docking scoring functions

Felsztyna I^a, Miguel V^a, Villarreal MA^b, García DA^a

a - IIBYT (CONICET-UNC), Departamento de Química, Cátedra de Química Biológica, Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba

b - Instituto de Investigaciones en Físico-Química de Córdoba (INFIQC-CONICET-UNC), Departamento de Matemática y Física, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba

Insect nervous system is the main target of the most used insecticides, as is the case of non-competitive antagonists (NCAs) that block GABA_A receptor (GABA-R). In insects, the homopentamer formed by the so-called Rdl subunit is the most representative type of GABA-R. Docking based virtual screening (VS) is a computational method that allows to explore libraries of chemical compounds in order to identify those which are most likely to bind to a protein target. We aim to validate Rdl structural models and scoring functions to carry out a VS of NCAs that bind to *Aedes aegypti* Rdl homopentamer (Rdl_{hom_o5}). Since there are no experimental 3D structures of any insect Rdl_{hom_o5}, we performed homology modeling to obtain our protein structure. GABA-R, as well as the rest of Cys-loop receptors, is a transmembrane ion channel whose conformation varies among three pharmacological states: open, closed and desensitized. Considering that the NCAs binding site is located inside the channel pore, and that its conformation could affect the docking calculation of the binding of these ligands, we obtained homology models based on Cys-loop receptors templates in the three pharmacological states. A set of ligands which are known to act as NCAs were docked in the proposed binding site of all our models, and the binding energy obtained by docking was compared with experimental data for each compound. Also, different docking scoring functions were used to select the combination of protein structure and scoring function that results in the best correlation with the experimental data. Although in previous NCAs binding studies an open channel template was chosen to model an insect Rdl_{hom_o5}, we obtained the best correlation to experimental data using a model based on a structure crystalized in the desensitized conformation (human GABA-R β3 homopentamer), using the Vinardo scoring function.

Contact: ivanfelsztyna@gmail.com

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Adaptive Biasing Force method applied to the study of the interaction of GABA_ARs and benzodiazepines

Amundarain MJ^a, Sampaio Dias AH^b, Caffarena ER^b, Costabel M^a

a - Grupo de Biofísica. Departamento de Física, Universidad Nacional del Sur (UNS), IFISUR (UNS-CONICET), Bahía Blanca, Argentina.

b - Programa de Computação Científica, Fundação Oswaldo Cruz

GABA_A receptors are the primary mediators of the fast inhibitory response in the central nervous system of mammals by selectively allowing the passage of Cl⁻ ions into the neurons. These members of the Cys-loop family are activated by different agonists, including γ -Aminobutyric acid, and modulated by a myriad of pharmacologically relevant compounds, such as b-carbolines, benzodiazepines, neurosteroids and alcohol.

In this research, we studied the interaction between diazepam, zolpidem and flumazenil, and the $\alpha_1\beta_2\gamma_2$ GABA_AR through the ABF method (Adaptive Biasing Force), an enhanced sampling technique, implemented in *NAMD2.12*. The initial structures were obtained from homology modeling (based on the β_3 homopentamer PDB ID: 4COF) and molecular docking. Also, simulations were performed on flumazenil bound to the recently published structure of a $\alpha_1\beta_2\gamma_2$ subtype (PDB ID: 6D6U). In ABF, the sampling is facilitated by the addition of a biasing force to the equations of motion in order to obtain, after enough sampling, a Hamiltonian lacking the average force acting on the reaction coordinate. The free energy along that coordinate is determined as the potential resultant from the force. Due to the high computational cost, we simulated only the extracellular domain of the α_1 and γ_2 subunits (the benzodiazepines cavity). We defined the reaction coordinate as the distance between the center of mass of the protein and the ligands, allowing it to vary between 10 and 40 Å, in a set of 15 windows of width 2 Å. Each window was simulated until energy convergence was obtained (<1 kcal/mol).

We estimated the dissociation free energy between the receptor and the ligands. Even though the results for the modeled receptor differ from experimental data, they fall within the expected values for initial structures obtained from molecular docking on homology models. Moreover, the binding free energy was correctly estimated for the system comprising flumazenil and the experimental structure.

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Computational prediction of nsSNPs effects on bovine GSTP1: an integrated protein function and structure approach to SNPs prioritization

Falomir-Lockhart A^a, Crespi J^a, Alvarez P^a, Villegas Castagnasso E^a, Giovambattista G^a, Rogberg Muñoz A^b

a - IGEVET - Instituto de Genética Veterinaria "Ing. Fernando Noel Dulout" (UNLP-CONICET La Plata), Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, Argentina - La Plata, Buenos Aires, Argentina.

b - Departamento de Producción Animal, Facultad de Agronomía, Universidad de Buenos Aires - Ciudad Autónoma de Buenos Aires, Argentina.

The glutathione S-transferases (GSTs) superfamily protects against reactive species and substances, minimizing oxidative stress. Additionally, it regulates the apoptotic processes through a non-enzymatic interaction with signaling kinases. Previous studies have shown that GSTP1 haplotypes produce differences on cellular proliferation and apoptosis. Therefore, analysis of the genetic variability and of the potential impact of amino acid variants on protein structure and function by cost-effective and reliable *in silico* methods could enhance our understanding of GSTP1 involvement in muscle redox state and apoptosis. In particular, *post mortem* conversion of animal muscle into meat involves apoptosis and could be used as a model of cellular extreme oxidative stress leading to programmed cell death. In this research, bovine GSTP1 gene was resequenced and fifteen exonic SNPs were detected, some of them leading to amino acids substitution (non synonymous SNPs). The possible impact of these mutations on protein structure and function was tested by a combination of tools based on evolutionary and structural information. Next, homology modeling was performed to predict and compare the 3D protein structures of unresolved amino acid sequences. Our results were consistent, suggesting that substitutions C15W, C48G, Q65H and L70Q may impact on GSTP1 structure and function. Moreover, haplotype analysis enabled the prioritization of three structures for further analysis. This is one of the first attempts to study residue variants in bovine GSTP1 protein using prioritization bioinformatics tools before conducting *in vitro* or *in vivo* experimental studies.

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How Recruitment mechanism contributes to patterning in *Drosophila* developing wing

Alvarez HA^{a,b}, Muñoz Nava L^c, Nahmad Bensusan M^c, Chara O^{d,b}

a - Department of Biological Sciences, Faculty of Exact Sciences, University of La Plata (UNLP), La Plata, Argentina

b - Systems Biology Group (SysBio), Institute of Physics of Liquids and Biological Systems (IFLYSIB), National Scientific and Technical Research Council (CONICET), University of La Plata (UNLP), La Plata, Argentina

c - Department of Physiology, Biophysics, and Neurosciences, CINVESTAV-IPN, Mexico City, Mexico

d - Center for Information Services and High Performance Computing (ZIH), Technische Universität Dresden, Dresden, Germany

How concentration patterns of signals that determine cell fates get established is a fundamental problem in developmental biology. In the developing wing of the fruit fly *Drosophila melanogaster* (called imaginal wing disc), the wing fate is defined by the expression of the selector gene vestigial (vg). The size of the Vg domain is determined through proliferation of Vg expressing cells near the dorsal-ventral boundary of the wing disc, and also through the propagation of the Vg pattern to neighboring cells by a process known as recruitment. However, the spatiotemporal contributions of these mechanisms to the final size of the wing have not yet been carefully examined. We quantitatively examine Vg expression along the dorsal-ventral (DV) axis in the wing disc and find that the Vg pattern overscales (*i.e.*, it expands more than predicted by tissue size). This overscaling phenotype can be recapitulated from simulations of a mathematical model that explicitly incorporates a recruitment mechanism. We developed a Cellular Potts Model of a growing tissue where the Vg concentration expressed in each cell is assumed as a function of its difference with the neighboring cells and encoded by an Ordinary Differential Equation.

The agreement between the experimental data and the modeling results here presented suggests that the proposed recruitment mechanism could, at least in part, explain the spatio-temporal pattern of Vg concentration.

SysVert: a new package for modelling tissues using vertex mode

Ceccarelli A^a, Borges A^a, Cura Costa E^a, Alvarez HA^a, Vidal V^a, Chara O^{b,a}

a - SysBio, Instituto de Física de Líquidos y Sistemas Biológicos (IFLYSIB) and Universidad Nacional de La Plata, Argentina

b - Center for Information Services and High Performance Computing, Technische Universität, Dresden, Alemania

In our group, we are developing *SysVert*, a new package encoded in Python to simulate tissues using vertex models. In these approximations, cells are modelled as polygons and tissue dynamics are governed by forces acting on the vertices of those polygons. Although other implementations exist, most of them have a drawback: the user needs to have certain knowledge of computer programming to successfully use them. In contrast, our package is designed to be user-friendly, with an intuitive graphical interface and it will be open source, to allow for a dynamic feedback between users and developers to, in turn, introduce new features and improvements in the package.

SysVert has several features that permit the user to simulate a wide arrange of tissues in different conditions. For example, the package allows the user to define cell types within a tissue and different rules of cell proliferation, apoptosis or topological rearrangements. Finally, the user of *SysVert* can define chemical signals like morphogens which can interact with the cells allowing multi-scaling systems biology strategies.

Here, we present simulations performed with a pre-release version of *SysVert*, as a proof of concept of the package capabilities

Deltamethrin and naringenin docking to modeled human voltage gated sodium channel 5 α subunit

Cosy Isasi S^a

a - Cátedra de Bioquímica y Biología molecular, Medicina, Facultad de Ciencias Médicas-UNC

Dilated cardiomyopathy (DCM) is a structural heart disease that causes dilatation of cardiac chambers and impairs cardiac contractility. Nav1.5, the predominant cardiac sodium channel alpha subunit has been involved in uninterrupted sodium currents causing cardiac arrhythmias previous to DCM, but the regulation of the sodium channel has yet to be fully understood. Established therapies consist of an ACE-inhibitor, a diuretic, and a beta-blocker, which leads to a decreased left systolic function. Pyrethroid insecticides block the inactivation of voltage-gated sodium channels (VGSC). Harmful effects studied on mammals and humans exclude cardiac arrhythmias. Objective: to evaluate in silico the possibility of interaction between deltamethrin, naringenin, hydroxytyrosol pinoresinol and inactivation region of VGSC 5a. Structures of ligands were obtained from ZINC database. Human hVGSC5a was homology-modeled (SWISSMODEL- Phyre2). Interaction-free energies (ΔG_i) were determined with Autodock Vina and Swiss Dock for restricted (rb) and unrestricted ligand binding (urb) to lysyl prolyl glutamine / arginine (KPQ / N), the conserved segment necessary for inactivation. The values obtained for deltamethrin, naringenin (urb) were -8, -7.4 Kcal, hydroxytyrosol and pinoresinol (rb) -5.88 y -6.22 Kcal. The results support the assumption that deltamethrin might impair hVGSC inactivation. Patients with arrhythmia chronically exposed to the insecticide (Chagas disease patients in an endemic area) would suffer a deterioration of their condition due to exposure. Naringenin binding opens the possibility to measure sodium currents on treated cells. Hydroxytyrosol and pinoresinol showed low affinities for KPQ but stronger binding to transmembrane segments. Molecular dynamics with virtual plasmalemma are ongoing.

Advances in the functional modulation of the NFS1/ACP- ISD11/ISCU/FXN Fe-S cluster mitochondrial supercomplex by molecular intervention through specific Trojan tutors

Pignataro MF^a, Correa A^b, Castro I^a, Santos J^a

a - IQUIFIB-Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, UBA. Junin 956, 1113 Ciudad de Buenos Aires

b - Institut Pasteur Montevideo, Uruguay

The development of molecules that can specifically bind to targets plays a major role in therapeutics and diagnostics, and also in basic and applied research. As an alternative to antibodies, the use of a different kind of specific molecules against various targets has been tested and characterized over the last years (1-2). The possibility of using them as molecular tutors, that promote conformational stabilization, appears as a promising strategy for the treatment of several diseases. In this work, we employed Sac7d variants (affitins), selected by ribosome display technology using Frataxin (FXN) as the target. The lack of FXN, an allosteric activator of NFS1/ACP-ISD11/ISCU Fe-S cluster supercomplex in mitochondria, leads to Friedreich's ataxia, a rare inherited disease that causes progressive nervous system damage. We performed four rounds of Ribosome Display using a Sac7d library, a DNA binding protein from *S. acidocaldarius*, and human FXN (wild-type) as the target. To test whether the selection was successful we (a) performed an ELISA to detect the binding of affitins to FXN and BSA (as a negative control) and (b) we investigated individual clones by subcloning the product of the last round of selection into a pFP1001 vector, transformed *E. coli* DH5 α F'IQ cells, induced affitin expression and performed an ELISA. Positive clones were sequenced. We found that the ratio of 450 OD of FXN to BSA was 1.6, when the optimal value should be around 8-10 (2) suggesting the need of at least one more round of selection to discard the BSA binders. The finding of any new specific affitin for FXN and some unstable variants will allow us to modulate not only its stability but also its dynamics and activity, leading in potential therapeutic agents.

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Analysis of the functional and structural stability of β -glucosidase BGLA from *Cytophaga Hutchinonii*

Garrone N^a, Iglesias Rando MR^a, Gorojovsky N^a, Craig PO^a

a - Departamento de Química Biológica, FCEyN, UBA - IQUIBICEN, CONICET

Cytophaga hutchinsonii is a gram-negative bacterium that can efficiently degrade crystalline cellulose through a combination of different cellulases. The enzymes involved in this process are potentially useful for biofuel production. BglA is a beta-glucosidase from this bacterium which produces the hydrolysis of cellobiose into glucose. The cloning and recombinant expression of this enzyme in *E.Coli* yields large amounts of soluble protein. It has a good activity and a low inhibition rate by glucose which makes it attractive for industrial purposes. However, it is somewhat sensitive to temperature. Here we present an analysis of the temperature dependence of the enzyme activity and its inactivation rate. The activity is optimal around 25°C but sharply decays above 30°C in an irreversible process. To evaluate the possibility of inactivation by the oxidation of catalytic important residues we studied how BglA's activity loss was affected in reducing conditions in the presence of DTT. We also present an analysis of the urea induced denaturation of this multidomain protein followed by intrinsic tryptophan fluorescence, ANS binding, CD signal and enzymatic activity. Perspectives for the thermal stabilization of this enzyme and its possible use in synthetic multienzymatic complexes (artificial cellulosomes) for improving lignocellulose degradation is discussed.

An improved method for the purification of human alpha synuclein

Rial Hawila MR^a, Gómez GE^a, Delfino JM^a

a - Departamento de Química Biológica e IQUIFIB (UBA-CONICET), Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires

Human α -synuclein (AS) is a 140-residue, intrinsically disordered protein (IDP) that can give rise to insoluble aggregates under pathological conditions. AS is the primary component of Lewy bodies, the hallmark of Parkinson's disease. With the aim of analyzing its various structural forms, it is essential to first obtain the protein in high purity. AS was expressed in *Escherichia coli* using plasmid pT7-7 (courtesy of Dr. Soledad Celej, CIQUIBIC, UN Córdoba-CONICET). Following transformation, BL21 electro competent cells were grown in LB medium with ampicillin (100 μ g/mL). Cells were induced with 1 mM IPTG (at $OD_{600\text{ nm}} \sim 0.70$), cultured for 4 h at 37 °C and harvested by centrifugation. The cell pellet was resuspended in lysis buffer (lysozyme in 50 mM Tris-HCl pH 7.4, 5 mM EDTA, 0.5% Triton X-100, 1 mM PMSF) and incubated for 1 h at 37 °C. This suspension was intermittently sonicated in an ice bath (10 s pulses). Thereafter, $MgCl_2$ (20 mM final concentration) and DNase (Sigma) were added and incubated for 1 h at room temperature. Metal ion sequestration was secured with 20 mM EDTA and cellular debris was removed by centrifugation. The supernatant was boiled for 20 min and further centrifuged. This new supernatant was exhaustively dialysed and loaded onto a pre-packed HiPrep 16/10 Phenyl FF (high sub) hydrophobic interaction column and washed with 2 M ammonium sulfate in 20 mM potassium phosphates buffer, 1 mM EGTA, pH 7.4. AS eluted with decreasing salt and was thoroughly dialysed again. Pooled fractions were concentrated using a 10 kDa Amicon® Ultra-15 filter device and loaded onto a HiLoad 16/60 Superdex® 200 prep grade SEC column. This was run in 20 mM potassium phosphates buffer, 1 mM EGTA, 50 mM NaCl, pH 7.4. Protein mass was confirmed by ESI MS, and CD spectra were collected at pH 4 or 7 to evaluate AS conformational plasticity. This experimental protocol yields functional AS devoid of proteolytic fragments.

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Binding properties of Sterol Carrier Protein 2 (SCP2) from *Yarrowia lipolytica* characterized using Laurdan

Gianotti A^a, Ferreyra R^a, Ermácora MR^a

a - Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes-CONICET

Sterol Carrier Protein 2 (SCP2) binds lipids with high affinity and broad specificity, and is present in all forms of life, as a stand-alone protein or as module in a large variety of multidomain proteins, playing different roles.

In this work we characterized the hydrophobicity, fluidity, and dipolar dynamics of the binding site of SCP2 from *Yarrowia lipolytica* using the environmentally-sensitive fluorescent probe Laurdan. The fluorescence properties of bound Laurdan revealed a binding site with an overall polarity similar to that of dichloromethane and an internal phase comparable to that of phospholipid membranes with coexisting solid-ordered and liquid-crystalline states. Moreover, the binding site of SCP2 can accommodate competitively more than one ligand, with micro and nanomolar dissociation constants.

Our findings are important for the characterization of SCP2 biological functions and the design of specific inhibitors.

Computer simulation study of CO and O₂ affinity of in neuroglobin mutants proposed as CO scavengers for treatment of intoxication with carbon monoxide

Navarro MB^a, Bringas M^b, Martí MA^c, Capece L^b

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

b - Departamento de Química Inorgánica, Analítica y Química Física - F.C.E.N. - U.B.A. (Argentina)

c - Departamento de Química Biológica, FCEyN, UBA - IQUIBICEN, CONICET

Neuroglobin (Ngb) is a hemoprotein belonging to the globin family, which is expressed in the nervous system of mammals, particularly in humans. Like other globins, such as human hemoglobin, is able to bind small ligands such as oxygen (O₂) or carbon monoxide (CO).

The latter, when bound to human hemoglobin, produces a blockage of the binding site for O₂, this being the main cause of death by poisoning worldwide. In a recent experimental work, a variant of human neuroglobin that could function as a CO scavenger in aerobic

conditions has been found. In this Ngb variant, the distal histidine (HisE7) was replaced by a glutamine residue. This variant has an increased affinity for CO and a decreased affinity for O₂, which, among other factors, makes it a potential candidate for this role.

In this work we combine classical molecular dynamics simulations with hybrid quantum mechanics - molecular mechanics (QM/MM) calculations to determine the molecular basis of the differential effect of this mutation in ligand affinity. We observe differences in the hydrogen-bond network in the distal cavity between wild type Ngb and H64Q Ngb that could explain the differences in ligand affinity.

Conserved linker length in double dsRBD proteins from plants restricts interdomain motion

Masali FC^a, Tabares LC^b, Rasia RM^a

a - Instituto de Biología Molecular y Celular de Rosario, IBR-CONICET-UNR, Rosario, Argentina

b - Institute for Integrative Biology of the Cell (I2BC), Department of Biochemistry, Biophysics and Structural Biology, Université Paris-Saclay, CEA, CNRS UMR 9198, F-91198 Gif-sur-Yvette, France

Multidomain proteins are ubiquitous in nature. Domain shuffling and duplication have helped organisms to achieve a wide range of functions by combining a limited number of individual modules. The domains in multidomain proteins are connected by sequences of aminoacids of varying length and rigidity. HYL1 is a protein from *Arabidopsis thaliana* that participates in microRNA biogenesis in plants whose precise function is not known at present. Analysis of HYL1 sequence showed a domain architecture consisting of two double stranded RNA binding domains (dsRBDs) connected by a 17-residue linker and followed by a long unstructured C-terminal dispensable for its function *in vivo*. In the present work we studied how the linker restricts the mobility between both domains. Inter domain distances measured by PELDOR on double labeled protein samples show a narrow distribution around 4 nm. NMR Paramagnetic Relaxation Enhancement (PRE) on single labeled protein samples shows an asymmetry in transient domain-domain interactions. By means of ensemble simulations and calculation of PREs we found that the domains explore a restricted conformational space. Sequence analysis of linkers between double dsRBD plant proteins shows a high conservation in linker length. Altogether our results put forward the functional importance of the linkers between dsRBD modules in multidomain proteins.

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Development and evaluation of non covalent coupling methods for the production of artificial cellulosomes

Iglesias Rando MR^a, Gorojovsky N^a, Goldbaum FA^b, Craig PO^a

a - Departamento de Química Biológica, FCEyN, UBA - IQUBICEN, CONICET

b - Fundacion Instituto Leloir-CONICET

Lignocellulose is the most abundant renewable resource on the planet and it is an excellent substrate for the production of biofuels. Its enzymatic degradation generates sugars that upon fermentation produce bioethanol. For an economically viable production of biofuels it is essential to develop new methods to increase the activity and stability of the enzymes involved in lignocellulose degradation. The cellulosomes of some anaerobic organisms represent the most efficient machinery for the degradation of lignocellulose. These multienzymatic complexes co-localize different cellulolytic enzymes and cellulose binding domains, increasing their degradation activity through enzymatic proximity and substrate targeting effects. Our goal is to develop artificial cellulosomes using an oligomeric protein scaffold that is highly stable and highly expressed in bacteria for the colocalization of cellulases, hemicellulases, beta-glucosidases and cellulose binding domains. For the assembly of these multienzymatic particles we use a non-covalent coupling strategy through high affinity heterodimeric modules complementary fused to the scaffold subunits and the target proteins. In this work we present a comparative analysis of two alternative approaches for non-covalent coupling of enzymes to our oligomeric scaffold using heterodimeric coiled coil peptides and cohesin/dockerin modules.

Advantages and disadvantages of each method regarding the expression level, solubility and aggregation tendency of the target and scaffold modules are presented. The analysis of the expression behavior of the isolated protein modules used as building blocks is also presented for comparison. We also show results for the functional analysis of cellulose binding domains and for some enzymes coupled to the scaffold as well as on their own. It is expected that this technology would be valuable to improve lignocellulose degradation.

First approximation to the study of a tandemly repeated protein: the case of ABA-1A from *Ascaris suum*

Rodríguez S^a, Bélgamo J^a, Kennedy MW^b, Córscico B^a, Franchini G^a

a - INIBIOLP, CCT-La Plata, CONICET, Facultad de Ciencias Médicas, UNLP, 60 y 120, La Plata

b - School of Life Sciences, University of Glasgow

Tandemly repetitive polyproteins (TRPs) are rare in nature. They are produced as large precursor polypeptides, comprising repeated units of similar or identical amino acid sequence that are post-translationally cleaved into a dozen or so copies of functionally similar proteins. The polyprotein allergens/antigens of nematodes (NPAs) belong to this family, are small, helix-rich proteins, and have no known structural counterparts in other phyla. The NPAs present in *Ascaris suum* (ABA) are cleaved posttranslationally into multiple ~15 kDa protein subunits which may have similar or different functions. It is important to note that they also represent a novel class of lipid binding proteins from helminths group. ABA-1A, a single subunit of this family of proteins, is found in high amounts both in the pseudocelomic fluid of adult worms and in the excretion/secretion products from all parasitic stages. Recently, the structure of ABA-1A has been solved showing two binding sites with different characteristics. The protein adopts a novel seven-helical fold comprising a long central helix that participates in two hollow four-helical bundles on either side (Meenan et al., 2011 doi:10.1371/journal.pntd.0001040). Although structural and functional characterisation has been performed on the single subunit, ABA-1A, there are no reports on the polyprotein array. In this project we aim at getting further insight into the unusual translation process of these proteins. To this end we designed, constructed and expressed a tandem protein composed of two ABA-1A subunits. Preliminary analysis using spectroscopic techniques (fluorescence and CD) have shown that there are no differences in the secondary and tertiary structure between the tandem protein and the ABA-1A subunit alone. These results would suggest that there are almost no interactions between subunits during polypeptide folding *in vivo* when it is being synthesized.

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Fluorescence of tyrosine dimers: properties and applications in the study of the cross-linking of proteins

Reid LO^a, Dántola ML^a, Thomas AH^a

a - Instituto de Investigaciones Físicoquímicas Teóricas y Aplicadas (INIFTA), Departamento de Química, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, CCT La Plata-CONICET. La Plata, Argentina.

One of the most important modifications of the oxidative damage of proteins is the covalent bond between two tyrosines (Tyr), yielding the tyrosine dimer or dityrosine (Tyr₂). This linkage can occur between two Tyr residues in the same molecule, or between two molecules,¹ the latter leading to a high molecular weight product.² Tyr₂ is increasingly used as a marker of aging, stress and pathogenesis. It was identified in many pathological manifestations.³

The phenol groups of Tyr₂ are much more acidic than that of Tyr and the corresponding pK_a value was determined to be 7.25. The two acid-base forms have well differentiated spectral features, with absorbance maxima of 283 and 315 nm for the acid and basic form, respectively. This means that when Tyr₂ is formed *in vivo* a new chromophore appears in the proteins, which is able to absorb, unlike natural amino acids, at wavelengths significantly present in solar radiation and artificial sources of light. The fluorescence of Tyr₂ is an unspecific marker of oxidative damage in proteins.^{4,5}

In this work we have studied the emission properties of the acid and the basic forms of Tyr₂ in aqueous solution. Steady-state and time-resolved fluorescence experiments were carried out and lifetimes, quantum yields and emission spectra were obtained under different experimental conditions.⁶ We have used the emission properties of Tyr₂ to investigate the photosensitized oligomerization of proteins *via* the formation of Tyr₂. As a model system, we have studied the oligomerization of human serum albumin photoinduced by pterin under UV-A irradiation.²

Contact: athomas@inifta.unlp.edu.ar

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Focusing on the Human Mitochondrial Desulfurase NFS1

Sewell E^{a,b}, Castro I^{a,b}, Santos J^{a,b}

a - Departamento de Fisiología y Biología Molecular y Celular. Facultad de Ciencias Exactas y Naturales. Universidad de Buenos Aires. C.A.B.A.

b - Instituto de Química y Fisicoquímica Biológicas, Dr. Alejandro Paladini (UBA-CONICET). C.A.B.A.

[Fe-S] cluster biosynthesis is a multifaceted mitochondrial process that requires the multiprotein supercomplex NFS1/ACP-ISD11/ISCU, with the positive allosteric modulation of FXN. It is worth noticing that dimerization of NFS1 is key for the proper activity, and therefore the complete active supercomplex is composed of two symmetrically organized molecules of each one of the four proteins. The involvement of each protein in this supercomplex is thought to have crucial consequences in its functioning. For instance, ACP-ISD11 heterodimer is known to stabilize the formation of the NFS1 dimer preventing aggregation, and desulfurase activity is heavily unpaired in its absence. On the other hand, ISCU has a modulatory effect on the desulfurase activity and it is proposed to work as a scaffold for the assembly of the [Fe-S] cluster itself. FXN, has a strong positive effect on the modulation of the activity and it is proposed to bind the iron required for the reaction. Finally, NFS1 is the one responsible for the desulfurase activity itself. By a reaction that involves NFS1 prosthetic group PLP, the enzyme converts free cysteine to free alanine and forms a persulfide bond with one of its own cysteine residues.

Focusing on the recombinant human NFS1 protein, it was produced and purified requiring strategies to avoid aggregation. Its activity was assessed in the presence of the other protein components of the supercomplex. Desulfurase activity was assessed by measuring the hydrogen sulfide produced during the reaction. Additionally, the activation capability of different FXN variants was studied. As the activity of the supercomplex heavily depends on the interactions between subunits, a NFS1 double mutant containing a single tryptophan (Trp97) was design to study binding equilibrium. Our goal is to accurately measure the shifts in the fluorescence signals due to interactions of the different subunits, as well as a broad characterization comparing with the wild-type NFS1.

Functional study of CRAC and CARC peptides derived from *E. coli* α -hemolysin

Cané L^a, Guzmán F^b, Maté S^a, Herlax V^a

a - INIBIOLP, CCT-La Plata, CONICET, Facultad de Ciencias Médicas, UNLP, 60 y 120, La Plata

b - Núcleo Biotecnológico Curauma

Escherichia coli alpha hemolysin (HlyA) is a pore-forming protein which belongs to the family of 'Repeat in toxins'(RTX). The CRAC domain refers to the **C**holesterol **R**ecognition/interaction **A**minoacid **C**onsensus sequence. The CARC domain is similar to the CRAC sequence, but exhibits the opposite orientation along the polypeptide chain.

The aims of this work were to study the participation of CRAC and CARC in the stabilization of HlyA monomers in membranes by their interaction with cholesterol, to evaluate the role of Y³⁴⁷ in the interaction with membrane, and finally to find a cytotoxic peptide for the construction of an immunotoxin.

On the basis of experimental data and structural predictions, six peptides derived from HlyA were synthesized: PEP 1: transmembrane domain described as hemolytically active; PEP 2: also a transmembrane domain which sequence corresponds to a cholesterol binding domain (CARC); PEP3: similar to PEP2 but with residue Y³⁴⁷ substituted by A; PEP4: similar to PEP2 but with a CRAC sequence; PEP5 and PEP6 correspond to CARC sequences located near the acylation sites.

Peptides were synthesized by the solid phase peptide synthesis method (Fmoc strategy), purified by HPLC (C-18 column), the molecular mass was determined by mass spectrometry and peptide structure by circular dichroism. The hemolytic activity of peptides was measured using human erythrocytes and inhibition of hemolytic activity assays were performed pre-incubating erythrocytes with peptides and then adding them to wild type toxin.

Results describe PEP2 as hemolytic, which is promising and encourage us to use it in the design of immunotoxins. PEP3 was found not to be hemolytic suggesting residue Y³⁴⁷ is fundamental for the interaction of HlyA with lipidic membranes. PEP4 was found not to be hemolytic, which implicates the CRAC sequence added was unfavorable for peptide activity. PEP 6 competes with HlyA for binding sites in erythrocytes.

Hexa/Pentacoordination transition in THB1 studied by computer simulation

Julió Plana L^a, Lecomte JTJ^b, Estrín DA^a, Capece L^a

a - Departamento de Química Inorgánica, Analítica y Química Física - F.C.E.N. - U.B.A. (Argentina)

b - Department of Biophysics, Johns Hopkins University, Baltimore, Maryland 21218, United States

THB1 from green alga *Chlamydomonas reinhardtii* belongs to group 1 truncated hemoglobins (TrHb1s). It is a monomeric hemoglobin that functions as nitric oxide dioxygenase. The heme in THB1 displays two axial ligands: His77 and Lys53. In order to be able to bind external ligands, THB1 Fe-Lys53 bonds need to be break. The displacement of Lys53 involves a conformational change, which includes the rotation of Lys53 side chain that ends pointing outside the protein cavity. Also, the protonation state of Lys53 change. A battery of biophysical and biochemical studies were done [1], emerging several questions. Regarding the Lys53 opening mechanism, it is not clear if the protonation of Lys53 occurs in the heme cavity or when Lys53 have moved to the solvent. Also, if Lys53 protonation take place inside the heme cavity, it is unknown if it is concerted with the decoordination or it is sequentially. In this work we have tried to shed light on the mechanism of Lys53 decoordination in THB1 by means of computer simulations. For this purpose, we performed extended molecular dynamics simulations for the wild type THB1 and selected mutants for pentacoordinated state. We have analyzed the global structural movements associated with the transition, which involve the E helix and residues Lys49 and Arg52. Additionally, umbrella sampling calculations were performed to obtain the free energy profiles of the Lys53 displacement process when Lys53 is neutral and when Lys53 is protonated. To clear up if the protonation inside the cavity is concerted with the decoordination, QM-MM restrained optimizations were done. The results from this work strongly suggest that the “in” to “out” transition involves the protonation of Lys53 inside the distal cavity, which is also the driving force for Fe-Lys53 bond cleavage. Additionally, several key aminoacids have been identified as molecular determinants for the Lys53 displacement process.

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Acknowledgments

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Impact of glycosylation in Immunoglobulin A structure and function as studied by molecular dynamics simulations

Lorenzo E^a, Mariño KV^b, Di Lella S^a

a - Departamento de Química Biológica, FCEyN, UBA - IQUBICEN, CONICET

b - Instituto de Biología y Medicina Experimental (IBYME - Conicet)

Antibodies or immunoglobulins are key components of the immune system. These glycoproteins are central to the humoral adaptive immune response: they act by binding to pathogens and toxins, and this process could allow their direct neutralization, or trigger the involvement of the complement system and/or phagocytic cells in order to eliminate the threat.

In particular, secretory IgA (sIgA) is the major immunoglobulin at mucosal sites, a glycoprotein consisting of a secretory component covalently attached to dimeric IgA with one joining (J) chain. Immunoglobulin A (IgA) plays an important role in maintaining a balance with the commensal bacterial flora, in protecting our mucosal surfaces, and in extending maternal immunity via breast feeding. sIgA contains several sites of glycosylation and alterations in their structure can interfere with its immune function in the gut. It is worth noting that IgA is heavily glycosylated, and the presence of these N- and/or O-glycans (depending on the subtype) may result of importance in maintaining the protein conformation.

The main purpose of this work is to evaluate the impact of glycosylation in the structure of monomeric IgA, and how structural changes induced by its glycans may possibly affect IgA physiological function, by means of classical molecular dynamics simulations. We started by identifying glycosylation sites in the Fab (antigen-binding fragment) of IgA, as provided by the structures deposited in the Protein Data Base (PDBid 5E8E). We then proceeded to simulate two simplified systems, corresponding to a region of the Fab in the presence or absence of N-glycosylation. With the use of computational tools, we analyzed how the presence of certain carbohydrates modulates the conformation of the Fab. Possible consequences of the role of glycosylation in the protein function are discussed.

Inactivation of *E. coli* α -hemolysin photoinduced by pterin

Reid LO^a, Dántola ML^a, Herlax V^b, Thomas AH^a

a - Instituto de Investigaciones Físicoquímicas Teóricas y Aplicadas (INIFTA), Departamento de Química, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, CCT La Plata-CONICET, La Plata, Argentina.

b - Instituto de Investigaciones Bioquímicas de La Plata (INIBIOLP), CCT-La Plata, CONICET, Facultad de Ciencias Médicas, Universidad Nacional de La Plata, La Plata, Argentina.

Pterins are a family of heterocyclic compounds widespread in living systems. These molecules are photochemically active under UV-A excitation (320– 400 nm), can fluoresce, undergo photooxidation to produce various products and generate reactive oxygen species. Due to the photochemical features of these compounds, they are potential photosensitizers of biomolecules, such as DNA¹ and proteins.²

α -Hemolysin (HlyA) is an exotoxin, member of the pore-forming Repeat in Toxin (RTX) family, secreted by some pathogenic strains of *Escherichia coli*. The mechanism of action of this toxin seems to involve three stages that ultimately lead to cell lysis: binding, insertion and oligomerization of the toxin within the membrane.³

The aim of this work is to investigate the capability of pterin (Ptr), the parent and unsubstituted compound of oxidized pterins, to photoinduce chemical changes and inactivation of HlyA. Air equilibrated aqueous solution of HlyA and Ptr were exposed to UV-A radiation for different periods of time and were analyzed by UV/visible spectrophotometry, fluorescence spectroscopy and gel electrophoresis (SDS-PAGE). The hemolytic activity of the irradiated toxin was analyzed by light scattering at 595 nm in time.

Results indicate that HlyA can be inactivated by Ptr through a photosensitized process. The photodamage to HlyA results in the oxidation of the toxin in at least two different and specific sites: tryptophan (Trp) and tyrosine (Tyr) residues. The Trp degradation results in a fast decrease of the fluorescence intensity, but the hemolytic activity remains constant until 10 minutes of irradiation. In addition, Tyr residues contribute to dimerization of the protein, since Tyr dimers were detected by fluorescence. The electrophoresis analysis indicates unequivocally that Ptr photoinduces cross-linking of HlyA.

Contact: laraoreid@inifta.unlp.edu.ar

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Initial studies of fatty acid binding proteins (FABP) from parasitic cestodes as novel therapeutic targets

Bélgamo J^a, Pórfido JL^a, Rodríguez S^a, Pérez M^b, Rosenzvit M^b, Brehm K^c, Córscico B^a, Franchini G^a

a - INIBIOLP, CCT-La Plata, CONICET, Facultad de Ciencias Médicas, UNLP, 60 y 120, La Plata

b - Instituto de Investigaciones en Microbiología y Parasitología Médica (IMPaM), Facultad de Medicina, Universidad de Buenos Aires (UBA)

c - Institut für Hygiene und Mikrobiologie, Universität Würzburg, Würzburg, Germany

Echinococcus granulosus and *Echinococcus multilocularis* are the causative agents of cystic and alveolar echinococcosis respectively, and are among the neglected tropical diseases prioritized by the WHO. The scarcity of anthelmintic drugs available and the emergence of resistant parasites, makes the discovery of new anthelmintic drugs mandatory. The analysis of tapeworm genomes, showed absence of genes for fatty acids and cholesterol *de novo* synthesis and high expression of lipid binding proteins that could be involved in lipid acquisition from host tissues. Among them, fatty acid binding proteins (FABPs), small cytoplasmic proteins expressed in a tissue specific manner in mammals, bind and transport fatty acids and retinoids, probably involved in signaling pathways trafficking and membrane synthesis. In this work we are analysing the recombinant expression and value of cestode' s FABPs as novel drug targets. *In silico* analysis of tapeworm genomes revealed the existence of at least five FABP coding genes in the genomes of *E. granulosus*, *E. multilocularis* and *T. solium*. The sequences from *E. multilocularis* FABPs were cloned, sequenced and compared with the information available at the databases. Analysis of expression shows that *E. multilocularis* FABPs' seem to be transcribed in a stage-specific manner. The isoforms tested for binding show that they bind fatty acids with an affinity comparable to the mammalian counterparts. An inhibitor of mammalian FABPs (HTS01037) was tested *in vitro* on some isoforms employing fluorescence based methodologies. Additionally, using an *in vivo* cysticercosis (*T. crassiceps*) model the effect of the FABP inhibitor was evaluated on *T. crassiceps* cisticerci. Preliminary results indicate that HTS01037 presents a strong effect on parasite viability. Altogether, these results suggest that FABP isoforms may play specific roles in different stages/ tissues, related to lipid metabolism of parasites and might be good therapeutic targets.

Inner dynamics of L-BABP molten globule measured by time resolved fluorescence anisotropy

Cifuentes-Samboni U^a, Salinas S^b, Perduca M^c, Mónaco HL^c, Montich G^a

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 - Centro de Excelencia en Productos y Procesos Córdoba-CONICET, Pabellón CEPROCOR

c - Università di Verona, Italia

Liver bile acid-binding protein, L-BABP, from avian liver belongs to a family of low molecular mass proteins (14-15 kDa) that bind fatty acids and other nonpolar ligands. Its three dimensional structure is typical of the entire protein family, i.e. a β -barrel consisting of ten antiparallel β -strands arranged in two orthogonal β -sheets enclosing an inner cavity where the ligand is bound. L-BABP can acquire different folding states as a function of pH: it is in the compact native state within the pH range 7-5. At pH 2.4, in the absence of salts, acquires the folding of a pre-molten globule. By adding salts or decreasing the pH to 1.5 in pure water L-BABP refolds partially and reach the molten globule state (Nolan et al. 2005).

Here we studied the rotational correlation time, Φ , of the single tryptophan residue, Trp6, to evaluate the inner dynamics of L-BABP in the native and partly unfolded states. To this end we measured the time resolved fluorescence anisotropy of Trp6. We consider that the measured correlation time, Φ_{exp} , has contributions from the hydrodynamic rotation of the protein, Φ_{rot} , plus the inner movement of the residue within the protein Φ_{in} .

Independent measurements of the hydrodynamic volume of native and partly unfolded L-BABP allowed us to calculate Φ_{rot} and consequently the value of Φ_{in} . We concluded that in the partly unfolded states at pH 2.4 and 1.5, Trp6 acquired a limited increase in mobility, far less than in the completely unfolded state in 8 M GndHCl.

We also measured the rotational correlation time of 1,6-diphenil-1,3,5-hexatriene, DPH, bound to L-BABP. We concluded that both in the native and in the partly unfolded states DPH does not have independent movements but rather moves solidary with the protein.

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Microscopical description of the cold denaturation of globular proteins

Espinosa YR^a, Carlevaro CM^a, Grigera JR^b

a - IFLYSIB, (CONICET-UNLP), Facultad de Ciencias Exactas, UNLP

b - Centro de Química Inorgánica, CONICET, Universidad Nacional de la Plata, B1900, La plata, Argentina

Cold denaturation of globular proteins is an intriguing phenomenon that deserves special attention. Thus, the hydrophobic effect is considered the main driving force for folding and protein stability, as well as the loss of the protein stability when its upon cooling. Experimental and theoretical evidence recognize the role played by the density of water and its temperature-dependence that are largely determined by the energetic and geometric features of H-bonds. Hence, there are those who argue that the model proposed by Frank and Evans in 1945 confirm that the H-bonds in the hydration shell of non-polar solutes are stronger and ordered than those in bulk water in the cold denaturation. However, the Muller's model and some experimental data indicate that the hydration shell is more disordered or more broken, than those in bulk water. Therefore, there is a discrepancy in the microscopical model for the cold denaturation. With the intention of understanding this phenomenon, we have chosen as alternative approach Molecular Dynamics simulations (MD) using the Gromacs-2018 package and analyzing the cold denaturation of the Yfh1 frataxin of *S. cerevisiae*. In this study, we created two system at 225 K and 1 bar. For the first system the protein was immersed into the box with water with a solid seed of Ice I_h in solid state. Meanwhile, the second system was hydrated by adding water molecules randomly in liquid state. After 1 μ s of simulation trajectories the first system was crystallized in Ice I_h (freezing), while the second system (without seed) remained in liquid state. Our MD simulations shows details of in the number of number of hydration shell H-bonds and the reorganization of water-water and water-proteins H-bonds during to cooling, showing a decrease in water density due to an increased in the fraction of the water molecules with a perfect tetrahedral coordination modify the solvent accessible surface area in the protein in dependence with hydrophobic effect.

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Molecular crowding modulates enzymatic activity and structure of β -Gal from *Kluyveromiceslactis*

Noe M^a, Clop P^a, Perillo MA^a, Nolan MV^a

a - IIBYT (CONICET - ICTA y Cátedra de Química Biológica, FCFyN, Universidad Nacional de Córdoba)

β -D-galactosidase [EC 3.2.1.23] (β -Gal) is a soluble enzyme capable of catalyzing lactose hydrolysis into its constitutive monosaccharides: glucose and galactose. β -Gal has been extensively studied because of its nutritional, biotechnological and therapeutic impact. Not only in the cellular milieu but also in situations of technological interest like during encapsulation, the activity of β -Gal has to be evaluated in crowded systems.

β -Gal from *kluyveromiceslactis* was historically isolated as a dimeric active protein of about 250 kDa. But, recent researches have described that in some conditions, like those generated by the presence of crowding conditions in the interior of a non-denaturing electrophoresis gel, a tetrameric form appears. This tetramer has lower activity than the dimeric state.

The objective of the present work was to evaluate the effect of molecular crowding on β -Gal structure and the relationship with its enzymatic activity modulation.

PEG⁶⁰⁰⁰, a non-charged highly water-soluble polymer with well-known effects on water dynamics was used to produce the crowded environment. The enzymatic reaction was evaluated by measuring kinetic parameters of β -Gal against an artificial substrate (ONPG). Protein conformation and thermal stability were analysed by fluorescence spectroscopy. Results showed that molecular crowding induced changes on kinetic parameters of β -Gal: at low molecular crowding agent concentration, an enhancement on enzymatic activity was observed, while at high crowding agent concentration, a qualitative change from a Michaelian to a sigmoidal behavior was observed. The studies on protein conformation showed that molecular crowding affected β -Gal structure: changes on fluorescence emission spectra and protein calorimetric profile were observed.

Changes in protein compactness and hydration could be the responsible for the qualitative change behaviour observed at the highest molecular crowding assayed.

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Molecular dynamic simulations of membrane insertion domain of apolipoprotein A-I dimer

Lynn MS^a, McCarthy A^b, Garda HA^a

a - INIBIOLP (Fac. de Cs. Mèdicas, UNLP-CONICET)

b - CREG (Fac. de Cs. Exactas, UNLP)

The sequence of the central region of apoA-I plays a key role in the reversible association of this protein with lipid membranes, a process that may be of great importance for the mechanism of cholesterol exchange between high density lipoproteins and cell membranes. Data obtained by our group allowed us to postulate a model in which ApoA-I is a dimer forming an intermolecular bundle of two pairs of central helices, inserted perpendicularly to the plane of the membrane. The conformation of the membrane insertion domain would be altered in apoA-I Δ K107. The deletion in the central region of the sequence of the protein would be responsible for the altered cellular responses and the high atherogenic risk present in patients carrying the mutation.

We performed molecular dynamic simulations and essential dynamics analysis of apoA-I and apoA-I Δ K107 dimers inserted in a POPC/CHOL membrane. In this work we present preliminary results of the dynamic behavior of the domain insertions of both proteins.

Pore dynamics in PIP aquaporin closed state

Zerbetto De Palma GG^a, Canessa Fortuna A^b, Aliperti L^c, Armentía L^b, Zeida A^d, Estrín DA^e, Alleva K^a

a - Instituto de Química y Fisicoquímica Biológicas (UBA-CONICET)

b - Universidad de Buenos Aires, Departamento de Fisicomatemática, Cátedra de Física.

c - Laboratorio de Fisiología de Proteínas, IQUBICEN y Facultad de Ciencias Exactas y Naturales, UBA, Buenos Aires, Argentina

d - Departamento de Bioquímica and Center for Free Radical and Biomedical Research, Facultad de Medicina, Universidad de la República, Montevideo, Uruguay.

e - Universidad de Buenos Aires-CONICET, DQIAQF-INQUIMAE, Facultad de Ciencias Exactas y Naturales

PIP are membrane proteins from the aquaporin family that facilitates water transport across plasma membrane in plants. The permeability of these channels are determined by the pore inner structure and regulatory elements in the protein structure. Previous reports pointed to a conserved histidine residue located in loopD as a key element of the gating mechanism, trapping loopD in a closed conformation when protonated at low pH (Tournaire-Roux *et al.*, Nature 2003, Törnroth-Horsefield *et al.*, Nature 2006). Additionally, by means of structural data obtained from the X-ray crystallography of a PIP2, it was suggested that pore lining hydrophobic residues in loopD are also involved in the gating mechanism. These hydrophobic residues are proposed to be inserted into a cavity near the entrance of the pore when the PIP channel is in a closed conformation, modifying the inner structure of the pore.

In this work we combine functional assays and classical molecular dynamics simulations of PIPs to elucidate the relationship between a pore lining hydrophobic residue in loopD (Leu 206 or Leu 197, in BvPIP2;2 or SoPIP2:1 respectively) and the pore inner structure in its vicinity.

Experiments expressing wild type or L206A BvPIP2;2 in *Xenopus* oocytes showed that mutant PIP exhibit less transport inhibition when tested at high intracellular proton concentration. Molecular dynamics simulations of SoPIP2;1 starting from crystal structure and BvPIP2;2 homology models (both in a closed conformation) show a larger pore radius and lower water chemical potential across the pore coordinate near the mutated residue. Intrinsic permeability and hydrogen bonds between water molecules crossing the pore and pore lining residues were also analyzed from simulations. Our results suggest that, when in a closed state, Ala PIP mutants have a less hindered water transit across the pore, providing experimental evidence about the fine details of pore closure promoted after loopD conformational change.

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Rescuing the rescuer: on interaction between ISD11 and the Mitochondrial Acyl Carrier protein.

Herrera MG^a, Pignataro MF^a, Noguera ME^a, Santos J^a
a - Instituto de Química y Físico-Química Biológicas (CONICET-UBA)

Iron-sulfur clusters are essential cofactors in many biochemical processes [1]. ISD11, one of the subunits of the protein complex that carries out the cluster assembly in mitochondria, is necessary for cysteine desulfurase NFS1 stability and function [2, 3]. Several authors have recently provided evidence showing that in the recombinant complex expressed in *E. coli*, ISD11 interacts with the acyl carrier protein (ACP) from this microorganism. This interaction may stabilize ISD11 which is a protein that tends to aggregate when it is isolated. Interestingly, in this complex, ACP interacts with ISD11, not only by electrostatic interactions, but also by means of a long chain acyl group which is bound to Ser36 of ACP by the phosphopantetheine moiety. This molecule intercalates in the core of ISD11 and interacts with the three alpha helices of ISD11 [4, 5]. Considering these results, we decided to evaluate if ISD11 is able to generate a stable complex with de mitochondrial mature human ACP (hACP) form, and this may favors ISD11 solubility and reduce its aggregation prone tendency. We carried out the co-expression of hACP and ISD11 in *E. coli*. This work showed that hACP and ISD11 recognize each other and form a soluble, structured and stable complex which is able to bind to the human NFS1 subunit modulating its activity. Also, we started to study the structure of the hACP-ISD11 complex by X-ray crystallography, obtaining promising results. These findings offer the opportunity of evaluating the mechanism of interaction between ISD11 and NFS1 by biophysical, computational and biochemical tools.

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Steady-state NTPase activity of Zika virus NS3 helicase and its coupling with mechanical work (unwinding activity)

Mikkelsen E^a, Gebhard LG^b, Cababie LA^a, Incicco JJ^a, Rossi RC^a, Gamarnik AV^c, Kaufman SB^a

a - Instituto de Química y Físicoquímica Biológicas y Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires-CONICET

b - Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes-CONICET

c - Fundación Instituto Leloir-CONICET

Zika virus (ZIKV) nonstructural protein 3 (NS3) unwinds double stranded RNA driven by the free energy derived from the hydrolysis of nucleoside triphosphates (NTPs).

In this work we present an initial characterization of the properties of the steady-state NTPase activity of ZIKV NS3 helicase. Initial rates of NTP hydrolysis were measured and the corresponding substrate curves for ATP, GTP, CTP and UTP were obtained. In all cases the resulting substrate curves were well described by hyperbolic functions whose parameter values (k_{cat} and K_M) were obtained by non-linear regression analysis. The order of specificity of NS3 for these nucleotides was evaluated according to the value of the ratio (k_{cat}/K_M).

Additionally, different nucleotides were tested as substrates for the RNA unwinding activity of NS3. Time courses of RNA unwinding reactions were carried-out and samples were resolved by polyacrilamide gel electrophoresis. Results indicate that NS3 acts as an energy transducer using any of the four nucleotides tested as substrates. That is, in all four cases NS3 couples its catalytic properties (NTPase activity) with the ability to perform the mechanical work of translocation along single stranded RNA and unwinding of double stranded RNA.

Stress-related α -synuclein's post-translational modifications: from etiology to early diagnosis of Parkinson's disease

Jimenez E^a, **Cavazzutti GF**^a, Toscani AM^a, Jovin TM^b, Urlaub H^b, Falomir Lockhart LJ^a

a - INIBIOLP, CCT-La Plata, CONICET, Facultad de Ciencias Médicas, UNLP, 60 y 120, La Plata

b - Max Planck Institute for Biophysical Chemistry

Parkinson's disease (PD) is a debilitating and incurable neurodegenerative disease that affects more than 1% of the population above 60 years-old. The combination of genetic predispositions and external factors, such as compounds that promote oxidative stress, promote the gain of toxic function of the protein alpha-Synuclein (aSyn), even in the absence of genetic mutations in its coding gene, SNCA, that ends up forming insoluble amyloid aggregates known as Lewy bodies. The identity of the toxic species responsible for PD remains elusive. In this context, we propose that post-translational modifications (PTMs), as a well-known source of variability of proteins' structure, function, and localization, may be responsible of triggering the pathological role of aSyn in neurons.

We have generated a series of oxidative modifications on recombinant aSyn with a photo-tunable method based on Ru complexes photosensitizer that promote selectively Tyr-residues oxidation through a radical mechanism. This method has been adapted to generate both covalent oligomers and nitrated monomers of aSyn. On the other hand, we have also generated catecholamine-modified and acetylated aSyn. We characterized these proteins variants by mass spectrometry to confirm PTMs positions and exposure of Tyr residues under different conformational states. Biochemical characterization is underway to analyze the effect of each PTM on amyloid aggregation, membrane binding and cytotoxicity in neuron-like cell cultures to evaluate their putative increase in toxicity.

Finally, the systematic and reproducible production of modified aSyn is being used to generate tools and methods, such as Nanobodies (VHHs) or MRM-MS, that could quantitate specific PTMs in circulating aSyn as early biomarkers of PD pathology.

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Structure, function and effect of bisphosphonates on *Trypanosoma cruzi* hypoxanthine phosphoribosyltransferase

Valsecchi WM^a, Rossi RC^b, Santos J^a, Delfino JM^b

a - Departamento de Química Biológica, IQUIFIB, Facultad de Farmacia y Bioquímica, UBA

b - Departamento de Química Biológica e IQUIFIB (UBA-CONICET), Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires

This study investigates the structure-function relationship and the effect of a family of bisphosphonates (BPs) on *T. cruzi* hypoxanthine phosphoribosyltransferase (*Tc*HPRT). This enzyme catalyzes the transfer of ribose-1-phosphate from phosphoribosyl pyrophosphate (PRPP) to hypoxanthine or guanine bases, yielding IMP or GMP, respectively, and has been proposed as a prime target for drugs aimed at treating parasitic diseases since its activity is essential for the survival of trypanosomatids.

Contrary to the long-standing claim that *Tc*HPRT is a monomer in solution, we have previously shown that the protein adopts a tetrameric arrangement. Interestingly, the proteolytic removal of the C-terminal region yields a dimer, showing enhanced activity¹. Here we present a novel kinetic analysis of the *Tc*HPRT activity, which is fully consistent with its inhibition induced by the set of BPs. The proposed kinetic scheme takes into account the occurrence of cooperativity -substrate binding exhibits a sigmoidal mode- and a partially ordered sequence for the binding of substrates and inhibitors.

Remarkably, BPs display a biphasic behavior: activating at low concentrations and inhibiting at high concentrations, showing a competitive fashion. Cooperativity of PRPP binding as well as the activating effect demonstrated by BPs is more pronounced for the tetramer than for the dimer. On the contrary, the inhibitory action is less marked for the tetramer.

Strikingly, the inhibitory effect of BPs is essentially null when assayed against human HPRT, a fact that might be greatly advantageous for the design of selective drugs. We also show here a structural comparison between both variants in order to underline possible reasons for such selective effect of BPs.

This new molecular knowledge becomes most relevant for the design of innovative parasite-targeted therapeutics.

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The effects of aluminum on the plasma membrane calcium pump depends on the biophysics of reconstitution

de Sautu M^a, Ferreira-Gomes M^a, Saffioti NA^a, Rossi RC^a, Rossi JP^a, Mangialavori I^a
a - Departamento de Química Biológica, IQUIFIB, Facultad de Farmacia y Bioquímica, UBA

Aluminium (Al^{3+}) is involved with the pathophysiology of neurodegenerative disorders, such as Parkinsonism dementia and Alzheimer's disease. The mechanisms that have been proposed to explain the toxicity of Al^{3+} are linked to changes in the cellular calcium homeostasis. PMCA is a P-ATPase involved in the regulation of the calcium homeostasis. Its function is to transport Ca^{2+} from cytoplasm towards the extracellular medium against the electrochemical gradient modulating the cytoplasmic Ca^{2+} concentration. In previous works, we already showed that Al^{3+} irreversibly inhibits Ca^{2+} -ATPase activity of PMCA by preventing the dephosphorylation of the pump and that AlCl_3 inhibits calcium efflux mediated by PMCA in HEK293T cells.

The aim of this work was to study if the effect of Al^{3+} on the protein is affected by the enzyme lipidic environment, since this would provide information of the distinctive effect that Al^{3+} would have depends on the lipidic composition of the cell membrane where the PMCA is located. To characterize this effect, we reconstituted the enzyme in different lipidic environment and evaluated its inhibition by Al^{3+} . In addition, to characterize if the differences observed were due to an effect of the lipidic environment on the enzyme structure or an Al^{3+} chelating effect, we measure the interaction of Al^{3+} with different lipidic structures.

In the isolated system, increasing concentration of acidic phospholipids decreased the Al^{3+} effect on PMCA. However, when PMCA is reconstituted with brain extract lipids, Al^{3+} increased its Ca^{2+} -ATPase activity. These results suggest that Al^{3+} affects PMCA both by its binding to the enzyme and modifying the biophysical characteristics of the pump lipid environment.

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The purified preparation of Spf1 P5-ATPase exhibits a Ca-stimulated ATPase activity not related to Spf1

Corradi GR^a, Mazzitelli LR^a, Petrovich GD^b, Grenon P^b, Adamo HP^b

a - IQUIFIB-Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, UBA, Junin 956, 1113 Ciudad de Buenos Aires

b - Instituto de Química y Fisicoquímica Biológicas y Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires-CONICET

The P5-ATPases are the most intriguing members of the large family of primary active transporters known as P-ATPases. Despite the fact that the putative transported substrate has not yet been identified, significant progress is being made toward the biochemical characterization of these proteins. The best characterized P5-ATPase is the Spf1 from *Saccharomyces cerevisiae*. We have previously shown that purified micellar preparations of recombinant His-tagged Spf1 hydrolyzes ATP and it forms the phosphoenzyme intermediate (EP) characteristic of the transport reaction cycle of P-ATPases. Moreover, we have shown that Ca²⁺ modulates Spf1 by decreasing the level of EP. Here we present results suggesting that at least part of the effect of Ca²⁺ is mediated by traces of contaminant proteins that co-purify with Spf1. These are as follows i) when the reaction media contained EGTA and no added Ca²⁺, the rate of Pi production from ATP decreased with time during the first 5 min ii) the addition of Ca²⁺ increased the ATPase between 0 to 5 fold depending on the preparation, and iii) Ca²⁺ stimulated the ATPase activity of the catalytic death mutant Spf1-D487N. The level of EP formed by Spf1 was higher in the presence of EGTA and decreased with the increase in the Ca²⁺ of the media. The magnitude of this Ca²⁺ effect on the EP level showed a positive correlation with the stimulation of the ATPase activity in each preparation of Spf1 tested. Analysis by mass spectrometry of the Spf1 preparation after SDS-PAGE detected the presence of several contaminant proteins. Altogether these results indicate that the activation by Ca²⁺ of a “contaminant” ATPase present in the purified preparation of Spf1 decreases the level of Spf1 EP. Thus the effect of Ca²⁺ on Spf1 may result from the activation of another protein interacting with Spf1 and not an intrinsic feature of the enzyme.

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Thermodynamic characterization of the folding process of *Psychromonas ingrahamii*'s frataxin (pFXN) by using calorimetry and circular dichroism

Batastini NA^a, González-Flecha FL^a, González-Lebrero RM^a, Román EA^a

a - IQUIFIB-Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, UBA, Junin 956, 1113 Ciudad de Buenos Aires

Despite proteins in a family share certain degree of sequence identity and structural conservation, the process by which each one reaches the native structure may be specifically and subtle regulated. In this sense, frataxin family showing a general structural conservation but different cellular roles between eukaryotes and prokaryotes, display several folding mechanism under the same conditions. Frataxins are around 7-10 kcal/mol stable but depending on the molecular species present, particular signatures in its stability modulation were observed. *Psychromonas ingrahamii*'s frataxin (pFXN) shows a pH dependence of the folding Gibbs free energy when going from pH 6 to 8.

The main goal of this work is to perform a thermodynamic characterization of the pFXN folding process and its pH and temperature dependencies. Here, using isothermal titration calorimetry (ITC) we measure the exchanged heat during pFXN folding as a function of pH and temperature, and parallelly, secondary structure changes were registered by far-UV ellipticity measurements.

In a first approach, the change in the measured heat was analyzed in terms of three components: the protonation of the native and denatured states, and the folding of the protein. The enthalpy change for this last process (dH_f) was modeled as a first order Taylor series of pH. Circular dichroism experiments yielded the native and denatured fractions of pFXN as a function of pH at different temperatures and the number of protons involved in the process. This analysis also allowed the calculation of the dependency of the folding entropy on pH. Our results points to an exhaustive characterization of the folding process of pFXN with a robust thermodynamic description of the process yielding not only the folding enthalpy and entropy but also their derivatives respect to the pH and the changes in heat capacity for the folding process and for basal states.

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A protein from poisonous snail eggs perforates cell membranes combining a lectin chain with a Pore-Forming Toxin

Giglio M^a, **Ituarte S^a**, Milesi V^b, Prieto ED^c, Dreon M^a, Maté S^a, Caramelo JJ^d, Heras H^a

a - Instituto de Investigaciones Bioquímicas de La Plata "Prof. Dr. Rodolfo R. Brenner", INIBIOLP. CONICET CCT La Plata

b - Instituto de Estudios Inmunológicos y Fisiopatológicos, IIFP. CONICET CCT La Plata - UNLP

c - Instituto de Investigaciones Físicoquímicas Teóricas y Aplicadas, INIFTA. CONICET CCT La Plata - UNLP

d - Instituto de Investigaciones Bioquímicas de Buenos Aires, IIBBA. CONICET- Fundación Instituto Leloir

Cellular membranes are crucial for life and animals have evolved many effective strategies for damaging them. We found that the reduced number of predators of the eggs from the invasive snails *Pomacea canaliculata* and *P. maculata* is largely explained by the occurrence of noxious defensive proteins. In particular, a 400-KDa tetrameric protein, named PV2, that triggers neurological symptoms in mice. The aim of this study was to look further into the structural aspects of this toxin in *P. maculata* (PmPV2), and its interaction with membranes.

Here we show that PmPV2 is a pore-forming toxin (PFT) combining two immune proteins: a tachylectin module linked to a membrane attack complex/perforin (MACPF) module. Homology modeling and bioinformatics indicated that the tachylectin domain belongs to the HydWA family, with a 6 blade β -propeller structure. The MACPF fold has the characteristic twisted and bent β -sheet core with two flanking transmembrane helixes and it is fused with a novel C-terminal accessory domain restricted to invertebrates MACPFs. Through Atomic Force Microscopy (AFM), we observed that PmPV2 inserts into POPC/Cho lipid bilayers and oligomerizes into ring-like structures compatible with pores, causing membrane thinning. We tested the effect of PmPV2 on intestinal cell cultures, since enterocytes would be the first cells the toxin would encounter when ingested by a predator. Viability of Caco-2 cell cultures decreased in a dose-dependent manner, while patch-clamp experiments indicated alterations in the plasma membrane conductance, compatible with pores of ~ 14 nm. The toxin has a remarkable structural stability in a wide range of pH values (2.0-10.0) as determined by fluorescence spectroscopy, small angle X ray scattering and circular dichroism suggesting it can withstand the passage through gut.

This study provides the first evidence into the molecular mechanism of membrane interaction and pore formation for an animal toxin with a MACPF domain.

Dehydron-functionalized His335 from a histidine kinase participates in phosphotransfer activity

Bortolotti A^a, Costa JG^a, Villalba JM^a, Fernández A^b, Cybulski L^a

a - Departamento de microbiología. Facultad de Ciencias Bioquímicas y Farmacéuticas. Universidad Nacional de Rosario. CONICET.

b - INQUISUR (CONICET-UNS)-Instituto de Química del Sur

DesK histidine kinase is an integral membrane thermosensor *in Bacillus*. It is the key protein of a regulatory pathway which controls the physical state of membrane lipids. Its intracellular domain catalyses the ATP-dependent auto-phosphorylation of His188, in a signal-regulated fashion. Once phosphorylated, it transfers the phosphate group to the Asp54 of the effector protein, DesR. Phosphorylated DesR is the active form of the protein that binds DNA, promoting expression of the desaturase gene.

Hydrogen bonds of the peptide bond which are not totally protected from water attack are called "dehydrons,". Dehydrons play an important role in the activation of catalytic residues because they function as activators of nucleophilic groups. This activation results from the induction of chemical basicity in interfacial water molecules, promoting deprotonation of adjacent nucleophiles.

Yap View 0.666 software was employed to identify dehydron bonds on the X-ray pdb crystal structures of the cytoplasmatic domain of DesK. His335 forms dehydron bonds with Gly339 and Ser332. Dehydron-functionalized His335 would act like nucleophile which delocalizes electrons promoting the phosphotransfer. Simple H335A and double H188V-H335V variants were constructed to prove this hypothesis. Both mutants lack kinase activity, but maintain parental phosphatase activity. To test the role of His 335 dehydrons, we constructed mutants G339V and S332V, which result in dehydration of hydrogen bonds His 335-G339 and His335-S332 and according to our hypothesis would result in deactivation of His335. These mutants showed no kinase activity, highlighting the critical role of dehydrons in functionalizing catalytic residues.

Doxycycline interacts with tau protein forming less toxic species: a biophysical approach

Medina L^a, Vera CC^a, González Lizarraga MF^a, Sequeira S^a, Parrales V^b, Bizat N^b, Ramos Souza Barbosa L^c, Raisman-Vozari R^b, Chehín R^a

a - IMMCA (Instituto de Investigación en Medicina Molecular y Celular Aplicada)

b - ICM - Institut du Cerveau et de la Moelle Epinière - Hôpital Pitié Salpêtrière

c - IFUSP, São Paulo, Brazil

Amyloid aggregation of specific proteins seems to be the common biological event involved in neuronal death in different neurodegenerative diseases. Interfering with this abnormal aggregation could slow down or stop neuronal death. However, despite the huge effort invested, traditional drug discovery strategies have fallen. The exploration of new uses for approved drugs provides a useful alternative to fill the gap between the increasing incidence of neurodegenerative diseases and the long-term assessment of classical drug discovery technologies.

Doxycycline, a second generation tetracycline, prevents neurodegeneration in animal models. We have been able to demonstrate the ability of this antibiotic to reshape alpha-synuclein oligomers into off-pathway non-toxic species, unable to destabilize biological membranes and thus, cell viability. Herein, we extended these studies to Tau, whose aggregation and phosphorylation are involved in neurodegeneration in Alzheimer's disease.

Using fluorescence, infrared and SAXS spectroscopy we analyzed tau conformational changes in order to understand the molecular events leading to aggregation. According with our results, heparin can induce tau aggregation with a classical sigmoidal behavior and the presence of doxycycline strongly inhibits the amyloid fibrils formation, although oligomeric species are still formed. Doxycycline does not inhibit the GSK3-beta activity, suggesting that the antibiotic may not affect the phosphorylation pattern. Since results suggest that doxycycline may form different tau oligomeric species, we are evaluating its effects in a *C. elegans* model expressing human tau and in SH-5YSY cells. We have also tested doxycycline effect in other protein aggregation models without the effects obtained with alpha-synuclein and Tau.

Our results strongly suggest that doxycycline could be a selective inhibitor of the main neurotoxic oligomeric species.

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Effects of the application of ultrasound on the thermodynamic properties of collagen present in meat industry waste

Velázquez DE^{a,b}, Latorre ME^{a,c}

a - Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina

b - Instituto de Física de Materiales Tandil (IFIMAT), Facultad de Ciencias Exactas, UNCPBA, Tandil, Argentina

c - Departamento de Tecnología y Calidad de los Alimentos, Facultad de Ciencias Veterinarias, UNCPBA, Tandil, Argentina

Collagen represents the most abundant structural protein in the body of mammals ($\approx 30\%$ of total proteins). Animal tissues, leathers, tendons and bones are considered waste of the meat industry, being tissues rich in collagen. The industry is concerned with reducing its waste and obtaining by-products. It is known that ultrasound treatment (US) affects the properties of cell membranes and tissue-associated proteins. There are several studies on the effects of the application of the US as a whole to the extraction treatment, chemical and/or thermal, of collagen from waste, however the effect of the US as pre-treatment on leather has not yet been studied. Changes in the physicochemical characteristics of the collagen present in leathers pre-treated with US could favor and/or improve the processes and/or characteristics of the obtained collagens (native and gelatin). In this work, the thermodynamic characteristics of the collagen present in pigs treated with US were studied. Different treatments were applied with US (40 kHz, Amp: 80%, On / Off: 20 "/ 20") during total times 0 (control); 1; 2 and 5 min. Differential scanning calorimetry (DSC) tests were carried out at 10 K/min. The obtained results indicate a decrease in enthalpy of total denaturation (J/g) with the increase in treatment time. At the same time, the curves showed differences in the temperatures of beginning and end of the process of collagen denaturation after different treatment times. From the information obtained from the thermodynamic analysis, this work aims to deepen knowledge about the chemical, rheological and functional properties of post-US collagen. The thermal characterization carried out provides relevant information on the efficient reuse of waste from the food sector.

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FapC: a functional amyloid from the opportunistic pathogen *Pseudomonas aeruginosa*

Siri M^a, Herrera M^a, Celej MS^a

a - CIQUIBIC, Departamento de Química Biológica, Facultad de Ciencias Químicas, CONICET, Universidad Nacional de Córdoba, Córdoba, Argentina.

Proteins can self-assemble into a minimum-energy fibrillar conformation known as amyloid. The thermodynamic stability of the amyloid state relative to the native structure decreases as a function of the length of the protein, presumably due to topological constraints associated with the packing of a long polypeptide chain into the fibril core. It has been proposed that proteins with 300-500 residues might have been selected to minimize the risk of amyloid formation (Knowles et al, Nat. Rev., 2014, 384.) This interpretation is based on the analysis of the thermodynamic stability of pathological amyloids, generally formed by relatively short peptides or proteins, or proteolytic fragments of larger precursors. However, different organisms use amyloids for functional purposes. FapC is the recently discovered protein responsible for the formation of the functional amyloid identified in *Pseudomonas*. This amyloid acts as an adherent and aggregation factor in the bacteria biofilm, providing mechanical robustness and resistance against environmental stressors. With the aim of understating the biological importance of the amyloid state in health and disease, we want to determine the conformational stability of the functional amyloid FapC.

FapC from the opportunist pathogen *P. aeruginosa* PAO1 was expressed and purified from *E. coli*. The recombinant protein formed fibrils under *in vitro* conditions, which were characterized by Thioflavin T fluorescence, FT-IR spectroscopy and electron microscopy. Conformational stability was evaluated by chemically induced equilibrium denaturation studies. Results will be discussed in comparison with reported information on pathological amyloids.

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Fibrillar aggregates of an amyloidogenic variant of apolipoprotein A-I. Structure and cellular reactivity

Gisonno RA^a, Prieto ED^b, Curto LM^c, Gorgojo JP^d, Rodriguez ME^d, Schinella GR^{a,e}, Tricerri MA^a, Ramella NA^a

a - INIBIOLP, CCT-La Plata, CONICET, Facultad de Ciencias Médicas, UNLP, 60 y 120, La Plata

b - INIFTA-CONICET, Fac. de Cs. Exactas, UNLP. Suc. 4-C.C. 16. (1900) La Plata, Argentina. Fac. de Ingeniería, UNLP, 1 y 47 (1900) La Plata, Argentina

c - Instituto de Química y Físicoquímica Biológicas y Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires-CONICET

d - Centro de Investigación y Desarrollo en Fermentaciones Industriales (CINDEFI)

e - Facultad de Ciencias Médicas, UNLP

Different protein conformations may be involved in the development of the clinical manifestations associated to human amyloidosis¹. Even though a fibrillar conformation is usually the signature of damage in the tissues of patients, it is not clear whether this specie is *per se* the cause or the consequence of the disease. Human apolipoprotein A-I (apoA-I)-derived amyloidosis is poorly known. About 20 naturally occurring mutations have been described in patients suffering multiple organ failure. The reason of apoA-I variants misfolding and aggregation is far to be known. We have previously incubated different natural variants under mild acidic conditions and in the presence of ligands as heparin, and shown that under short periods and low protein concentrations mainly oligomeric species are obtained². Here we set up to characterize the folding of a natural variant (apoA-I_{Lys107-0}) which induces amyloidosis plus severe atherosclerosis. With the hypothesis that a pro-inflammatory micro environment could favor protein misfolding, we oxidized this variant under controlled amounts of H₂O₂ and incubated by 30 days at pH 6.0. LC-MS/MS mass spec analysis indicated oxidation of Met 148 and Circular dichroism analysis showed a loss in the alpha helical structure. Transmission electron and atomic force microscopy confirmed the presence of fibrils which strongly bind the amyloid-indicative probe Thioflavin T. Interestingly, this conformation was reactive to induce the formation of neutrophil extracellular traps (NETs) from healthy donor. Our results suggest that the fibrillar conformation of this variant may elicit a pro inflammatory cellular response which participate in the severity of the clinical chronic landscape.

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Function and stability: desing and characterization of human frataxin variants

Castro I^a, Sewell E^a, Santos J^a

a - Instituto de Química y Físicoquímica Biológicas (UBA-CONICET)

Frataxin (FXN) is a mitochondrial protein involved in [Fe-S] cluster formation, it is an allosteric activator of the protein complex NFS1/ACP-ISD11/ISCU. FXN deficient expression results in Friedreich's ataxia. This deficient expression is caused by a decrease in the *fxn* gene transcription because of the amplification of repetitive sequences in the first intron, or by the presence of mutations that lead to FXN variants with functional deficiency or decreased stability, with more tendency to aggregation or with altered internal mobility.

Here, we show results concerning the design and study of a set of human FXN variants. Our goal was to find FXN functional variants with increased thermodynamic stability *in vitro* and decreased tendency to degradation in the cell. We used the stability predictors FOLDX, Dynamut and PoPMuSiC and we chose eight point-mutants which are predicted as more stable than the wild-type protein. We discarded mutants involving positions that may affect the interaction with the protein complex NFS1/ACP-ISD11/ISCU and the positions located near reported pathological mutations. The recombinant variants were successfully expressed in *E. coli* and purified to develop the functional and structural characterization.

Also, we studied the effect of the combination of a stabilizing point mutation with the mutation K147R. Although the mutation K147R was reported that inhibit its ubiquitination and degradation by the proteasome, it is thermodynamically less stable than the wild-type protein. We observed that double mutant is more stable than the FXN K147R and it is functional.

IsTRP, an unusual thioredoxin

Pórfido JL^a, Bellanda M^b, Sturlese M^c, Baggio C^b, Vignaga S^b, Bisio H^d, Salinas G^e

a - Institut Pasteur Montevideo, Uruguay

b - Dipartimento di Scienze Chimiche, Università degli Studi di Padova

c - Dipartimento di Scienze del Farmaco, Università degli Studi di Padova

d - Département de microbiologie et médecine moléculaire, Faculté de Médecine, Université de Genève

e - Departamento de Biociencias, Facultad de Química, Universidad de la República/Institut Pasteur de Montevideo

The thioredoxin fold is broadly distributed in nature and provides a scaffold for many proteins with diverse functions. It is the founding member of the thioredoxin superfamily, which includes thioredoxins (Trx), glutaredoxins (Grx), disulphide isomerases, etc. The structure of Trx consists of the basic Trx domain (one β -sheet, built with 4 β -strands, and 3 α -helices that connect those strands) plus an additional β -strand and α -helix at the N-terminus. They have a redox active motif defined as CXXC, typically CGPC. In our lab, an atypical Trx has been described, which lacks redox activity and has, instead, the ability to bind iron-sulfur clusters (ISC). The ligand is a Fe₂S₂ cluster bound through homodimerization by both cysteines from each monomer, independently of glutathione or other low molecular weight thiols. Due to its properties, this protein has been named IsTRP (Iron-sulfur thioredoxin-related protein). It is worth mentioning that it is exclusively encoded in the genomes of cestodes of the order Cyclophyllidea, which are pathogens for humans as well as for other animals.

The structure of IsTRP, determined by NMR, clearly belongs to a Trx with some unusual characteristics. Particularly, when comparing it with redox active Trx, it can be seen that IsTRP has a more flexible active site and a more compact and hydrophobic core. Those properties could be related to the loss of redox activity and the acquisition of ISC-binding properties. We have identified some key residues in IsTRP that could explain its unique properties and we are currently evaluating different mutants in those residues in order to determine whether the redox activity can be restored.

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Multiparametric analysis of Pyrenyl-maleimide labeled human apolipoprotein A-I cystein mutants.

Tárraga WA^a, Gonzalez MC^a, Falomir Lockhart LJ^a, Garda HA^a

a - INIBIOLP, CCT-La Plata, CONICET, Facultad de Ciencias Médicas, UNLP, 60 y 120, La Plata

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.

ApoA-I is composed of several amphipathic alpha-helices. In water solution, they form a bundle with poorly characterized tertiary and quaternary structures. Depending on the concentration, apo A-I self-aggregates to form dimers and oligomers of higher orders. It also interacts with phospholipids and forms discoidal HDL (dHDL).

The aim of the present study is to obtain information on the apoA-I self-aggregation in solution important for understanding the mechanisms of HDL generation. Six cysteine mutants (K107C, K133C F104C, L137C, K226C and F225C) were specifically designed and labeled with pyrenyl-maleimide in positions corresponding to hydrophilic and hydrophobic faces of helices 4, 5 and 10. The monomer and excimer fluorescence of the labeled proteins were registered as a function of total apoA-I concentration; and several mathematical models were developed and compared to evaluate dissociation constants (Kds) corresponding to the different oligomerization events.

The labeled mutants were stable in solution as indicated by tryptophan fluorescence. With the exception of F104C, they were biologically active since they can interact with phospholipids to form dHDL. Fluorescence emission spectra of pyrene showed excimer formation only in the case of labeled F225C, K133C and K226C mutants, indicating the participation of helices 5 and 10 in the contact regions during certain oligomerization. Changes in p-value of monomer emission also reported conformational changes during apoA-I oligomerization.

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NAD⁺ inhibits GAPDH aggregation by preventing nitrosative stress-induced conformational changes

Muñoz Sosa CJ^a, Romero JM^a, Curtino JA^a, Carrizo ME^a

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

Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is a multifunctional protein involved in cell death processes frequently associated with oxidative/nitrosative stress. S-nitrosylation of GAPDH facilitates its binding to the E3-ubiquitin-ligase Siah1, which has a nuclear localization signal that promotes the entrance of the protein complex to the nucleus causing apoptosis. GOSPEL (GAPDH's Competitor Of Siah1 Protein Enhances Life) protein interacts with GAPDH and interferes with the binding between GAPDH and Siah1, inhibiting their apoptotic effect.

Oxidative/nitrosative stress also induces the aggregation of GAPDH in vitro, which is in accordance with the presence of the enzyme in insoluble aggregates found in some neurodegenerative diseases.

Evidence provided by our laboratory (1) indicates that in the presence of nitric oxide (NO) GOSPEL co-aggregates with GAPDH increasing its aggregation rate. GAPDH Cys152 plays an essential role in this process since their S-nitrosylation initiates the oxidative modification that triggers the formation of disulfide-bonded aggregates. Both GAPDH aggregation and GAPDH-GOSPEL co-aggregation were inhibited by NAD⁺.

Here we present preliminary circular dichroism studies indicating that NAD⁺ inhibits the conformational changes induced by the NO donor NOR3. We also report the X-ray structure of GAPDH treated with NOR3 in the presence of NAD⁺. In addition to the NAD⁺ density the difference map exhibits a positive density connected to the SH of Cys152 that could only be attributed to NO. These results suggest that NAD⁺ could be inhibiting the NOR3-induced aggregation by stabilizing NO-GAPDH conformation.

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Structural and kinetic stability of *Pomacea* egg carotenoproteins

Pasquevich MY^{a,b}, Heras H^{c,b}

a - Cátedra de Bioquímica y Biología Molecular, Facultad de Ciencias Médicas, UNLP

b - Instituto de Investigaciones Bioquímicas de La Plata "Profesor Doctor Rodolfo R. Brenner" (UNLP-CONICET)

c - Cátedra de Química Biológica, Facultad de Ciencias Naturales y Museo, UNLP

The freshwater snails of *Pomacea* genus deposit pigmented eggs above the water. Pigmentation is provided by abundant oligomeric carotenoproteins which supply nutrients and photoprotection to the growing embryo. Homologous egg carotenoproteins have evolved different defensive roles. For instance, in the invasive canaliculata clade, PcOvo and PmPV1, provide bright reddish colors, a warning coloration advertising the presence of toxins in the eggs; on the contrary, in the non-invasive bridgesii clade, PsSC with a pale color (non-warning signal), have a remarkable lectin activity not found in the canaliculata clade. The aim of our work is to begin to unveil the structure-function evolution of *Pomacea* carotenoproteins. We studied the structural stability and the resistance to proteolysis of PpPV1, a pale *P. patula* carotenoprotein member of the flagellate clade (the most basal of the genus) and compare it with the other clades of *Pomacea*. The stability of PpPV1 was studied against pH, temperature and guanidinium chloride (GdnHCl), using spectrophotometry and spectrofluorometry; the resistance to proteolysis was determined by proteinase K and *in vitro* simulated gastrointestinal digestions. PpPV1 remained stable over temperatures up to 85°C and over a wide range of pH (4.0-12.0), with slight alterations at pH 2.0. The unfolding equilibrium to GdnHCl showed that while 50% of PmPV1 and PcOVO are unfolded at 2.7 M, basal PpPV1 do so at 5.4 M. The oligomer was resistant to proteolysis, a feature that, together with the resistance to SDS denaturation, is shared by kinetically stable proteins. The study revealed that all *Pomacea* carotenoproteins share similar resistance to thermal denaturation, extreme pHs and proteolysis. However, PpPV1 has a remarkable greater resistance to chemical denaturation than its homologs. The loss of stability along evolution could have contributed to the gain of new functions, such as acquiring a strong warning coloration in the most derived species.

Structural and mechanistic dissection of the syncytial respiratory virus nucleocapsid and its interaction with the RNA polymerase cofactor phosphoprotein

Salgueiro M^a, Alvarez Paggi D^a, Esperante SA^a, Oliveira GA^b, de Prat Gay G^a

a - Fundación Instituto Leloir and IIBBA-CONICET

b - Biochemistry and Molecular Genetics Department, University of Virginia

Infection by respiratory syncytial virus (RSV) is the major cause of lower respiratory disease, particularly in infants. Genome replication and transcription of RSV is operated through a macromolecular complex composed of the RNA dependent RNA polymerase (L), a phosphoprotein cofactor (P), the nucleocapsid protein (N), and the transcription anti-termination factor (M2-1). RSV genome consists of a single negative RNA strand coated by the N protein forming the nucleocapsid. P interacts with the nucleocapsid, potentially eliciting a conformational transition that partially exposes RNA for L to carry out RNA synthesis. We produced recombinant N, which is expressed as decameric rings with tightly bound RNA. We investigated the stability of the N-Rings and found that low Gdm.Cl concentrations cause RNA dissociation and concomitant irreversible aggregation of the protein moiety. Increase in temperature causes irreversible aggregation of the N-Rings, but these are highly resistant to urea denaturation where 4.5 M concentration and long incubations are required to expose the otherwise inaccessible RNA, while maintaining the decameric arrangement. We next focused on the N-P interaction by electrophoretic mobility assays and show that they interact with a stoichiometry of one N-Ring per P-Tetramer. Fluorescence spectroscopy show a first binding event with a low molecularity and small anisotropy change followed by a large anisotropy change in excess of N, presumably corresponding to a high molecularity oligomer. We started a structural characterization of the species by cryoelectron microscopy and found that the N-Rings can form 9-11mers, and show a preliminar near atomic resolution.

We also present negative staining electron microscopy of the N-Ptet complex showing heterogeneous particles, due to the disorder present in P. Our results constitute the basis of a structure and mechanistic dissection of the assembly of the RNA polymerase complex of this virus.

Thermal storage of β -galactosidase immobilized in Inclusion Bodies

Flores SS^a, Perillo MA^a, Nolan MV^a, Sanchez J^a

a - IIBYT (CONICET - ICTA y Cátedra de Química Biológica, FCEfYN, Universidad Nacional de Córdoba)

In our laboratory we produce a recombinant β -Gal in *E.coli*. In controlled conditions we favor the overexpression of β -Gal rearranged in the formed of inclusion bodies ($IBs_{\beta-Gal}$). Interestingly, the $IBs_{\beta-Gal}$ exhibits not only higher activity but also higher resistance to temperature and pH inactivation with respect to the soluble β -Gal.

As $IBs_{\beta-Gal}$ represent an immobilized protein sample we are studying the properties of this naturally occurring structures as a reusable catalytic device. Therefore, the storage temperature of $IBs_{\beta-Gal}$ is critical in its reusability achievement. In this work we evaluate three storage temperatures (ST) on the structure-function relationship of $IBs_{\beta-Gal}$. We demonstrate that the ST modulates the recovery of $IB_{\beta-Gal}$ catalytic activity measured at 37°C. At ST=4°C the activity decreases, but in frozen conditions the activity grows up vs the conservation time. At ST=-20°C we measured the lowest activities. Surprisingly at ST=-80°C the highest $IB_{\beta-Gal}$ activities were obtained. In full hydrated conditions (4°C) the enzyme desorbed from IBs. In contrast, the non-hydrated (frozen) conditions prevent desorption process and consequently the enzyme activity lost. Moreover, the freezing rate, which affects the crystal structure of water, seems to determine the enzyme integrity. At ST=-20°C the bigger size of ice crystals could be related to certain unfolding of a protein population within IBs. Conversely, the small crystal structures (ST=-80°C) preserved a high-quality conformation concomitantly with a $IB_{\beta-gal}$ restructuring, The intrinsic fluorescence parameters demonstrate that I_{max} shifts to lower values as the ST decrease from de desorbed β -Gal ($I_{max} = 349$ nm) to the $IBs_{\beta-Gal}$ sample kept at -80°C ($I_{max,-80} = 341$ nm). We propose that the best storage condition is at -80°C where the microorganism contamination is prevented and the freezing rate would improve β -Gal catalytic activity.

Acknowledgments

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Ubiquitin-Pterin fluorescent adduct formation under UV-A radiation

Reid LO^a, Dántola ML^a, Marin ML^b, Lhiaubet V^b, Thomas AH^a

a - Instituto de Investigaciones Físicoquímicas Teóricas y Aplicadas (INIFTA), UNLP, La Plata, Argentina

b - Instituto de Tecnología Química UPV-CSIC, Valencia, España

Different compounds are able to induce photosensitivity as a result from exposure to certain molecules and light; these includes phototoxic and photoallergic reactions. The photoallergy normally involves a covalent binding between proteins and photosensitizer agents leading to the formation of a complete photoantigen, which may trigger a hypersensitivity reaction due to a cell-mediated immune response.

Pterins belong to a family of heterocyclic compounds present in a wide range of living systems and this compounds are able to photosensitize damage in proteins, DNA and their components by Type I (electron -transfer) and Type II (singlet oxygen) mechanisms.^{1,2}

Therefore, given the biological and medical relevance of the photosensitizing properties of pterins, the aim of this work is to study if pterin (Ptr), the parent and unsubstituted compound of oxidized pterins, is able to generate photoadducts with proteins and establish its photoallergic potencial. For this study, aqueous acidic solution of Ubiquitin (Ub) and Ptr were irradiated ($I_{\text{ex}}=350$ nm) at room temperature. Ub was used as a model protein given that is a small (8.5 kDa) regulatory protein, which has only one Tyrosine (Tyr) residue and none Tryptophan residue. The irradiated solution were analyzed by UV/visible spectrophotometry, HPLC, fluorescence spectroscopy and SDS-PAGE.

Under UV-A radiation Ptr is able to form an adduct with Ub, and this reaction is much more efficient in the absence of O₂. The spectroscopic analysis reveals that the emission and the excitation spectrum are similar to those corresponding to Ptr, as well as the fluorescence lifetime. On the other hand, as a consequence of the photosensitized process, the protein suffers oligomerization mediated by Tyr dimers, and also a fragmentation can occur, which is dependent of the oxygen concentration in the atmosphere.

Contact: ldantola@inifta.unlp.edu.ar

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Zn(II) affinity optimization during the natural evolution of NDM carbapenemase

Delmonti J^a, Palacios AR^a, Vila AJ^a

a - Instituto de Biología Molecular y Celular de Rosario, IBR-CONICET-UNR, Rosario, Argentina

The clinical efficacy of carbapenems, last resort antibiotic used against multiresistant bacteria, is threatened by the dissemination of genes coding for potent carbapenemases, enzymes which hydrolyse and inactivate these drugs. Among them, New Delhi Metallo- β -lactamase (NDM), a Zn(II)-dependent enzyme produced by *Enterobacteriaceae*, has experienced the fastest and widest geographical spread. Up to date, 24 natural variants of NDM have been reported in clinics. NDM variants differ by a few mutations outside the active site, with substitution M154L being the most frequent. It has been reported this mutation enhances resistance upon Zn(II) limiting conditions without imparting protein stabilization. Moreover, all double mutants containing M154L substitution have shown the highest resistance under zinc deprivation conditions. Between them, the variant NDM-15 with mutations M154L and A233V was the less susceptible to metal depletion.

In the present work, we aim to study changes in biochemical and biophysics characteristics produce by the substitution M154L. We focus on alleles NDM-15 (M154L A233V) and NDM-4 (M154L). Although these variants have not shown differences in steady state activity, they differ in the accumulation of key reaction intermediates formed during carbapenems hydrolysis. UV-Vis spectroscopy and paramagnetic NMR studies with Co(II) substituted variants have not reflected changes on active site. Based on the idea that the alteration in the hydrolysis mechanism of carbapenems can be a consequence of improving the Zn(II) binding affinity, we performed competition experiments with Zn(II) chromophoric chelators. We prove that substitution M154L impacts only on the first binding event, without changing the affinity of the second Zn(II) ion. Overall, these results suggest that optimization of protein Zn(II) affinity is driving the natural evolution of NDM.

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Metal fluoride effects on the Plasma Membrane Ca²⁺ ATPase: Characterization of the fluoride-stabilized phosphoenzyme analogues

Saffioti NA^a, Riesco AS^a, de Sautu M^a, Rossi RC^a, Berlin J^b, Ferreira-Gomes M^a, Rossi JP^a, Mangialavori I^c

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

b - Department of Pharmacology, Physiology and Neuroscience, New Jersey Medical School, Rutgers University, Newark, NJ, 07103, USA.

c - Instituto de Química y Físicoquímica Biológicas y Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires-CONICET

The Plasma Membrane Ca²⁺ Pump (PMCA) is one of the most important participants in cytoplasmic Ca²⁺ regulation, and belongs to the P-type ATPases family (P-ATPases). The Albers and Post model postulates that P-ATPases exist in two main conformations E₁ and E₂ that can be phosphorylated forming E₁-P and E₂-P. Recently, the first structural model of PMCA was obtained by cryo-electronmicroscopy¹. In the context of progresses in the study of PMCA structure, new methods to stabilize this protein in different conformations are necessary in order to investigate the relationship between its structure and function.

In this work we studied the effects of fluoride complexes of aluminium (AlF_x), beryllium (BeF_x) and magnesium (MgF_x) on PMCA by means of measurement of enzyme activity, and employing fluorescent or hydrophobic photoactivatable probes. These complexes stabilize different states of the phosphorylated intermediates in other P-ATPases, but they have never been tested on PMCA. Our results show that the three complexes behaved as slow reversible inhibitors of Ca²⁺-ATPase and phosphatase activities by preventing phosphorylation from ATP. The inhibition was not competitive with Ca²⁺ in the case of AlF_x and MgF_x, in contrast with results observed in other P-ATPases. The affinities for AlF_x, BeF_x and MgF_x increased slightly when PMCA was activated by calmodulin. On the other hand, the water content in the nucleotide binding site increased as a result of the conformational change produced by fluoride complexes binding. This effect occurred also when PMCA was phosphorylated from ATP, which allowed to measure the concentration of the phosphorylated intermediate in real time. In summary, our results show that these fluoride complexes are a useful to reveal the properties of different phosphorylated intermediates that are involved in the mechanism of hydrolysis of ATP by the PMCA.

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Activation of the membrane fluidity sensor, Ire1, is modulated by a transmembrane zipper of polar residues

Almada JC^a, Dunayevich P^b, Colman Lerner A^b, Cybulski L^a

a - Departamento de microbiología. Facultad de Ciencias Bioquímicas y Farmacéuticas. Universidad Nacional de Rosario. CONICET.

b - Instituto de Fisiología Biología Molecular y Neurociencias (IFIBYNE). Universidad Nacional de Buenos Aires. CONICET.

Receptor kinases play a key role in transmitting signals: they are essential for detecting physicochemical stimuli in the environment and for activating adaptive cellular responses. Our research studies the molecular biophysical activation mechanism of the protein kinase *Inositol-required enzyme 1* (Ire1), which locates in the endoplasmic reticulum (ER) of eukaryotic organisms.

The folding capacity of proteins in the ER is monitored by a signaling network called Unfolded Protein Response (UPR). Ire1 is an ER transmembrane sensor which is activated by two different stimuli: 1) by misfolded proteins and 2) by a decrease in the fluidity of the ER membrane.

The activation of Ire1 promotes dimerization which stimulates its activity. Ire1 cleaves an mRNA that codes for a transcription factor that induces the expression of 381 genes involved in the biosynthesis of lipids and chaperones.

We focus on responding how the transmembrane segment (TMS) can detect changes in membrane fluidity? It has a group of polar hydrogen-bond forming amino acids that are located on the same face of the TMS, as a heptad zipper and they could form reversible inter-helix hydrogen-bonds.

We used CRISPr Cas9 technology to introduce mutations in the potential zipper to either reinforce or to weaken it. We found that introduction of additional hydrogen-bond forming amino acids to reinforce the zipper face resulting in active proteins in the absence of a stimulus. On the contrary, replacing hydrogen-bond forming amino acids from the original zipper to alanines weaken it, resulting in inactive proteins regardless of the stimulus.

To sum up, we identified a potential hydrogen-bond zipper located in the TMS of Ire1, which could be responsible for sensing eukaryotic membrane fluidity. The activation mechanism would imply a dimerization through the hydrophilic face delimited by the zipper of polar residues forming hydrogen bonds in less fluid membranes, which would result in activation of the protein.

Conserved proline residues reveal conformational dynamics as the basis for oligomerization and aggregation routes in the syncytial respiratory virus NS1 interferon response inhibitor

Conci J^a, Oliveira GA^b, Alvarez Paggi D^a, Duarte Pagani T^b, Esperante SA^a, Borkosky S^a, Alonso L^a, Borges R^b, de Prat Gay G^a

a - Fundación Instituto Leloir

b - Instituto de Biofísica Carlos Chagas Filho (IBCCF). Federal University of Rio de Janeiro. Brazil

Respiratory syncytial virus is the only member among mononegavirales with a distinctive innate immunity inhibition response that relies on two non-structural proteins, NS1 and NS2 with no known homologues. NS1 displays several activities reported in connection with blocking immune response that must rely on a large number of binding partners. We have previously investigated its folding and quasi-spontaneous oligomerization route that yields soluble and regular spherical particles of 30 nm (NS1SOs). We tackle the analysis conformational dynamics determinants behind the oligomerization and a newly described ionic strength dependent aggregation route. Single and double replacement of conserved proline residues (P67A and P81A) show local and long range effects on the dynamics, which led us to tackle an experimental analysis of stability, oligomerization, and aggregation routes. P81A mutant displays a more rigid dynamics, and is less prone to oligomerization and aggregation, and shows the largest stabilization towards chemical denaturation. Overall, P67A is less stable and more prone to oligomerization and aggregation. The double mutant shows different compensation of the oligomerization and aggregation routes compared to stability to unfolding, while unfolding kinetics is governed by P67. Changes in the denaturation slope, m value, reports differences in exposure of surface area between folded and unfolded states, where the largest change is observed for the P81A mutant, consistent with a more rigid folded state with lower accessibility to the solvent. Residual structure of the unfolded state is evidenced by a large fluorescence transition after the global tertiary and secondary structure unfolding took place. The insoluble aggregation competes with the soluble NS1SOs route, suggesting two plausible species in the crowded cellular environment that may bring together many of the binding partners reported.

Does *Protein A* mirror image exist in solution?

Vila JA^a, Martin OA^a, Scheraga HA^b, Vorobjev Y^c

a - Grupo de Bioinformática Estructural-IMASL-CONICET-UNSL.

b - Baker Laboratory of Chemistry

c - Russian Academy of Sciences

There is abundant theoretical evidence indicating that a mirror image of *Protein A* may occur during the protein folding process. However, as to whether such mirror image exists in solution is an unsolved problem. Here we provide sound theoretical evidence indicating that the use of a mutant of *Protein A*, namely Q10H, could be used to detect the mirror image conformation in solution. Indeed, our results indicate that the native conformation of the *Protein A* should have a pKa, for the QH10 mutant, at ~6.1, while the mirror-image conformation should have a pKa close to ~7.3. In addition, evidence is provided indicating the tautomeric distribution of H10 must also change between the native and mirror conformations. Although this may not be completely relevant for the purpose of determining whether the *Protein A* mirror image exists in solution, it could provide valuable information to validate the pKa findings.

Acknowledgments

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Elucidating the *cis/trans* autophosphorylation mechanism of the DosS sensor histidine kinase from *Mycobacterium tuberculosis*

Olivieri FA^a, Dodes Traian MM^a, Martí MA^a, Wetzler DE^a

a - Departamento de Química Biológica, FCEyN, UBA - IQUIBICEN, CONICET

Two-component systems (TCSs), comprising histidine kinases (HKs) and response regulators, empower bacteria to sense and adapt to diverse environmental stimuli. The TCS DosS-DosT/DosR from *Mycobacterium tuberculosis* controls the entry of the bacillus into a latent, dormant state, that renders antibiotics inefficient while reducing clinical manifestations of the disease. DosS and DosT are heme-histidine kinases which can be activated by hypoxia or by the presence of NO or CO. When active, these HKs undergo autophosphorylation in a conserved histidine residue and then transfer the phosphate to an aspartic residue in the DosR regulator, which induces the expression of the dormancy regulon.

As most TCSs, DosS and DosT consist of a sensor domain and a kinase core (KC) with an ATP binding domain (ABD) and a dimerization histidine phosphate accepting domain (DHp). Structurally, dimeric HKs can autophosphorylate in a *cis* (intramonomer) or *trans* (intermonomer) mechanism. It has been posited that the key determinant is the loop that connects two alpha-helices at the base of the DHP domain four-helix bundle.

As the KC tertiary structures of DosS and DosT have not been solved yet, we elucidated the *cis/trans* mechanism performing a biochemical approach. For this purpose, we engineered DosS mutants without sensor domains, which are known to be constitutively active. One of these mutants also had two point-mutations that inhibit its ability to bind ATP, but still allows it to act as phosphate acceptor and another mutant lacks the phosphorylatable histidine but can bind ATP and act as a phosphate donor.

Employing these constructs we elucidated that DosS undergoes autophosphorylation by a *trans* mechanism. Considering this result together with the available structural information for DosS and DosT and other HKs, we have constructed a homology model that will allow us to study the phosphorylation and activation mechanisms through classical and quantum molecular dynamics.

Acknowledgments

CONICET, ANPCYT, UBA

New approaches in the characterization of RESP18HD-insulin binding

Toledo PL^a, Torkko JM^b, Müller A^b, Solimena M^b, Ermácora MR^c

a - UNQ

b - German Center for Diabetes Research (DZD e.V.)

c - IMBICE (CONICET-CICPBA)

ICA512/IA-2/PTPRN is a protein tyrosine phosphatase enriched in secretory granules (SGs) of the pancreatic β -cells and other neuroendocrine cells. In previous studies, the involvement of this protein in the biogenesis, trafficking and exocytosis of insulin SGs, as well as in β -cell proliferation was studied. In this work, we focused in the extracellular domain Glucocorticoid-responsive regulated endocrine-specific protein 18 homologous domain (RESP18HD) and its potential role in the insulin SG cargo targeting. RESP18HD has distinct regions: cleavable ER translocation signal (aa 1-34), N-terminal Cys-rich motif (aa 35-90) and intrinsically disordered region in the C-terminal part of the domain (aa 91-131). RESP18HD is necessary and sufficient for the sorting of ICA512 to SGs of rat insulinoma INS-1 cells and it binds with high-affinity to insulin and proinsulin. In previous a work, we observed the formation of complexes between insulin and RESP18HD (aa 35-131) demonstrated by co-aggregation *in vitro*. To study aggregation phenomena, complexes of insulin and RESP18HD, formed spontaneously, were analyzed by TEM and by tioflavin T assay. The complexes were amorphous and did not form fibrillar arrangements. We have observed that the Cys-rich motif contains sufficient information for SG targeting, by fluorescence microscopy of RESP18HD-TQ2 tagged fusion protein variant in INS-1 cells. Moreover, Cys-rich motif did not form spontaneously aggregates with insulin. For that reason, we studied if the presence of this motif affects insulin fibrillar formation induced by heat and stirring. Fibrillar structures were no observed, confirming the binding between Cys-rich motif and insulin by a mechanism no related to aggregation. Given these results, it is necessary to continue the binding characterization and it relation with insulin SG cargo targeting.

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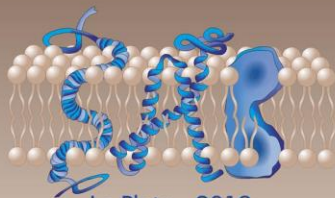
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