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Juan Pablo Acierno

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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 San Luis and to the XLVIII Annual Meeting of the Argentinean Biophysical Society (SAB).

Since its foundation in 1972, SAB has steadily maintained annual meetings 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, for the fourth consecutive year, SAB supports the "Meeting of Young Biophysicists" 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, researchers, fellows and students.

During the three days meeting we will enjoy a scientific programme that includes four Plenary Conferences, five Symposia and three Lightning Talks Sessions, 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 researchers, organized by fellows, works have been selected among over 145 poster contributions to be presented as oral communications within each symposium or during the lightening talks' sessions.

San Luis is a province near the geographical center of Argentina, known for rugged hills and the mild climate of the dry Pampas grasslands. In the northwest is Sierra de las Quijadas National Park, whose ochre-colored sandstone landforms include canyons and the huge amphitheater-shaped Potrero de los Funes. Also to be found here are preserved dinosaur footprints, tortoises and peregrine falcons. Beyond this touristic information it is pleasure to welcome all of you to the National University of San Luis (UNSL) where this meeting will be held. The UNSL was founded in 1973 but it possesses a rich historical legacy whose emergency point dates back to 1939 with the foundation of the National University of Cuyo.

In order to carry out the XLVIII Annual Meeting, we have had the financial aid of the National Agency of Scientific and Technological Promotion (ANPCyT), the National Council of Scientific and Technological Research (CONICET), the National University of San Luis (UNSL), the School of Physics, Mathematics and Natural Science of the UNSL, the School of Chemistry, Biochemistry and Pharmacy of the UNSL, of the School of Health Science of the UNSL, the Company of Biologists, the Institute of Applied Mathematics San Luis (IMASL), the Multidisciplinary Institute of Biological Research San Luis (IMIBIO-SL), and of the SAB. We have also received the collaboration of private sponsors, such as Avanti Polar Lipids. We would like to thank the coordinators of the Symposia, Conferences, Lightening Talks 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.

Last, but not least, 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 (last year) 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 once again the words by Dr. Bernardo Alberto Houssay, Argentine winner of the Nobel Prize in Physiology and Medicine in 1947 and founder of CONICET:

"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
XLVIII Annual Meeting SAB

Scientific programme

Wednesday, November 27

8:30 – 10:00 h *Registration*

9:30 – 10:00 h Opening Ceremony

Jorge Vila, IMASL-CONICET UNSL, San Luis, Argentina.

10:00 – 11:00 h ***Opening Lecture***

Chair: Ricardo D. Enriz

Chromatin as a self-returning random walk

Igal Szleifer, Northwestern University, USA.

11:30 – 12:00 h ***Lightning talks***

Chair: Jorge Vila

Liposomes can both enhance or reduce drugs penetration through the skin. Dolores Carrer, Instituto de Investigaciones Médicas Mercedes y Martín Ferreyra (INIMEC), Córdoba, Argentina.

Polymeric liposomes with phospholipids from lung surfactant as a potential delivery system for pulmonary tissue**.** Nadia Chiaramoni, Instituto Multidisciplinario de Biología Celular (IMBICE), Universidad Nacional de Quilmes, Quilmes, Argentina.

Responsive soft-nanoarchitectures as novel substrates with tunable mechanical properties. Catalina von Bilderling, Instituto de

Investigaciones Físico-químicas Teóricas y Aplicadas (INIFTA), Universidad de Buenos Aires, Buenos Aires, Argentina.

Can nano-bubbles permeabilize cell membranes? M. Silvina Moyano, Universidad Nacional de Cuyo, Mendoza, Argentina.

Water state/structure inside nanopores modulates hydrolytic enzymatic catalysis. Inés Burgos, Instituto de Investigaciones Biológicas y Tecnológicas (IIByT), Universidad Nacional de Córdoba, Córdoba, Argentina.

Multi scale modeling of thiol overoxidation by hydrogen peroxide: low molecular weight and protein thiol. Jonathan Semelak, Instituto de Química Física de los Materiales, Medio Ambiente y Energía (INQUIMAE), Universidad de Buenos Aires, Buenos Aires, Argentina.

12:00 – 13:30 h *Lunch*

13:30 – 15:00 h Symposium 1 – Molecular modeling in Biophysics

Chair: Sebastián Andujar

Using Polyamine Concentration to Trigger Drug Release from Polymer Hydrogel Films**.** Gabriel Longo, Instituto de Investigaciones Físico-químicas Teóricas y Aplicadas (INIFTA), Universidad Nacional de La Plata, La Plata, Argentina.

Membrane binding strength vs. pore formation cost - What drives the membrane permeation of cargoes functionalized with cell-penetrating-peptides? Mario Del Pópolo, Instituto Interdisciplinario de Ciencias Básicas (ICB), Universidad Nacional de Cuyo, Mendoza, Argentina.

***Multi scale QM-MM modeling of chemical reactivity in complex environments*.** Darío Estrín, Instituto de Química Física de los Materiales, Medio Ambiente y Energía (INQUIMAE), Universidad de Buenos Aires, Buenos Aires, Argentina.

Theoretical studies of novel antifouling templates for specific capture of target proteins. Estefanía Solveyra, Northwestern University, USA.

Study of clusters in cylindrical polyelectrolyte solutions on basis to the counter ion condensation theory. Jorge Bertolotto, Universidad Nacional de La Plata, La Plata, Argentina.

15:00 – 15:30 h *Coffee break*

15:30 – 17:00 h Symposium 2 – Computational biophysics: developments and applications of biomedical interest

Chair: Mario Del Pópolo

***Polimeric micelles as drug nanocarriers: insights from simulations*.** Mónica Pickholz, Instituto de Física de Buenos Aires (IFIBA), Universidad de Buenos Aires, Buenos Aires, Argentina.

Simulations of the optical activity of voltage sensitive probes in polarized model membranes. Vanesa Galassi, Instituto Interdisciplinario de Ciencias Básicas (ICB), Universidad Nacional de Cuyo, Mendoza, Argentina.

Combining in-silico and experimental methods for the design of drugs specific to tumor-associated macrophages. Eliana Asciutto, Escuela de Ciencia y Tecnología, Universidad Nacional de San Martín, San Martín, Argentina.

Molecular Simulations in South America: Coming of Age. Sergio Pantano, Instituto Pasteur de Montevideo, Montevideo, Uruguay.

Exploring the similarities of reduced consciousness and unconscious states through functional connectivity: balancing data with model. Carla Pallavicini, Instituto de Física de Buenos Aires (IFIBA), Universidad de Buenos Aires, Buenos Aires, Argentina.

17:00 – 19:00 h ***Poster Session A (odd ID)***

19:00 – 20:00 h ***Welcome Reception Cocktail***

Thursday, November 28

8:30 – 9:00 h *Registration*

9:00 – 10:00 h ***Gregorio Weber Conference***

Chair: M. Soledad Celej

Aquaporins: current understanding and new challenges. Gabriela Amodeo, Instituto de Biodiversidad y Biología Experimental y Aplicada (IBBEA), Universidad de Buenos Aires, Buenos Aires, Argentina.

10:00 – 10:30 h *Coffee break*

10:30 – 12:00 h ***Symposium 3 – Glycobiophysics***

Chair: Santiago Di Lella

N-*glycan structures, lectin domains, and glycoprotein's fate in the secretory pathway.* Cecilia D´Alessio, Instituto de Investigaciones Bioquímicas de Buenos Aires (IIBBA), Fundación Instituto Leloir (FIL), Universidad de Buenos Aires, Buenos Aires, Argentina.

***SIRAH late harvest: Adding glycans to coarse-grain molecular dynamics simulations*.** Pablo Garay, Instituto Pasteur de Montevideo, Montevideo, Uruguay.

***Glycobiological axis in adipose tissue: structural, biochemical and functional characterization of Galectin-12*.** Alejandro Cagnoni, Instituto de Biología y Medicina Experimental (IBYME), Buenos Aires, Argentina.

Effect of anesthetics in viscoelasticity and aggregation kinetics of glycated erythrocytes. Marcus Vinícius Batista da Silva, Universidad Nacional de Rosario, Rosario, Argentina.

Molecular characterization and Cryo-EM structure of the hemocyanin of the invasive freshwater snail Pomacea canaliculata. Ignacio Chiumiento, Instituto de Investigaciones Bioquímicas de La Plata (INIBIOLP), Universidad Nacional de La Plata, La Plata, Argentina.

12:00 – 12:30 h National Systems

Chair: Lía Pietrasanta

Eleven years of the National Systems of Large Instruments, Facilities and Databases. Gabriela Gorjón and Gastón Mayada Fabbri, Dirección Nacional de Planificación de Recursos Físicos, Subsecretaría de Coordinación Institucional, Secretaría de Articulación Científico Tecnológica, Argentina.

12:30 – 14:00 h *Lunch*

14:00 – 15:30 h ***Symposium 4 - Young Researchers***

Chairs: M. Julia Amundarain & Ezequiel Frigini

Incyclinide, a non-antibiotic tetracycline, prevents α-synuclein aggregation and disrupts fibrillary forms of the protein**.** M. Florencia González Lizarraga, Instituto de Investigación en Medicina Molecular y Celular Aplicada (IMMCA), San Miguel de Tucumán, Argentina.

Characterization of the differential interaction of cationic and anionic PAMAM dendrimers with hydrophobic and hydrophilic drugs. Daniela Igartúa, Instituto Multidisciplinario de Biología Celular (IMBICE), Universidad Nacional de Quilmes, Quilmes, 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.

Water dipolar relaxation in crowded protein systems**.** Agustín Mangiarotti, Instituto de Investigaciones Médicas Mercedes y Martín Ferreyra (INIMEC), Córdoba, Argentina.

15:30 – 16:00 h *Coffee break*

16:00 – 16:45 h ***2018 SAB Award Lecture***

Chair: Lía Pietrasanta

Structural Consolidation of Human Frataxin: Global Impact of the C-Terminal Region Local Dynamics**.** Santiago E. Faraj, Instituto de Química y Fisicoquímica Biológicas (IQUIFIB), Universidad de Buenos Aires, Buenos Aires, Argentina.

16:45 – 18:00 h Lightning talks

Chair: Francisco Garibotto

Dialkyl-guanidines as a novel series of Rac1 GTPase inhibitors: an interdisciplinary approach. Diana Wetzler, Instituto de Química Biológica de la Facultad de Ciencias Exactas y Naturales (IQUIBICEN), Universidad de Buenos Aires, Buenos Aires, Argentina.

Structural and functional characterization of short versions of BlaR1 and MecR1 of Staphylococcus aureus. Damila Mihovilcevic, Instituto de Biología Molecular y Celular de Rosario (IBR), Universidad Nacional de Rosario, Rosario, Argentina.

***On the conformational transition between the fully folded and locally unfolded substates of an atypical 2-Cys peroxiredoxin*.** Sebastián Vázquez, Instituto Multidisciplinario de Biología Celular (IMBICE), Universidad Nacional de Quilmes, Quilmes, Argentina.

***A proposed reaction mechanism for maize monomeric glyoxalase I*.** Javier González, Instituto de Bionanotecnología del NOA (INBIONATEC), Universidad Nacional de Santiago del Estero, Santiago del Estero, Argentina.

***An NMR-active chemical probe to measure stereospecific methionine sulfoxide reductase activity in vitro and in vivo*.** Carolina Sánchez-López, Instituto de Biología Molecular y Celular de Rosario (IBR), Universidad Nacional de Rosario, Rosario, Argentina.

Ligand-modulated transport rather than long range protein allostery couple the gates and active sites in human proteasomes. Horacio Botti, Universidad de la República, Montevideo, Uruguay.

Photoinduced Protein Nitration Method by Sensitizer Tris(bipyridine)-Ruthenium (II) Chloride Complex. Ezequiel Giménez, Instituto de Investigaciones Bioquímicas de La Plata (INIBIOLP), Universidad Nacional de La Plata, La Plata, Argentina.

18:00 – 20:00 h **SAB Assembly Meeting**

Friday, November 29

8:30 – 9:00 h *Registration*

9:00 – 10:00 h **Plenary Lecture**

Chair: Osvaldo Martin

Probing mechanisms of regulation of PI3K-family enzyme complexes using hydrogen/deuterium exchange mass spectrometry. Roger Williams, MRC Laboratory of Molecular Biology, Cambridge, United Kingdom.

10:00 – 10:30 h *Coffee break*

10:30 – 11:30 h **Plenary Lecture**

Chair: Sergio Pantano

The Unified Coarse Grained Model of biological macromolecules: principles and applications. Adam Liwo, Faculty of Chemistry, University of Gdánsk, Gdánsk, Poland.

11:30 – 12:30 h ***Lightning talks***

Chair: Mario Del Pópolo

Effect of ethanol on yeast membranes. Darío Genovese, Centro de Investigaciones en Química Biológica de Córdoba (CIQUIBIC), Universidad Nacional de Córdoba, Córdoba, Argentina.

Bases of pH sensing by a transmembrane Histidine kinase. Ana Bortolotti, Universidad Nacional de Rosario, Rosario, Argentina.

Structural properties of apolipoprotein A1 associated with evolutionary constrains. Tomás Masson, Instituto de Biotecnología y Biología Molecular (IBBM), Universidad Nacional de La Plata, La Plata, Argentina.

Nanopatterned surface for studying protein-blood cell interactions. Nicolás Saffioti, Instituto de Nanosistemas (INS), Universidad Nacional de General San Martín, San Martín, Argentina.

The role of Cu^+ and ATP on the reaction cycle of a Cu^+ transport ATPase from *Legionella Pneumophila.* M. Agueda Placenti, Instituto de Química y Fisicoquímica Biológicas (IQUIFIB), Universidad de Buenos Aires, Buenos Aires, Argentina.

Determination of the biochemical and structural modifications in the cytoplasm of bovine oocytes during* in vitro *maturation by Raman microscopy. Luis Jiménez, Instituto de Química del Noroeste Argentino (INQUINOA), San Miguel de Tucumán, Tucumán, Argentina.

12:30 – 14:00 h *Lunch*

14:00 – 16:00 h ***Poster Session B (even ID)***

16:00 – 17:30 h ***Symposium 5 – Proteins and Membranes: Structure and Function***

Chairs: Ricardo D. Enriz & M. Soledad Celej

***Mechanism of action of small cationic peptides with antibacterial activity. Theoretical and experimental study*.** Adriana Garro, Instituto

Multidisciplinario de Investigaciones Biológicas (IMIBIO), Universidad Nacional de San Luis, San Luis, Argentina.

***Structural studies of TRIM7 B30.2 domain and its interaction with glycogenin*.** M. Elena Carrizo, Centro de Investigaciones en Química Biológica de Córdoba (CIQUIBIC), Universidad Nacional de Córdoba, Córdoba, Argentina.

Molecular mechanism of protein transport via the Twin-Arginine Translocon (Tat). Fernanda Rodríguez, Instituto de Procesos Biotecnológicos y Químicos (IPROBYQ), Rosario, Argentina.

***Correlation between the hydration of acyl chains and phosphate groups in lipid bilayers: Effect of phase state, head group, chain length, double bonds and carbonyl groups*.** Antonio Rosa, Centro de Investigación en Biofísica Aplicada y Alimentos (CIBAAL), Universidad Nacional de Santiago del Estero, Santiago del Estero, Argentina.

Changes in biophysical properties of membranes containing sphingomyelins with very long chain PUFA during cooling. Alejandro Peñalba, Instituto de Investigaciones Bioquímicas de Bahía Blanca (INIBIBB), Bahía Blanca, Argentina.

17:30 – 18:00 h Closing Ceremony and Ponce Hornos Awards

Chairs: José María Delfino & M. Soledad Celej

Plenary Lectures

Chromatin as a self-returning random walk

Szleifer I[a], Huang K[a]
a - Northwestern University

As a complex of interphase DNA, RNA and proteins, chromatin stores our genetic and epigenetic information and regulates gene expression. How chromatin folds into functional structures has become an emerging puzzle in biology today. With the textbook view of chromatin folding based on the 30nm fiber being challenged, it has been proposed that chromatin has an irregular 10nm nucleosome polymer structure whose folding philosophy is elusive at the single-cell level. Here, we introduce a self-returning random walk to describe chromatin folding in single cells. Based on a simple folding algorithm, our model unifies the high contact frequency discovered by genomic techniques, and the high structural heterogeneity revealed by imaging techniques, which two chromatin properties we theoretically prove to be irreconcilable within a fractal chain framework. Our model provides a holistic view of genomic organization, in which the functional and packing units are liquid-tree-like domains, linked and isolated by stretched out, transcriptionally active DNA to form a secondary structure of chromatin that further folds into a “3D forest” under confinement. The model pivots a wide array of experimental observations and suggests the existence of universal chromatin folding principles for a disordered chromatin fiber to avoid entanglement and fulfill its biological functions.

The Unified Coarse Grained Model of biological macromolecules: principles and applications

Liwo A[a], Czaplewski C[a], Sieradzan AK[a], Lubecka EA[b], Karczyńska AS[a], Lipska AG[a], Golon Ł[a], Giełdoń A[a], Krupa P[c], Mozolewska MA[d], Makowski M[a], Samsonov SA[a], Ślusarz R[a], Ślusarz M[a], Bojarski KK[a], Sikorska C[a], Zięba K[a]

a - Faculty of Chemistry, University of Gdańsk, Gdańsk, Poland

b - Institute of Physics, Faculty of Mathematics, Physics, and Informatics, University of Gdańsk, Gdańsk, Poland

c - Institute of Physics, Polish Academy of Sciences, Warsaw, Poland

d - Institute of Computer Science, Polish Academy of Sciences, Warsaw, Poland

Molecular simulations have an established position in the studies of biological systems, from structure prediction/determination to drug design. However, despite an unprecedented growth of computer power, biological timescales are still beyond reach of all-atom simulations for larger systems. On the other hand, coarse-grained approaches enable us to extend the time- and size-scale of simulations by several orders of magnitude compared to all-atom approaches. The construction of the respective effective energy functions requires, however, a lot of care. A physics-based coarse-grained energy function originates from the potential of mean force (PMF) of a system under study, in which the degrees of freedom omitted when passing to the coarse-grained representation are averaged out. In our approach, we express the PMF as a sum of Kubo cluster cumulant functions, termed PMF factors, which pertain to smaller sections of the system, which can be identified with particular energy terms, this ensuring transferability. The PMF factors are expanded into cluster-cumulant series, of which only the lowest-order terms are kept. To derive the analytical formulas for the cluster cumulants, atomistic-detailed interaction energies are expressed in terms of atom-atom distances which are, in turn, expressed as functions of collective rotation angles, λ, about the virtual-bond axes, which are averaged over. The coarse-grained energy terms thus determined include the atomistic details in an implicit manner, hence, they are scale-consistent. Based on this formalism, we developed the Unified Coarse Grained Model of biological macromolecules, which comprises polypeptide chains (UNRES, containing 2 sites per residue), nucleic acids (NARES-2P, 2 sites per residue), and polysaccharides (SUGRES-1P, 1 site per residue). Owing to the rigorous physics-based scale-consistent derivation of the energy function, the model performs well in the simulation of structure, dynamics, and thermodynamics of the systems under study despite aggressive coarse graining. The software package are available from www.unres.pl. In this talk, theory and applications of the model in free, as well as bioinformatics- and data-assisted modeling will be discussed, including our participation in the Community Wide Experiment on the Critical Assessment of Techniques for Protein Structure Prediction (CASP).

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Probing mechanisms of regulation of PI3K-family enzyme complexes using hydrogen/deuterium exchange mass spectrometry

Williams R[a]
a - MRC Laboratory of Molecular Biology, Cambridge, UK

Anabolic and catabolic pathways are tuned to meet the demands of changing environments. Members of the phosphatidylinositol 3-kinase (PI3K) family of enzymes evolved from a common ancestor and are present in all eukaryotes, and they maintain homeostasis in a changing environment. PI3Ks and the protein kinase mTOR are members of this enzyme family, and they regulate response to nutrition and growth factors. In eukaryotic cells, the eIF2alpha kinase GCN2 is activated by amino acid starvation to initiate the Integrated Stress Response (ISR) that inhibits general protein translation, while the protein kinase mTORC1 is activated to promote protein synthesis when cells are fed. These nutrition-sensing pathways are linked through their influence on autophagy and lysosomal sorting, processes that are regulated by the VPS34-PI3K complexes. While mTORC1 is activated by amino acid abundance and promotes protein translation, nutrient stress activates GCN2, which initiates the Integrated Stress Response (ISR) and inhibits general translation. Our work on GCN2, mTORC1 and VPS34 complexes is aimed at understanding the structural mechanisms that regulate these complexes in control of growth, pathogenesis and cancer. While X- ray crystallography and electron cryo-microscopy (cryo-EM) provide a wealth of information regarding mechanisms of regulation of these enzymes, they have limitations. They may not provide the structures needed, and even when structures are obtained, information regarding the dynamics of the structures is typically indirect. We have applied hydrogen/deuterium exchange mass spectrometry (HDX-MS) to characterise protein/protein interactions, protein/membrane interactions and the conformational changes that accompany these interactions. HDX-MS has shown how mTOR-containing and PI3K-containing complexes interact with membranes and how their conformations change when activated on membranes. It has also shown how ribosomes interact with and activate GCN2.

Aquaporins: current understanding and new challenges

Amodeo G[a,b]
a - Departamento de Biodiversidad y Biología Experimental, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires
b - Instituto de Biodiversidad y Biología Experimental y Aplicada (IBBEA) - UBA-CONICET

Aquaporins (AQPs) are small integral membrane channels that have challenged water exchange capacity across biological membranes throughout all kingdoms. Despite they all share common structural features (assembled as homo/heterotetramers in which each monomer has its own permeable pathway), their multiple isoforms are distinguished for a high diversity in cell localization, transport selectivity (e.g. small solutes vs gases) as well as their regulatory properties. The invaluable number of high-resolution X-ray structures of AQPs along with molecular dynamics simulation studies has contributed to understand clue structural mechanisms by which water flux through AQPs is controlled. This has paved the road for genetic and physiological approaches and, all this combination is helping to close the “structure-function gap”, confirming their specific contribution both in the animal and plant field. The aim of our work is to contribute to the study of plant AQPs addressing regulatory mechanisms that can rapidly adjust water membrane permeability (Pf). In particular, plant plasma membrane intrinsic proteins (PIPs) seems to play an important role in controlling (Pf). These PIP aquaporins also represent a highly abundant and conserved subfamily divided into two subgroups: PIP1 and PIP2. In terms of their function, all PIPs show capacity to rapidly adjust the Pf by means of a gating response. In our work, we specifically addressed the cytosolic acidification as a stimuli that favors the close state. Many PIP1s also show another feature: they fail to reach the PM when expressed alone, but they can succeed if they are coexpressed with a PIP2. Therefore, in terms of activity, PIP aquaporins can rapidly adjust membrane water permeability by means of two mechanisms: channel gating and channel translocation of PIP subunits (PIP1 and PIP2, organized -or not- in mixed tetramers). Evidences indicate that these mechanisms are not only highly conserved among species but their juxtaposition enhances the dynamics of the response. In this sense our findings also contribute to describe regulatory mechanisms that cannot be attributed to independent monomers.

Finally, the functional properties of this interaction and physiological consequences are addressed in order to understand the relevance of the cell-to-cell pathway in the plant hydraulic dynamics not only as a physiological challenge but also as a response to adverse plant environmental conditions.

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Structural Consolidation of Human Frataxin: Global Impact of the C-Terminal Region Local Dynamics

Faraj SE[a,b], Santos J[c]
a - CONICET – Universidad de Buenos Aires. Instituto de Química y Fisicoquímica Biológicas "Prof. Alejandro C. Paladini" (IQUIFIB). Buenos Aires, Argentina.
b - Universidad de Buenos Aires. Facultad de Farmacia y Bioquímica. Departamento de Química Biológica. Buenos Aires, Argentina.
c - Departamento de Fisiología y Biología Molecular y Celular, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires. Instituto de Biociencias, Biotecnología y Biomedicina (iB3)

Human Frataxin is a small mitochondrial protein involved in iron-sulfur cluster synthesis, whose deficiency causes the neurodegenerative disease Friedreich's Ataxia. This work addresses the study of the main structural determinants of the dynamics and the heterogeneity of the native ensemble. We focused on defining the role of the C-terminal region (CTR) of Frataxin in the folding/unfolding mechanism and identifying possible correlations between local and global stability and flexibility.

Using complementary spectroscopic, chemical and biophysical approaches, we analyzed wild-type human Frataxin and a set of rationally designed CTR mutants, including two highly destabilized disease-associated variants. Our results show that the modification of the CTR interactions affects the dynamics and stability to different extents.

Besides, we studied Frataxin folding and unfolding reactions. We found evidence of the existence of an intermediate state in the folding route with significant secondary structure content but relatively low compactness, compared with the native ensemble. Experiments revealed that the CTR is essentially unstructured in the most compact transition state, which is not altered by the complete truncation of the CTR.

Furthermore, we investigated local fluctuations of the CTR by means of cysteine modification experiments performed on L→C replacement mutants. The assessment of locally focused dynamics indicates that the conformation of the CTR fluctuates in the seconds to minutes timescale, with partial independence from global unfolding events. Chemical modification results cannot be explained in terms of the global unfolding reaction alone, given that free energy differences for local unfolding were found to be significantly lower than those for the global unfolding reaction.

Altogether, these results provide experimental evidence of local phenomena with global effects and contribute to understanding how global and local stability are linked to protein dynamics.

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Symposia

Multi scale QM-MM modeling of chemical reactivity in complex environments

Estrin D[a], Foglia N[a], Semelak J[a], Zeida A[b]
a - Departamento de Química Inorgánica, Analítica y Química Física, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires / INQUIMAE-CONICET
b - 5 Departamento de Bioquímica, Facultad de Medicina, Universidad de la República

Computational techniques for modeling chemical reactivity in complex environments have emerged during the last decades as an important tool to complement experimental information, providing atomic resolution insight into the reaction mechanisms, as well as thermodynamic and kinetic information. An elegant way to deal with this issue consists in employing multi level quantum classical schemes (QM-MM). We will present in this talk an overview of our group QM-MM implementation, which combines a density functional theory (DFT) description of the QM subsystem using Gaussian basis sets with a classical description of the environment using the Amber force field. The implementation, called LIO, has been effficiently adapted for using graphical process units (GPU). A key issue in the study of reaction mechanisms is the definition of a suitable reaction coordinate. In this context, we will show an implementation of a nudge elastic band scheme which allow us to judge the quality of a given reaction coordinate. We will illustrate this approach by analyzing the mechanism of the reduction of ferric heme proteins by nitric oxide. In addition, we will show results of a study of the reactions of thiols with peroxides, which are very relevant in redox biochemistry, both in aqueous and protein environments.

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Membrane binding strength vs. pore formation cost - What drives the membrane permeation of cargoes functionalized with cell-penetrating-peptides?

Del Pópolo M[a]
a - ICB-CONICET & Facultad de Ciencias Exactas y Naturales UNCUYO

Cell-penetrating-peptides (CPPs) are notoriously effective at traversing lipid bilayers, even when they are mostly composed of positively charged amino-acids that make the peptide highly hydrophilic. Not only that; attaching CPP molecules to membrane-impermeable cargoes, such as water-soluble proteins and nanoparticles, confer these cargoes a remarkable membrane-permeation capacity. The effect of boosting the cellular uptake of a cargo by chemical functionalization with CPPs is known as the “CPP-effect”, and the molecular basis of this well-stablished phenomenon are still unknown. In this presentation, we will discuss how cell-penetrating-peptides assist the transport of nanoparticles and non-CPP peptides across a lipid bilayer. We will first present a simple phenomenological model that highlights the relevant kinetic and thermodynamic parameters controlling the membrane translocation process. Coarse-grained Molecular Dynamics simulations will then be used to compute free-energy profiles for the insertion of the CPP, the cargo, and the CPP-cargo complex into a lipid bilayer containing 50% of anionic lipids. We will then vary the concentration of negatively charged lipids, and evaluate how the surface charge density affects the adsorption and insertion energies of each of the cargoes. Inspired by recent reports claiming that fatty acids promote the membrane permeation of CPPs, we will also explore the effect of palmitic acid on the cargo’s insertion free-energy profile. On the whole, we find that CPPs increase the diffusion rate of the cargo across the membrane by altering the balance between the binding energy to the lipid surface, and the energy cost for creating the transition state configuration to jump across the membrane. Recognizing the key role of adsorption and activation energies allows us to explain why, and to what extent, increasing the negative surface charge density and the concentration of floppy carboxylic acids in the membrane enhance the CPP effect.

Using Polyamine Concentration to Trigger Drug Release from Polymer Hydrogel Films

Longo G[a]
a - Instituto de Investigaciones Fisicoquímicas Teóricas y Aplicadas (INIFTA), UNLP-CONICET, La Plata, Argentina.

Hydrogels of cross-linked polymer chains are generally biocompatible and have highly tunable properties. They can be engineered to respond to biological stimuli such as changes in the concentration of a chemical species. Because of these properties, responsive materials based in polymer hydrogel are frequently considered for biomedical applications. Using Statistical Mechanics and computer simulation results, in this talk we will discuss the interaction of weak polyacid hydrogel films with polyamine solutions. Polyamines such as putrescine spermidine and spermine are essential for cell growth and required in many inter/intra-cellular processes. Near tumor cells the concentration of these polyamines is relatively higher than around healthy cells, which can be key in increasing the speed at which tumors spread. We will show that responsive hydrogels can incorporate large quantities of polyamines, which is relevant in the context of current therapies that are based on reducing polyamine concentration to slow down tumor spread. In addition, polymer hydrogels can release a therapeutic drug upon polyamine adsorption.

Combining in-silico and experimental methods for the design of drugs specific to tumor-associated macrophages

Asciutto EK[a], Scodeller P[b]
a - Escuela de Ciencia y Tecnología, Universidad Nacional de San Martín
b - University of Tartu

Macrophages are immune system cells with crucial roles in cancer. Anti-inflammatory tumor macrophages (called "M2") are highly pro-tumor, and can be found in the lymph nodes in early stages of the disease, so that a probe that is directed to them will have immediate diagnostic applications. From a recent identification of a peptidic motif that accumulates and internalizes in M2 TAMS by binding selectively to the mouse mannose CD206/MRC1 receptor, we have modeled the human receptor-ligand complex and determined the interactions that govern the specific affinity, and the ligand binding domain.
From these results, we have designed a new ligand in a head to tail cyclized version, that binds and internalizes in M2 with the important property of activating only in tumor and not in healthy tissues.

Polimeric micelles as drug nanocarriers: insights from simulations

Pickholz M[a], Albano J[a]
a - Departamento de Física, FCEyN, UBA - IFIBA, CONICET

A wide range of nanoparticles have been developed used for both , therapeutic and diagnosis. Nowadays, these kind of particles have a significant impact on healthcare. In this direction, systems based on biocompatible amphiphilic block coplymers are interesting materiales to developed drug delivery systems. Depending on block length and the hydrophilic hydrophobic balance, they can assembly in different structures, such as micelles and polymersomes. Computer simulations, such as Molecular Dynamics simulations, are a very powerfull tool to understand biomolecular processes. In this direction, Coarse Grain models - where atoms are grouped in specific sites - allow simulation time and length scales of the systems beyond what is achievable with traditional atomistic models. Here, we developed a coarse-grained model of sumatriptan suitable for extensive molecular dynamics simulations. We then tested the sumatriptan model in a micellar environment along with experimental characterization of sumatriptan-loaded micelles. The simulation results showed good agreement with photon correlation spectroscopy and electrophoretic mobility experiments.

Simulations of the optical activity of voltage sensitive probes in polarized model membranes

Galassi V[a], Sosa M[a], Del Pópolo M[a]
a - Facultad de Ciencias Exactas y Naturales-Universidad Nacional de Cuyo

Voltage sensitive dyes (VSD) are widely used to determine membrane potential both in imaging diagnosis field and in scientific research. However, their application is limited by their sensibility and rate of response [1].

We are developing a thorough computational study of optically active molecules sensitive to membrane potential. As sample cases, we chose di-3-ANEPPDHQ, widely used for *in vitro* determinations [2]; and indocyanine green (ICG), an infrared fluorescent dye with FDA approval for medical use [3]. The mechanism of response of these chromophores is basically electrochromic, i.e. the stationary electronic states responsible for the optical activity (light absorbance and fluorescence emission) are sensitive to the external electric field. Thus, the challenge in this kind of study is to combine the electronic description of the dye, prohibitive over a couple of hundreds atoms, with the complex condensed phase of biological systems, which is in turn determinant of the external field magnitude and fluctuations.

We built atomistic models for the probes embedded in polarized membrane bilayers, and performed enhanced sampling calculations to determine the potential of mean force for membrane insertion, in order to establish the energetics of the partition process and to deconvolute the configurational contribution to the optical response.

We appealed to the time-dependent resolution of Kohn-Sham equations (TD-DFT) and hybrid potentials from quantum mechanics and molecular mechanics (QM/MM) to calculate the absorption spectra of the VSDs. We obtained the spectral shifts corresponding to different configurations of the dye in the bilayer: adsorbed and inserted, and differential response to the transmembrane potential. The long-term objective is to develop a methodology that allows the study and molecular optimization of CSVs.

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Molecular Simulations in South America: Coming of Age

Pantano S[a]
a - Institut Pasteur Montevideo, Uruguay

Despite the worldwide-recognized solid level in theoretical (bio)physics and (bio)chemistry of South American scientists, the establishment of (bio)molecular simulations as a scientific area has taken a long time, partially because of a low scientific integration between South-American scientists (1).

In this short presentation, I will briefly consider some of the reasons for this problem a current continental initiative aimed at ameliorating this situation.

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Glycobiological axis in adipose tissue: structural, biochemical and functional characterization of Galectin-12

Cagnoni AJ[a], Maller SM[a], Banoud N[b], Sigaut L[c], Pérez Sáez JM[a], Pietrasanta L[c], Croci DO[a,b], Di Lella S[d], Sundblad V[a], Rabinovich GA[a], Mariño KV[a]
a - Insituto de Biología y Medicina Experimental (IBYME - Conicet)
b - Instituto de Histologia y Embriologia de Mendoza Dr. Mario H. Burgos, IHEM
c - Centro de Microscopías Avanzadas (CMA), Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires
d - Departamento de Química Biológica- FCEN- UBA/ IQUIBICEN-CONICET

Lectins, endogenous glycan-binding proteins, can decipher and convey glycan-containing information into biologically relevant cellular programs. Galectins are a family of soluble lectins defined by a common structural fold and a conserved carbohydrate recognition domain (CRD), capable of recognizing N- and O-glycans bearing the disaccharide *N*-acetyllactosamine. By interacting with cell surface glycoconjugates, these lectins control a myriad of cellular processes including endocytosis and cell signaling cascades, leading to regulation of cellular activation, proliferation, migration, and survival. Based on their molecular architecture, galectins have been classified into three different structural groups: (i) proto-type galectins, which contain a single CRD and are capable of dimerizing; (ii) chimera-type galectins, with a single CRD, which are able to form pentamers; and (iii) tandem-repeat type galectins, with two CRDs of different nature in the same polypeptidic chain. Glycan specificity of each member of the family is dictated by differences in the architecture and dynamics of the ligand-binding groove (LBG). While some galectin members are ubiquitously expressed, other galectins show preferential expression in certain tissues or cell types. Among these, galectin-12 (Gal-12) is preferentially localized in adipose tissue and plays a key role in adipocyte differentiation, lipolysis and glucose homeostasis. Compared to other galectins, Gal-12 has been historically out of the spotlight due to the lack of publically available crystal structure and the difficulties for its recombinant expression. In spite of these drawbacks, we have studied the biochemical and biophysical determinants of their structure, glycan specificity and subcellular localization and identified its pro-angiogenic function within adipose tissue. Surprisingly, and distinct from other members of the galectin family, Gal-12 revealed preferential recognition of 3-fucosylated *N*-acetyllactosamine (LacNAc) structures, that are highly represented in endothelial cells. Using complementary experimental and theoretical approaches, our studies unveil atomistic, biochemical and topological features of a hypoxia-regulated lectin in adipose tissue that controls endothelial cell function via recognition of 3-fucosylated glycans.

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SIRAH late harvest: Adding glycans to coarse-grain molecular dynamics simulations

Garay P[a,b], Pantano S[b]
a - Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina
b - Grupo de Simulaciones Biomolecuales, Institut Pasteur Montevideo, Uruguay

Glycans are one of the most structurally and functionally diverse molecules in nature. Their structures range from simple mono or disaccharides to more complex structures like chains, branched structures, oligosaccharides or polysaccharides, and could bond to other biological molecules like proteins and lipids to form glycoproteins and glycolipids. One of the principal tools to complement experimental studies is the use of molecular dynamics (MD) simulation. Nevertheless, the computational cost of simulating large glycosylated proteins or chains with realistically long polysaccharides with polymerization degrees in the order of 10^5 poses significant challenges to fully atomistic MD simulations. A workaround to this limitation is the development of coarse grain (CG) models. CG representations reduce the number of atoms in the system making them less computational demanding but still preserving salient features of the molecules of interest. Our group develops the SIRAH force field (***S****outhamerican* ***I****nitiative for a* ***R****apid and* ***A****ccurate* ***H****amiltonian,* www.sirahff.com) which contains parameters for DNA, proteins, solvents, ions, and lipids.

Here, show the development of the first glycan model at the CG level that allows simulations of polysaccharides and/or glycosylated proteins in an unbiased and consistent manner. Moreover, we provide a fully generalizable coarse-graining rule that can be expanded to virtually any six-membered ring (pyranoses) including functionalizations and ramifications. Examples of applications include oligosaccharides, cellulose fibers, pentobiose binding to the carbohydrate-binding module (CBM), and antibody recognition by N-glycosylated proteins.

Ours results from cellobiose, deca-b-D-Glcp 1-4, cellulose I alfa and beta, and pentobiose binding to CBM demonstrate that with this parametrization the same molecule can have different behaviors depending on the study system. The result from the N-glycosylation shows that is possible to use this post-translational modification in different situations.

These new moieties considerably expand the range of molecular systems amenable to be studied with CG force field.

N-glycan structures, lectin domains, and glycoprotein's fate in the secretory pathway

D'Alessio C[a,b]
a - Facultad de Ciencias Exactas y Naturales, UBA
b - Instituto de Investigaciones Bioquímicas de Buenos Aires (IIBBA-CONICET) y Fundación Instituto Leloir

N-glycans transferred to proteins are remodeled in the endoplasmic reticulum (ER) producing structures that determine the fate of the glycoproteins within the secretory pathway. Glucosidase II (GII) is a key player in *N*-glycan processing as it removes the two inner glucose residues from the glycan transferred to proteins during *N*-glycosylation and the glucose residue added back to not yet properly folded proteins during the quality control of glycoprotein folding in the ER. GII is a heterodimer whose alpha subunit bears the catalytic site while its beta subunit enhances deglucosylation activity through its C-terminal Mannose-6-phosphate (M6P) receptor homology (MRH) domain. A family of glycan receptors bearing MRH domains, including CD-MPR & CI-MPR (responsible for delivering acidic hydrolases with M6P signal to lysosomes), *N*-acetylglucosamine-1-phosphotransferase g subunit (responsible of generating the M6P signal), OS-9 (involved in the glycoprotein degradation pathway) and GII beta subunit recognize subtle differences in the *N*-glycan structures. Comparison of their structures showed a similar overall fold and identified conserved residues critical for the structural integrity of the carbohydrate binding pocket. Nonetheless, each one has its unique substrate specificity and its binding defines if the protein will continue in the folding process, will be delivered to lysosomes or will be degraded in proteasomes. In the present work, we show the effects on GII activity of swapping its own GII beta MRH domain for those MRH domains present in other lectins of the secretory pathway

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Single-Molecule Studies of the Dynamics of NS3h Helicase from the Dengue Virus

Amrein F[a], Gebhard L[b], Arunajadai S[c], Kaplan A[d], Gamarnik A[b], Bustamante C[e], Kaufman S[a]

a - Departamento de Química Biológica e IQUIFIB (UBA-CONICET), Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires
b - Fundacion 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 they show patterns with a certain periodicity which is manifested both in step-size and dwell times.

The former comprises discrete movements of the protein along the RNA strand, and the latter corresponds to periods during which the motor carries out 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

A step detecting algorithm was 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) there are two different populations of steps of different sizes, denominated steps and substeps for the larger and smaller size respectively, 2) the mechanical step-size value obtained was 10 bp, independent of ATP concentration, which strongly correlates with the NS3h site-size value for the RNA (10 to 11 nt), 3) the average value of the substep-size was 3 bp, with an apparent hyperbolic dependence with ATP concentration, 4) the unwinding dwell times are distributed into two distinct populations, and 5) dwell time values followed a decreasing hyperbolic function with the ATP concentration for both populations

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Characterization of the differential interaction of cationic and anionic PAMAM dendrimers with hydrophobic and hydrophilic drugs

Igartúa D[a], Martinez C[a], Chiaramoni N[a], Alonso S[a], Prieto J[a]
a - Laboratorio de Biomembranas (LBM), GBEyB, UNQ-IMBICE-CONICET

Dendrimers are polymers that consist of a core, branches covalently bonded to this core and a surface with multiple terminal groups. Due to their properties, the dendrimers are widely used in the area of nanomedicine. In this work, we used polyamidoamine (PAMAM) dendrimers with ethylenediamine core of generation 4.0 (DG4.0, cationic) and generation 4.5 (DG4.5, anionic). To characterize the ability of both dendrimers to interact with drugs, in this work we studied the formation of complexes with the hydrophobic drugs carbamazepine (CBZ) and curcumin (CUR), and with the hydrophilic drug tacrine (TAC). We developed a protocol to obtain complexes with each type of dendrimers and different drugs. In the case of hydrophobic drugs, different dendrimer-drug ratios were evaluated to increase solubility; while for the hydrophilic drug, only a high ratio was evaluated to ensure interaction. To address the characterization of these complexes, we studied the load capacity (moles of drug per mole of dendrimer) and the ability to increase the water solubility and stability of drugs.

Both dendrimers increased CBZ and CUR solubility. Regarding interaction with TAC, they also increased its solubility although it was not significant. In the case of CBZ, dendrimer-mediated solubilization would be due to the formation of complexes by encapsulating the drug in the internal pockets, involving hydrogen and hydrophobic bridge interactions. In the case of CUR, dendrimer-mediated solubilization could be due to the formation of complexes both by encapsulation in the internal pockets and by anchoring to their surface groups. In the case of TAC, although there was no significant increase in solubility, it was shown that the anionic terminal groups of DG4.5 were able to anchor the cationic amine group of this drug. These results show that the drug-dendrimer interaction is highly dependent on the chemical structure and size of the drug, and the type of dendrimer.

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Incyclinide, a non-antibiotic tetracycline, prevents α-synuclein aggregation and disrupts fibrillary forms of the protein

González Lizarraga MF[a], Ávila CL[a], Ploper D[a], Socías B[a], Pereira M[b], Michel P[b], Raisman-Vozari R[b], Pietrasanta L[c], 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 - Centro de Microscopías Avanzadas. IFIBA-DF FCEN-UBA

Parkinson's disease (PD) is a progressive neurodegenerative illness with age being the main risk factor for its development. The increase in longevity in most Western countries imposes the urgency of finding a disease-modifying approach for its treatment. Several promising molecules targeting PD pathogenic pathways have been proposed but with limited success. Targeted pathways include notably those regulating α-synuclein aggregation, oxidative stress and neuroinflammation. Due to the multifactorial characteristics of the disease, a multitarget drug with efficient activity against these processes is required. With this in mind, we previously demonstrated that the tetracycline doxycycline (DOX) reshapes oligomeric species of the PD protein α-synuclein reducing their toxicity, seeding capacity and propensity to form toxic fibrillary species. In addition, DOX showed anti-inflammatory and neuroprotective effects in PD models. However, the antibiotic activity of DOX represents a possible hurdle for its repositioning in long-term treatments. Thus, we sought to find a non-antibiotic DOX analog with potent anti-amyloidogenic properties, making this drug an ideal candidate for repurposing to treat PD and conceivably other amyloid-associated disorders. In order to detect putative anti-amyloidogenic ready to use molecules, we used cheminformatic methods to extract a novel structural motif capable of interacting with cross-β structures (Cbeta-IM) and screened a number of pre-existing compounds using this strategy. Incyclinide was selected among tetracyclines because i) it contains this motif in a planar structure, ii) crosses the BBB, and iii) is available for repurposing. Using a combination of biophysical techniques (fluorescence and infrared spectroscopy, second order scattering, different microscopes) together with cell biology approaches, we characterized its impact against α-synuclein toxic aggregates. Incyclinide had an exceptional ability to reshape α-synuclein oligomers. Moreover, Incyclinide was able to disrupt mature fibrils and the disassembled species did not trigger a neuroinflammatory response by microglial cells. The anti-amyloidogenic and anti-inflammatory properties of Incyclinide, together with its ability to cross the BBB, position Incyclinide as an ideal drug to be repurposed in PD. We also propose the Cbeta-IM as a molecular signature to be exploited for identifying novel drugs of interest for neuroprotection in PD.

Water dipolar relaxation in crowded protein systems

Mangiarotti A[a], Bagatolli LA[b,a]
a - Instituto de Investigación Médica Mercedes y Martín Ferreyra (INIMEC), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)
b - Departamento de Química Biológica Ranwel Caputto, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba

Recent results of our group revealed a strong coupling between the intracellular water dynamics and the ATP activity during glycolytic oscillations in yeasts [1,2]. Moreover, the integrity of cytoskeleton has proven to be crucial in the regulation of this process [2]. These results cannot be interpreted under the vision of the dominant cell model, but fit properly with the Association-Induction Hypothesis (AIH) developed by G.N. Ling [3]. Concisely, the AIH proposes that fluctuations in the chemical activity of central metabolites (e.g. ATP, hormones, divalent ions) during active metabolic processes modulate the conformational states of cytoskeletal proteins (e.g., actin) through association and inductive effects. This in turn controls the binding affinity of intracellular water and monovalent ions for proteins changing emergent properties of the cell interior and, therefore, the way in which numerous molecular actors perform in the cell.

Concerning the state of water in the cell, the AIH incorporates a subsidiary theory called Polarized-Oriented Multilayer Theory of Cell Water (POM) (4). It states that protein backbones, through the carbonyl and imino groups of the amide bond, offers a properly-spaced alternating negative-positive (called N-P) sites that orient an polarize a layer of oppositely-oriented water dipoles that in turn adsorbs, orients, and polarizes, another layer of oppositely-oriented water dipoles and so on. This constitutes a dynamic structure of multiple layers of polarized-oriented water dipoles that is cooperatively modulated by metabolic activity (e.g. levels of ATP) (3,4).

We are currently working with experimental models to test the AIH and its subsidiary theory POM. We use crowded protein and polymers as models, which are studied with fluorescent probes that respond to the extent of water dipolar relaxation. By employing spectroscopic techniques and fluorescence microscopy, we analysed the organization and dynamics of these crowded environments and its relationship with the protein/polymer structure.

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Mechanism of action of small cationic peptides with antibacterial activity. Theoretical and experimental study.

Garro A[a], Lopez Cascales JJ[b], Enriz R[a]
a - Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis; Instituto Multidisciplinario de Investigaciones Biológicas (IMIBIO-SL CONICET).
b - Grupo de Bioinformatica y Macromoleculas (BioMac), Area de Química Física, Universidad Politécnica de Cartagena

The increase in microbial resistance to antibiotics due to their excessive use during recent decades means that new therapeutical strategies are necessary. Thus, there is a real need for a next generation of new antibacterial agents. This event resulted in the identification of novel molecules, which could result useful for a future development. Among them, some natural peptides have been recently reported as antimicrobial compounds. We reported a series of small peptides possessing strong antibacterial activity against a broad spectrum of pathogens microorganisms (1-3). To better understand the mechanism of action of these cationic peptides we performed theoretical calculations using MD simulations and experimental measurements (circular dichroism CD), infrared spectroscopy (ATR-IR) and Dynamic light scattering (DLS), to evaluate the possible mechanism of action of these cationic peptides).

Our results indicate that the first stage of the action mechanism of small cationic peptides with antimicrobial activity is ruled by electrostatic interactions between the peptide and the pathogen cell membrane. Thus, an increase in its activity could be expected with an increase in the positive charge on the peptide. By contrast, the opposite behavior has been observed when the charge increases to reach a critical value, beyond which the activity falls. We evaluate the perturbation effects in a cell membrane model for two small cationic peptides with similar length and morphology but with different cationic charges. The thermodynamic study associated with the insertion of these peptides into the membrane and the perturbing effects on the bilayer structure provide valuable insights into the molecular action mechanism associated with the charge of these small cationic peptides. Our theoretical and experimental results provide an explanation (at the molecular level) of why charged peptides are not able to reach the threshold concentration necessary to produce the deformation of the membrane, that is, its subsequent biological activity.

Contact: adgarro@unsl.edu.ar

Molecular mechanism of protein transport via the Twin-Arginine Translocon (Tat)

Rodriguez F[a], Bertoni F[a], Subils T[a]
a - Instituto de Procesos Biotecnológicos y Químicos (IPROBYQ-CONICET)

The twin-arginine translocation (Tat) pathway has the remarkable ability of translocating folded proteins across membranes. The Tat pathway is found in bacteria, archaea and plant chloroplasts, and is required for important bacterial cellular processes including respiratory and photosynthetic energy metabolism, cell division, cell motility, quorum sensing, heavy metal resistance, iron acquisition, and biofilm formation. In *Escherichia coli* the Tat translocon consists of three membrane proteins: TatA, TatB and TatC. In *Bacilllus subtilis* it only has two components: TatA and TatC. Experimental evidence suggests that a TatBC complex binds to the signal peptide of the substrate protein. This binding event triggers the assembly of TatA with the TatBC-substrate complex, and the substrate protein is then translocated probably via TatA. The Tat pathway appears to employ a unique mechanism to allow the passage of structured substrates without losing the membrane impermeability to ions. How this is achieved by the translocon is not clear. Here we report molecular and structural studies of Tat components aimed at unveiling their molecular mechanism of action.

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Structural studies of TRIM7 B30.2 domain and its interaction with glycogenin

Carrizo ME[a], Muñoz Sosa C[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.

TRIM family of proteins comprises more than 80 members encoded in the human genome that participate in diverse cellular processes. They share a multimodular architecture with a conserved N-terminal tripartite motif (TRIM) and a variable C-terminal region. The TRIM motif includes a RING (Really Interesting New Gene) finger domain, one or two B-box domains, and a coiled-coil region. The RING finger is a zinc-binding domain that confers E3 ubiquitin ligase activity. B-boxes are also zinc-binding motifs but with a less well characterized function. The coiled-coil domain, instead, is mainly involved in dimerization/oligomerization of TRIM proteins. The C-terminal region can contain one or more domains that appear to mediate the recognition of E3 ubiquitin ligase substrates. The most common of these C-terminal domains is the B30.2, which is present in at least 30 human TRIM family members as well as in other unrelated proteins.

Human TRIM7 displays the canonical TRIM motif followed by a B30.2 C-terminal domain. It was first identified in a yeast two-hybrid screen for proteins that interact with glycogenin, the autoglucosyltransferase responsible for the initiation of glycogen biosynthesis. It has also shown to be an E3 ubiquitin ligase that play a key role in the c-Jun/AP-1 activation pathway via Ras. Its depletion reduced c-Jun transcriptional activity while its knockdown decreased the growth of lung tumors driven by Ras. A correlation between increased TRIM7 expression level and tumor size has also been described in hepatocellular carcinoma patients. In addition, recent studies have revealed that TRIM7 promotes glycogen accumulation in skeletal muscle when overexpressed and that it has antiviral properties dependent on its ubiquitin ligase activity and the presence of the B30.2 domain.

As these evidences suggest, TRIM7 is gaining increasing interest due to its potential as a therapeutic target. In this regard, B30.2 domain may be particularly important since specificity determinants of many TRIM proteins lie in this domain. In order to contribute to the understanding of TRIM7 mechanisms of action, we have solved the crystal structure of its B30.2 domain. Here we present the structural characterization of this domain and provide evidences for a putative binding interface through the analysis of sequence conservation and the effect of different mutations on the interaction with glycogenin, so far the only protein known to interact with TRIM7 B30.2 domain.

Oral Communications

Study of clusters in cylindrical polyelectrolyte solutions on basis to the counter ion condensation theory

Bertolotto JA[a], Umazano JP[a]
a - Facultad de Ciencias Exactas y Naturales. UNLPam.

The formation of parallel clusters of rods in hexagonal array has been studied by Ray y Manning[1] on basis to the counter ion condensation theory. That work constitutes an original and conceptually very important advance, which allows analyzing the formation of aggregates as a function of the macroion charge and the medium ionic strength. However, the theoretical study carried out by these authors requires some mathematical approximations. This approximations allow that the free energy to be expressed in three regions (near, intermediate and distant), approximations that lead to not very realistic interaction potentials. The size of the clusters and the distance between macroions predicted in their work are considerably smaller than the experimental values obtained by light scattering and electrical birefringence. In this paper, Ray and Manning's work is reviewed and more exact mathematical developments are applied. This improves the theoretical predictions and shows that the discrepancies found by these authors are not due to a deficiency of their model but to the errors due to the mathematical approximations used.

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Theoretical studies of novel antifouling templates for specific capture of target proteins

Gonzalez Solveyra E[a], Szleifer I[a]
a - Department of Biomedical Engineering, Northwestern University, Evanston, IL, USA.

Single particle reconstruction (SPR) analysis of cryoelectron microscopy (cryoEM) data is a rapidly growing method for determining the near-atomic molecular structures of biological samples. Affinity grids have been the most widely used approach to address the sample preparation challenge. Unfortunately, this method suffers from the limitation of having the affinity ligands in a fixed orientation near the grid surface, leading to preferred orientations of the captured protein that limits the random orientations needed for SPR.

In this work, our theoretical methods are focused on the rational design of multivalent rod-like affinity capture bottle-brush polymers that can be used to harvest the target protein from bulk solution. There are two major requirements that must be addressed: 1) that the rod-shaped template avoids non-specific protein adsorption (i.e., exhibits antifouling properties) and 2) that the affinity ligand binds specifically to the target protein without imposing a preferred orientation with respect to the template. To that end, we resort to a molecular theory (MT) that has been shown to provide accurate predictions for protein adsorption and ligand-receptor binding on surfaces and interfaces. We perform systematic studies of the surface modification of nanoconstructs of different geometry (planar, cylindrical, spherical) with PEG polymer brushes of variable chain length and molecular architecture bearing end-modified affinity ligands. We explore the system parameters required for optimal fouling resistance of untagged proteins and specific capture of tagged proteins. The resistance of these constructs against non-specific protein adsorption is studied using a non-tagged coarse-grained lysozyme model, while the binding of target proteins to the affinity rod-like templates is modeled using His-tag green fluorescent protein (His-GFP) as the targeted molecule.

MT calculations provide the lysozyme adsorption isotherms (i.e., amount of adsorbed protein) and characterize the His-GFP ligand-receptor binding as a function of several design parameters, including polymer chain length, polymer architecture, affinity ligand surface density, cylinder size, and solution conditions (pH, lysozyme & His-GFP concentrations, ionic strength). We also explore the effects of using polymer mixtures consisting of shorter PEG chains (for increased antifouling performance) and longer PEG spacers end-functionalized with an affinity ligand (for specific targeting).

Acknowldegments

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Exploring the similarities of reduced consciousness and unconscious states through functional connectivity: balancing data with model

Pallavicini C[a,b], Sanz Perl Y[a], Pérez Ipiña I[c], Zamberlan F[a], Issoglio F[d], Sitt J[e], Laufs H[f], Tagliazucchi E[a]

a - Departamento de Fisica, FCEN-UBA and IFIBA, CONICET-UBA
b - Laboratorio de Investigaciones Aplicadas a las Neurociencias - FLENI
c - Departamento de Física, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires
d - Departamento de Química Biológica, FCEyN, UBA - IQUIBICEN, CONICET
e - ICM - Brain and Spine Institute, France
f - Neurology Department University of Kiel, Germany

Consciousness can be defined as the capability of having a subjective experience, or having a point of view: a sense of being a distinct entity capable of agency, sentience, narrative identity in time. Conversely, unconscious states reflect the loss of these attributes, therefore the transitions between these states present an excellent framework to study neural correlates of these emerging properties and understanding of consciousness. In this work we study the similarities and differences of a set of reduced-consciousness (RC) and unconsciousness (UC) states and their contrast with wakefulness (W). Analyzing functional magnetic resonance images (fMRI) we were able to compute the functional connectivity (FC) over 90 brain areas in non-rem sleep (NREM), sedation and anesthesia with propofol, and patients in vegetative states. We used this information to train and evaluate random forest classifiers and explore generalizations amongst the FC of the six different states studied. Continuing, we replicate the RC, UC and W states using a semi-empirical model which combines the fMRI data, in vivo estimates of structural connectivity, and anatomically-informed priors that constrain the independent variation of regional activation. We found most similarities amongst sleeping and propofol treated individuals, both in the FC data and the model, except for NREM stage 1 which did not generalize with other RC and UC states. We were also able to trace brain areas associated with commonalities in FC.The present work has effectively contrasted mathematical modelling with empirical data in the pursuit of comprehending the mechanisms behind RC and UC and thus, complementarily learning what lies beneath the conscious waking states.

Molecular characterization and Cryo-EM structure of the hemocyanin of the invasive freshwater snail *Pomacea canaliculata*

Chiumiento IR[a], Brola TR[a], Ituarte S[a], Sun J[b], Qiu J[c], Otero LH[d], Heras H[a], Dreon MS[a]
a - INIBIOLP, CCT-La Plata, CONICET, Facultad de Ciencias Médicas, UNLP, 60 y 120, La Plata
b - Department of Ocean Science, Hong Kong University of Science and Technology, Hong Kong, China.
c - Department of Biology, Hong Kong Baptist University, Hong Kong, China
d - Fundación Instituto Leloir and IIBBA-CONICET

Hemocyanins (HC) are the respiratory proteins found in most mollusks and arthropods. Probably due to their huge size and glycan moieties molluscan HC can be used as immunostimulants. Nevertheless, structural knowledge of these proteins is scarce. In this work we studied the molecular assembly, glycosylation pattern and gene structure of the HC from the gastropod *P. canaliculata* (PcH). Through a proteomic and genomic approach, we identified 4 gene sequences of PcH subunits. Each subunit has the typical architecture of a gastropod HC, which is a string of eight globular functional units (FUs) of about 50 kDa each. Correspondingly, genes are organized in eight structurally related coding regions, with linker introns of variable length. Of special relevance for evolutionary considerations are introns interrupting regions that encode a discrete FU, a feature not found in other mollusks. Although the positioning of the FUs linker introns is highly conserved among mollusks, introns within FUs show no relationship neither in location nor phase. A preliminary low-resolution model of its structure was achieved by single-particle cryo-electron microscopy. PcH structure shows a cylindrical rearrangement assembled by di, tri, and tetra-decamers with an internal collar structure. PcH is N-glycosylated with oligo- or high mannose and hybrid-type structures, and complex-type N-linked glycans, without sialic acid. It is also decorated by terminal β-N-GlcNAc residues and nonreducing terminal α-GalNAc. This glycosylation profile, with an abundance of N-linked glycans, is similar to that of *Megathura crenulata* HC. In addition, polyclonal antibodies against PcH do not cross-react with other gastropod HCs, thus highlighting structural differences among them and opening a research avenue on the immunostimulant activity of PcH and the role that carbohydrates play in it.

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Effect of anesthetics in viscoelasticity and aggregation kinetics of glycated erythrocytes

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

Erythrocyte membrane present negative surface electric charges because of the sialic acid in glycocalyx. When blood flow is slower or null, the erythrocyte aggregates are similar to stacks of coins (rouleaux). However, in diabetes the erythrocyte charge can be reduced by glycation favoring formation of anomalous erythrocyte aggregation (clusters) and alteration of viscoelastic parameters. This effect could be increased in surgical procedures when the patient is under anesthetic drugs such as *propofol* (P), *remifentanilo* (R) and *vecuronium bromide* (V). To evaluate the possible effect of these anesthetics, erythrocytes from healthy donors were incubated with glucose solutions (glycated, G) or phosphate buffer solution (control, C) for 5 hours. After that, incubations were carried out with the different anesthetics and their combinations for 30 min (*propofol* at 4 µg/mL whole blood, *remifentanilo* at 10 ng/mL plasma, *vecuronium bromide* at 0.15 µg/mL plasma). After washing with PBS, the RBCs were suspended in autologous plasma at 40% and erythrocyte viscoelastic parameters were assessed (Elastic Modulus (µ), Surface Viscosity (η_m) and Deformability Index (DI)) with an Erythrocyte Rheometer from samples *in vitro* glycated and treated with P, R and V. Aggregation kinetics was also evaluated using an Optical Chip Aggregometer. When comparing between groups (G or C), Aggregation Amplitude (Amp) for G was higher than the respective not glycated treatment, meaning that glycation might increase the aggregation rate. Inside each group, Amp for PV treatment was increased when compared to C (not glycated), and PRG was higher than G. When analyzing viscoelastic parameters, µ was altered only in the glycated group for the PG, RG and RVG treatments when compared to G. The η_m was lower than C in the not glycated group, being these difference significant for R and PV treatment. There was also a variation in this parameter when comparing G and VG. The DI showed no significant differences. In conclusion, anesthetics could affect the aggregation kinetics and viscoelastic parameters after glycation and/or anesthetic treatment of RBCs. These results are very useful for prevention of complication in surgical procedures.

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Changes in biophysical properties of membranes containing sphingomyelins with very long chain PUFA during cooling

Peñalva DA[a], Hozbor F[b], Aveldaño M[a], Antollini S[a]
a - Instituto de Investigaciones Bioquímicas de Bahía Blanca (INIBIBB-CONICET)
b - INTA Balcarce

Although the molecular changes that affect the spermatozoa of economically important animals, such as bull and ram, are of great research interest, changes regarding plasma membrane lipid organization as temperature decreases are still poorly understood. Gametes from these mammals contain sphingomyelin (SM) species very long chain n-3 polyunsaturated fatty acids (VLCPUFA). (e.g., 30:6 SM, 32:6 SM and 34:6 SM in ram), while in most mammalian cells SM typically contains saturated and monounsaturated acyl chains (C14 to C24). Also, these spermatozoa have three subclasses of choline glycerophospholipids (CGP) with docosahexaenoic acid (22:6n-3) ester-bound at the *sn-2* position of glycerol, the *sn-1* position being occupied by a C16 hydrophobic chain bound by an ester, ether o vinyl ether linkage. The aim of this study was to gain information about the supramolecular structural organization of these atypical lipids and their changes in the segregation, topological and thermodynamic coexistence during cooling. After isolation of these SM species and CGP subclasses from ram sperm by a combination of chromatographic techniques, large and giant unilamellar vesicles (LUVs and GUVs) were prepared and examined by fluorescence spectroscopy and microscopy. Whereas the 3 subclasses of CGP remained in the liquid crystalline state, the SM species 30:6 SM, 32:6 SM and 34:6 SM showed gel-liquid crystalline transition temperatures within the 40 °C - 5 °C range (5, 15 y 29 °C, respectively). In ternary PC:Cholesterol:SM systems, 32:6 SM and 34:6 SM showed propensity to promote cholesterol-SM domain formation during cooling, although at different temperatures and rates from those of 16:0 SM, here used as a positive control. In GUVs containing 32:6 SM, the lateral lipid segregation and the process of dye efflux started at comparable temperatures. This coincidence did not occur in GUVs containing 16:0 SM. Thus, whereas at physiological temperatures VLCPUFA-containing SM molecular species are in a fluid state and are not involved in cholesterol-rich domains, this state is deeply altered at the low temperatures at which these gametes are usually preserved, thereby affecting their membrane stability.

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Correlation between the hydration of acyl chains and phosphate groups in lipid bilayers: Effect of phase state, head group, chain length, double bonds and carbonyl groups

Rosa AS[a], Cejas JdP[a], Disalvo EA[a], Frías MdlÁ[a]
a - CIBAAL Centro de Investigación en Biofísica Aplicada y Alimentos CONICET UNSE

This work demonstrates by means of FTIR-ATR analysis that water molecules intercalate at different extents in the acyl chain region of lipid membranes in correlation with the hydration of the phosphate groups. The evolution of water, asymmetric CH_2 and symmetric PO_2^- bands were inspected at different water vapor pressures. This correlation is sensible to the chain length, the presence of double bonds and the phase state of the lipid membrane. The presence of carbonyl groups modifies the profile of hydration of the two regions as observed from the comparison of DMPC and 14:0 Diether PC.

The present analysis shows that the phosphate hydration is coupled to CH2 hydration and that it´s modulated by double bonds, CO groups and in some extent by the acyl chain length for each membrane phase state.

The different water populations in lipid interphases would give arrangements with different free energy states that could drive the interaction of biological effectors with membranes.[1]

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Lightning Talks

Determination of the biochemical and structural modifications in the cytoplasm of bovine oocytes during *in vitro* maturation by Raman microscopy

Jimenez LE[a], Álvarez RM[a], Roldán-Olarte M[b]
a - INQUINOA (CONICET-UNT) Instituto de Química del Noroeste Argentino, Ayacucho 471, San Miguel de Tucumán, CP 4000, Tucumán, Argentina
b - INSIBIO (CONICET-UNT) Instituto Superior de Investigaciones Biológicas, Chacabuco 461 - San Miguel de Tucumán,CP 4000, Tucuman, Argentina

In recent years, studies to solve problems associated with assisted reproduction in bovines have become very important. The quality of oocytes is a determinant factor in the efficiency of *in vitro* reproduction process, therefore, being be able to identify competent oocytes for fecundation would be very important. During maturation, the complete oocyte undergoes biochemical and morphological changes, which can be separated in 2 different events, the nuclear and cytoplasmic maturation, synchronized with each other. The first, consists of chromosomal separation during the resumption of the first meiotic division, while the other event takes place with the reorganization of organelles and accumulation of mRNA, proteins, and lipids. The aim of this work is to study by Raman microscopy the cytoplasmic biochemical changes in bovine oocytes throughout *in vitro* maturation process. Immatures oocytes (IMM) and others that were 6, 18, and 22 hours (6H-MIV, 18H-MIV and 22H-MIV) under *in vitro* maturation conditions were studied. For a more precise analysis of the changes in the cytoplasm during *in vitro* maturation, the oocyte's zona pellucida was removed using an enzymatic method. The spectra of the 6H-MIV group showed the intensities of the sugar bands decreased while the intensities of the lipids signals increased considerably, with respect to IMM spectrum. This suggests that sugar is used as energy source to increment the number of lipids drops, whose main component are triglycerides. The 18H-MIV spectra showed no spectral differences regarding the lipid and protein content when compared to the 6H-MIV group. The intensities of the characteristic protein bands appeared increased in the 22H-MIV spectra with respect to the other 3 groups. Indicating that the accumulation of proteins takes place toward the end of the maturation process. These results show that Raman spectroscopy can be used as a powerful analytical tool to analyze oocyte quality.

Acknowldegments

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Effect of ethanol on yeast membranes

Genovese DM[a], Valdez-Taubas J[a], Wilke N[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.

Yeasts endure changing environmental conditions, often going through various stress situations. Given the broad applications of yeast in the industry, adaptation of yeast to stress conditions is an active research area. It has been reported that membrane fluidity is affected by environmental stresses, and that it changes as the culture ages. Furthermore, it was shown in a baker´s yeast strain, that ethanol increases membrane fluidity.

We aimed to study membrane fluidity upon ethanol (EtOH) stress in a laboratory strain of *Saccharomyces cerevisiae* yeast (BY4741), and compare it with a mutant that lacks ergosterol (erg6Δ), and a bread yeast. For this, we determined the fluorescence spectra of Laurdan before and after adding increasing levels of EtOH to the culture, and determined the Generalized Polarization (GP) of the Laurdan included in the cell membranes. GP varies from -1 (fluid membranes) to 1 (rigid membranes) depending on the dynamics of dipolar relaxation of the water molecules surrounding the Laurdan molecule.

We determined the GP value for BY4741 cultures at different stages of growth, and found no significant differences, being in all cases about 0.2. Surprisingly, erg6Δ also presented a similar GP value, suggesting that the absence of ergosterol is supplemented by its precursor Zymosterol. Baker´s yeast showed a slightly lower GP value (0.1), thus pointing to less rigid membranes.

The GP value was also determined in BY4741 at a single cell level with optical microscopy. The probe was observed only at the plasma membrane in some cells, or in all membranes in others and the GP value depended on the cell under study, finding an average value of 0.05.

When lipidic probes that mark disordered-phase regions were used (such as Rho-PE, FM4-64, and others), the mark on plasma membrane of BY4741 was heterogeneously distributed, showing patchwork-like patterns. Addition of 5% EtOH promoted no change in these patchwork aspect, while higher EtOH levels lead to the inclusion of the fluorescent probes to the cell interior.

Regarding the GP value, when 5% EtOH was added to cultures of all analyzed strains no changes were observed, while 20% EtOH decreased it temporarily, recovering the initial value after some seconds.

We conclude that both, the patchwork aspect of the plasma membrane and the high GP value found in yeast membranes appears as a constant property, which not easily changes. Therefore, it is very likely a necessary condition that has to be conserved.

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Liposomes can both enhance or reduce drugs penetration through the skin

Carrer DC[a], Peralta MF[a], Guzmán ML[b], Olivera ME[b]
a - Instituto de Investigación Médica Mercedes y Martín Ferreyra (INIMEC), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)
b - 2UNITEFA - CONICET, Pharmaceutical Sciences Department, School of Chemistry, National University of Córdoba

The adequate formulation of topical vehicles to treat skin diseases is particularly complex. A desirable formulation should enhance the accumulation of the active drugs in the target tissue (the skin), while avoiding the penetration enhancement to be so large that the drugs reach the systemic circulation in toxic amounts. We have evaluated the transcutaneous penetration of three drugs chosen for their widely variable physicochemical properties: Amphotericin B, Imiquimod and Indole. We incorporated the drugs in fluid or ultra-flexible liposomes. Ultra-flexible liposomes produced enhancement of drug penetration into/through human skin in all cases in comparison with fluid liposomes without detergent, regardless of drug molecular weight. At the same time, our results indicate that liposomes can impede the transcutaneous penetration of molecules, in particular small ones.

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Polymeric liposomes with phospholipids from lung surfactant as a potential delivery system for pulmonary tissue.

Chiaramoni N[a], Alonso S[a]
a - Laboratorio de Biomembranas (LBM), GBEyB, UNQ-IMBICE-CONICET

Liposomes have been widely used for many applications. In the last decades, polymeric lipids have been studied as an option to increase liposome stability. (Temprana et al. 2012).

In this work we used 1,2-bis(10,12-tricosadiynoyl)-sn-glycero-3-phosphocholine (DC8,9PC), a photopolymerizable lipid. When this diacetylenic lipid is in a molar ratio of 1:1 with DMPC, it forms polymers upon UV irradiation, increasing liposome stability (Chiaramoni et al., 2010). Lung surfactant is a complex with the specific function of reduces surface tension at the pulmonary air-liquid interface. Regarding its composition, about 80% and 10% of phospholipids are PC and PG, respectively, with minor contributions of PE and others. PC species are distributed in PC16:0/16:0 (DPPC), PC16:0/16:1 (POPC) and PC16:0/14:0 (PMPC) (Bernhard, 2016).

In this work, we combined lipids to form polymers but with de addition of DPPC and PMPC in order to direct these formulations to lung tissue. The hypothesis is that the presence of lipids that belong to surfactant will improve polymer interaction with pulmonary tissue.

Several formulations were obtained by combining DC8,9PC, DMPC, DPPC and PMPC. Afterwards, they were polymerised by UV irradiation, and polymerisation efficiency was determined in order to select those formulations that were more efficient.

For characterisation, particle size was determined by DLS and morphology by TEM. Membrane hydrophobicity was studied with the probe merocyanine 540. Cell viability was also assessed by the MTT method.

We conclude that those formulations with higher amount of DPPC instead of PMPC presented a more rigid surface, giving as a result enhanced polymerisation. None of the formulations presented toxic effects in cell culture

These formulations will be combined with mucolytic molecules for further assays.

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The role of Cu^+ and ATP on the reaction cycle of a Cu^+ transport ATPase from *Legionella Pneumophila*

Placenti MA[a], Roman EA[b,a], González Flecha L[a], González-Lebrero RM[a]
a - IQUIFIB-Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, UBA. Junin 956, 1113 Ciudad de Buenos Aires
b - Facultad de Ciencias Exactas y Naturales, UBA

P-type ATPases form 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 transport of transition metal ions, playing a key role in the regulation of their intracellular concentration. Cu^+ transport ATPases are the most widespread and conserved members of this subfamily among different species. In humans, mutations of these proteins are the direct cause of Menkes and Wilson diseases. Based on some functional and structural evidence, it was postulated that these proteins may have a unique specific mechanism, different from that of the most studied P-ATPases.

The aim of our work is to characterize the kinetics of the Cu^+ transport ATPase from *Legionella pneumophila* (*Lp*CopA) in order to elucidate its functional mechanism.

We evaluated the role of ATP and Cu^+ in the reaction cycle of *Lp*CopA by measuring steady state ATPase activity in presence of different concentrations of these natural ligands of the protein. We observed that ATPase activity rises as ATP concentration increases with a functional dependence that can be described by a sum of two hyperboles. On the other hand, the increment on Cu^+ concentration in the reaction media produces an increment of ATPase activity that can be described by an hyperbola plus a linear function. It is noteworthy that the ATPase activity can be measured even in the absence of Cu^+ .

From the analysis of the effects observed we formulated a minimum kinetic model that considers: two enzyme conformations with different affinities for ATP, enzyme phosphorylation and binding of at least two Cu^+ ions. This model is compatible with the structural information available for this enzyme and the reaction cycle models for the most characterized P-Type ATPases.

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Ligand-modulated transport rather than long range protein allostery couple the gates and active sites in human proteasomes

Botti H[a,b,c], Hugo M[d], Radi R[c,e], Grune T[f]

a - 1 Laboratorio de Biofísica Integrativa, Departamento de Biofísica, Facultad de Medicina, Universidad de la República

b - 2 Semillero Interdisciplinario Ciencias de la Información y de Datos Aplicadas a la Salud Humana (CIDASH), Espacio Interdisciplinario, Universidad de la República

c - 3 Centro de Investigaciones Biomédicas (CEINBIO), Espacio Interdisciplinario, Universidad de la República

d - Unidad de Investigación, Hospital Universitario Santa Cristina

e - 5 Departamento de Bioquímica, Facultad de Medicina, Universidad de la República

f - 4 Department of Molecular Toxicology (MTOX)

Human proteasomes are a group of 750 -2500 KDa protein complexes composed of a catalytic core and none, one or two regulatory particles. They are well known for their role in protein quality control, proteostasis and immune peptide processing. The complexity of even the smallest part, the catalytic core (CC also known as 20S proteasomes), is both structural and functional: all proteasomes combine transporter characteristics and self-confined peptide hydrolyzing/splicing multienzymatic systems (each core has two copies of 3 different active sites). Already published phenomena to be explained includes: sigmoid curves of product accumulation over time and apparent active site to active site and gate to active site interactions while using typical small size substrates. Also, a previous statistical model of proteasome kinetics supports that the behavior of the CC is better explained if multiple allosteric sites acting from the outside and inside of the particle were involved. We herein report a different behavior and build, estimate and select multiple models compatible with the observed data. Models are inferred for CC and 26S proteasomes in the presence of modulators and bortezomib, a drug currently used in the treatment of multiple myeloma. Our results show that once the complex enzymatic chemistry of the proteasome is fully acknowledged, model quality is enhanced and long range and outside-active site allosteric communication is no longer needed. Conflicts of Interest: the authors declare no conflicts of interest.

Contact: hbottialsina@gmail.com

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Responsive soft-nanoarchitectures as novel substrates with tunable mechanical properties

von Bilderling C[a,b], Giussi JM[b], Maza E[b], Cortez ML[b], Mijangos C[c], Pietrasanta L[d], Azzaroni O[b]

a - Departamento de Física, FCEN-UBA
b - Instituto de Investigaciones Fisicoquímicas Teóricas y Aplicadas, INIFTA. CONICET CCT La Plata – UNLP
c - Instituto de Ciencia y Tecnología de Polímeros, CSIC
d - Centro de Microscopías Avanzadas. IFIBA-DF FCEN-UBA

The design of responsive surfaces with tunable mechanical properties, such as stiffness or adhesion, holds interesting potential for applications in biomedical research, tissue engineering and cell adhesion manipulation. Nano/micro hydrogels are colloidal architectures that can incorporate large amounts of water thus changing their mechanical properties in response to specific stimuli such as temperature, pH, salt concentration or solvent nature. Poly (N-isopropylacrylamide) (PNIPAm) is one of the most studied thermoresponsive polymers: at 32°C PNIPAm-based architectures in aqueous solution experiment a drastic deswelling due to their lower critical solution temperature (LCST).

In this work, we present novel substrates designed and fabricated with two kinds of PNIPAm based thermosensitive nanoarchitectures: *spheres and pillars*. Nanomechanical properties of the substrates were characterized by Quantitative Nanoscale Mechanical-Atomic Force Microscopy (QNM-AFM).

PNIPAm nanospheres (microgels) containing a pH sensitive monomer, were LBL assembed with poly(diallyldimethylammonium chloride) (PDADMAC). Microgel based films were found to decouple conflicting properties: they exhibit an increase in hydrophobicity, stiffness and adhesion properties upon switching the temperature from below to above the LCST, while the permeability of redox probes through the film remained unchanged.

PNIPAm based nanopillars surfaces were also investigated, showing an abrupt change in wettability, topological and mechanical properties when increasing the temperature above the LCST. Interestingly, these surface properties were amplified through the incorporation of Fe_3O_4 nanoparticles. The incorporation of magnetic nanoparticles into the nanopillars sharply increased their stiffness and hydrophobicity above the LCST, whether their magnetic response resulted proportional to the amount of incorporated nanoparticles.

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Can nano-bubbles permeabilise cell membranes?

Moyano MS[a], Ferguson M[a], Del Pópolo M[a]
a - Facultad de Ciencias Exactas y Naturales (FCEN), UNCUYO, Mendoza, Argentina

The mechanical properties of cells have been studied for decades, with the intent to understand the underlying physical and chemical processes required to fulfil their basic functions. The presence of defects in cell membranes, such as pores and domain borders, plays an important role in many cellular processes, such as drug delivery and ion transport. Computer simulations have demonstrated themselves to be a powerful tool in the study of membranes and their defects. In this investigation we use computational models to simulate the behaviour of transient pores in lipid membranes, while in the vicinity of nano-bubbles. We employ a classical, coarse-grained force field, MARTINI, to simulate a 1,2-Dioleoyl-sn-glycero-3-phosphocholine (DOPC) lipid bilayer in water. Simulations of the lipid-water system are performed using the molecular dynamics (MD) code, LAMMPS. We use the Plumed tool to introduce a bias in the simulations in order to induce the formation of transmembrane pores. Other computational tools are used to analyse the structural and energetic aspects of the simulated systems. In our simulations, we observe a drastic reduction in the local lipid density upon the formation of pores. By comparing systems both in the absence and in the presence of a nano-bubble, we are able to estimate the difference in energy cost for opening transmembrane pores of different sizes. We find that the presence of a nano-bubble in the proximity of the membrane leads to a reduction in the energy required to open the pore. In addition, the pore formed has a greater radius, and therefore volume, than that formed in the absence of the nano-bubble. We conclude that nano-bubbles promote the formation of hydrophobic membrane pores which is expected to enhance membrane permeability.

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Multi scale modeling of thiol overoxidation by hydrogen peroxide: low molecular weight and protein thiol

Semelak J[a], Battistini F[b], Zeida A[c], Trujillo M[c], Estrin D[c]
a - Universidad de Buenos Aires-CONICET, DQIAQF-INQUIMAE, Facultad de Ciencias Exactas y Naturales
b - IRB-BSC Joint Research Program on Computational Biology
c - Departamento de Bioquímica and Center for Free Radical and Biomedical Research, Facultad de Medicina, Universidad de la República, Montevideo, Uruguay.

The reaction of peroxiredoxines with two-electron oxidants as hydrogen peroxide is usually accelerated by a factor of 10000 in comparison with the analogous reaction in solution. The first step of the reaction yields the protein with its key cisteyne residue oxidated as a sulfenic acid (RSOH) that is reduced in a next step, recovering the original thiol (RSH) and so the catalityc activity. However, given the conditions, the sulfenic acid can react with another equivalent of oxidant to form a sulfinic acid (RSO_2H). This process is called overoxidation and is irreversible. In order to study wheter the overoxidation is a catalyzed process or not and how fast it is in comparision with other possible reactions, we performed a computanial study as follows: First, we performed relatively long classical molecular dinamics of the protein in the sulfenic form to equilibrate it in its may be new more stable conformation, monitoring the RMSD. Secondly, we equilibrated the protein with the substrate in its active site. Finally, we performed a free energy calculation employing the Umbrella Sampling method, using a quantuum mechanics molecular mechanics scheme. This way, the system was able to exert chemical reactivity at a reasonible low computational cost. For compartion we also studied the analogous reaction in aqueos solution and compared the free energy profiles. Our results suggest that some residues that play a key role during the oxidation of peroxiredoxine, are not that relevant in the overoxidation process. Moreover, no significant changes were observed between the reaction in protein and in solution, suggesting that the process is not assisted by the enzymatic microenvironment.

Nanopatterned surface for studying protein-blood cell interactions

Saffioti NA[a], Leal Denis MF[b], Guglielmotti V[a], Ross Beraldi AM[a], Herlax VV[c], Schwarzbaum P[b], Pallarola D[a]

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

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

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

The Uropathogenic Escherichia coli (UPEC) is the main etiological factor of urinary infections[1]. UPEC produces the α-hemolysin (HlyA) toxin which plays an important role during the infection process[2]. HlyA exerts sublytic signaling on red blood cells (RBC) which involves Ca^{2+} entrance and may ultimately lead to hemolysis. Although HlyA was shown to form oligomers in the cell membrane, the role of oligomerization in toxin activity remains controversial. To address this question, we are exposing human RBC to custom-built nanopatterned surfaces where HlyA can be attached at precise locations and densities. As a first approach, we tested the best conditions for toxin attachment using linkers and continuous gold surfaces.

First, the gold surface was modified with cystamine. Then, it reacted with a polyethylene glycol polymer linker that has an N-hydroxysuccinimide ester on one end. The linker has a maleimide group on the other end which reacts with HlyA in a third step. The protein attachment was followed using a quartz microbalance. When HlyA was added to the surface we detected a significant change in resonance frequency if the linker previously reacted with the gold in a non-aqueous medium. This change does not revert with washing indicating covalent binding of the protein.

To measure the HlyA activity, RBC were loaded with Fluo 4 and incubated on surfaces functionalized with HlyA or Pro-HlyA (a precursor of HlyA without hemolytic activity). The surfaces with HlyA elicited Ca^{2+} entrance in 3,6 ± 0.24 % of total cells compared with 0.62 ± 0.1 % in the Pro-HlyA bound surfaces. This would indicate that the protein bound to the surface keeps its activity. The low number of affected RBC is related to low protein stability and loss activity during the binding process.

Future experiments will be directed towards increasing protein stability in the binding process and employment of non-continuous surfaces to modify toxin density, controlling the extent of oligomerization, and its effect on HlyA activity.

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On the conformational transition between the fully folded and locally unfolded substates of an atypical 2-Cys peroxiredoxin

Vazquez DS[a], Zeida A[b], Ferrer-Sueta G[b], Agudelo WA[c], Montes M[d], Santos J[e]
a - Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes, Grupo Vinculado de Biología Estructural y Biotecnología – IMBICE-CONICET-CCT La Plata
b - Lab. Fisicoquímica Biológica, Instituto de Química Biológica, Facultad de Ciencias, y Centro de Investigaciones Biomédicas, Universidad de la República, Montevideo, Uruguay
c - Fundación Instituto de Inmunología de Colombia
d - Departamento de Química Biológica e IQUIFIB (UBA-CONICET), Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires
e - Departamento de Fisiología y Biología Molecular y Celular. Facultad de Ciencias Exactas y Naturales. Universidad de Buenos Aires. C.A.B.A.

The thiol peroxidase from *Escherichia coli* (*Ec*TPx) is a peroxiredoxin that catalyses the reduction of different hydroperoxides. During the catalytic cycle of *Ec*TPx, two cysteine residues are involved, the peroxidatic cysteine (C_P) that is first oxidized to sulfenic acid by the substrate, and the resolving cysteine (C_R) which condenses with the sulfenic acid species of C_P to form a disulfide bond. A native conformational change in *Ec*TPx, denominated fully folded (FF) to locally unfolded (LU) transition, involving a partial unfolding of αH2 and αH3 helices (two non contiguous secondary structure elements), must occur to enable the formation of the disulfide bond since the catalytic cysteines are at 12 Å apart in the active-ready FF conformational substate. To elucidate this crucial process, the mechanism of the FF→LU and the LU→FF transitions, the forward and back steps in the catalytic cycle, respectively, were studied using molecular dynamics simulations in different oxidation and protonation states of C_P. Due to helix-coil transitions usually involve overcoming high energy barriers, thus conventional molecular dynamics (MD) simulations become insufficient to study this kind of conformational rearrangements. To surpass this obstacle, we employ the accelerated molecular dynamics scheme, an enhanced sampling technique that extends the effective simulation time scale to long microseconds. Preliminary results suggest that the FF→LU transition has a higher associated energy barrier than the refolding LU→FF process in agreement with the experimental low catalytic rate constant of EcTPx. Furthermore, *in silico* designed point-mutants of the αH3 helix enhanced locally unfolding events, suggesting that the native interactions in the active site of the FF native substate are not optimized for fully speed-up the conformational transition.

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Photoinduced Protein Nitration Method by Sensitizer Tris(bipyridine)-Ruthenium(II) Chloride Complex

Giménez E[a], Cavazzutti GF[a], Toscani AM[b], Jovin TM[c], Urlaub H[d], Falomir Lockhart LJ[e,a]
a - Instituto de Investigaciones Bioquímicas de La Plata (INIBIOLP), CCT- La Plata, CONICET. Facultad de Ciencias Médicas. Universidad Nacional de La Plata.
b - Facultad de Cs. Médicas, UNLP
c - Laboratory for Cellular Dynamics, Max Planck Institute for Biophysical Chemistry
d - Group of Bioanalytical Mass Spectrometry, Max Planck Institute for Biophysical Chemistry
e - Departamento de Cs. Biológicas, Facultad de Ciencias Exactas, UNLP

The addition of a -NO2 group to the side chain of proteins is a well-known post translational modification (PTM) related, not only to physiological processes, but also to a wide range of pathologies triggered or promoted by oxidative stress, including the aging process. The -NO2 group is mainly added to Tyrosine (Tyr) residues, but can also be incorporated to side chains of Tryptophan, Phenylalanine and Cysteine. Since this PTM is a common feature in several chronic diseases and disorders, in vitro studies have employed chemical reagents such as tetranitromethane and peroxynitrite as nitrative agents to model oxidative conditions. However, photochemical methods have aroused as powerful alternatives, in which different photosensitizers, such as rivoflavin and pterins, together with light, trigger radical reactions involved in oxidative processes but in a more controllable way. In this regard, Tris(bipyridine)-Ruthenium(II) Chloride complex (Ru(bpy)3Cl2) has been successfully used as sensitizer in photoinduced crosslinking reactions of unfolded proteins (PICUP), particularly with alpha-Synuclein (Borsarelli et al, Free Rad. Biol. Med., 2012). Despite its
proven potential as photosensitizer, it has not been tested for protein nitration. Therefore, we have adapted PICUP protocols to favors nitration over crosslinking in photochemical reactions induced by Ruthenium complexes, ammonium persulfate and light in order to systematically produce recombinant proteins with these types of PTMs for further characterization.

Our work first focused on the characterization and optimization of conditions for free Tyr residues nitration, including putative side reactions and interference by other amino acids. Then we evaluated the modification of different model proteins and its effect over their activity employing spectroscopic techniques and mass spectrometry. Supported by our results, we propose a novel method to massively produce nitrated proteins, which could be employed to investigate the consequences of introducing -NO2 on proteins as a model for oxidative stress-related pathologies in vitro.

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An NMR-active chemical probe to measure stereospecific methionine sulfoxide reductase activity *in vitro* and *in vivo*.

Sánchez-López C[a], Lombardo V[b,a], Biglione FA[a], Manta B[c], Binolfi A[a,d]
a - Instituto de Biología Molecular y Celular de Rosario, IBR-CONICET-UNR, Rosario, Argentina
b - Centro de Estudios Interdisciplinarios (CEI), Universidad Nacional de Rosario
c - Division of Genetics, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School
d - Plataforma Argentina de Biología Estructural y Metabolómica (PLABEM)

Oxidation of methionines is a hallmark of oxidative stress[1] but it is now emerging as a post-translational modification capable of regulating protein activity and cellular processes.[2,3] The oxidation of methionine side chains is a stereoselective oxidation of sulphur giving rise to the diastereomers S- and R-methionine sulfoxide (MetOx). This covalent modification can be reversed by the stereospecific enzymes MSRA and MSRB that specifically reduce MetOx S- and R-diastereomers, respectively. The stereospecific activity of these enzymes has been widely studied *in vitro*, nevertheless the analytical tools available to study their activities in native environments are scarce. The few available are limited to analyze just the activity of a single reductase and they do not allow discriminating between MSRA and MSRB in the same reaction. In this work, we developed a MetOx-derived small molecule called CarMetOx, as an NMR-active chemical probe to monitor stereospecific MSRs activity in native environments. We exploited the idea that sulfoxides display unique NMR signals that include different sets of resonances for their S- and R-diastereomers that are readily identified in the NMR spectra.[4] The progressive disappearance of cross-peaks corresponding to each diastereomer of ^{15}N-labelled CarMetOx can be analyzed by NMR to describe MSRA and MSRB enzymatic activity *in vitro* and *in vivo*. Reduction kinetics of an *in vitro* mixture of S- and R-diastereomers of CarMetOx spiked with pure MSRA or MSRB, showed that our chemical probe behaves like an oxidized protein substrate and allowed us to unambiguously assign each diastereoisomer. Studies in cell extracts demonstrated that CarMetOx is a good biosensor to monitor the activity of endogenous bacterial and mammalian MSRA and MSRB. Lastly, we tested CarmetOx in developing zebrafish embryos as a proof-of-principle for its *in vivo* use. Real-time NMR measurements of CarMetOx microinjected in the entire organism showed endogenous enzymatic MSRs activity and the analysis of each diastereomer demonstrated that zebrafish MSRAs enzymes are more efficient than MSRBs. Overall, we introduce CarMetOx, a novel NMR-based biosensor to study MSRs activities in native environments and at the same time introduces an analytical tool to explore the structural roles and functions of biomolecules in multicellular organisms by NMR.

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Bases of pH sensing by a transmembrane Histidine kinase

Bortolotti A[a], Almada JC[a], Vázquez DB[a], Inda M[a], Ruysschaert JM[b], Cybulski LE[a]
a - Departamento de microbiología. Facultad de Ciencias Bioquímicas y Farmacéuticas. Universidad Nacional de Rosario. CONICET.
b - Structure et Fonction des Membranes Biologiques (SFMB), Universite Libre de Bruxelles, Bruxelles, Belgium.

Changes in environmental conditions, like temperature or pH, represent challenges which not only primitive but also modern organisms face. Several systems have emerged to detect and adapt to changing conditions; nevertheless, the molecular bases of detection and signaling are still obscure for most of the systems.

The two component system DesK-DesR contributes to maintain lipid homeostasis in *Bacillus subtilis*. DesK has been identified as a transmembrane thermosensor that triggers synthesis of unsaturated fatty acids (UFAs) at low temperatures. Since Bacillus lives in the soil, where the pH may vary; we analyzed the activation of the DesK/R pathway at different pH and found that at acidic pH UFA synthesis is inhibited. A highly charged linker region containing basic and acidic residues connects the transmembrane domain with the cytoplasmic catalytic domain of DesK. We propose that this linker is responsible for pH sensing. At neutral pH, this charged region folds into a helix by salt bridge formation within the helix in the register i+4, and promotes the kinase state. At low pH, protonation of glutamate residues of the linker disrupts these bonds, which results in helix destabilization and interruption of signaling. This pH- dependent conformational change of the linker allows DesK to functions as a pH sensor. In acidic conditions, Bacillus decreases the content of UFA and other fatty acids with low melting point and increases the content of higher melting point fatty acids. This rearrangament in the lipid profile results in an increase of membrane lipid rigidity. We propose this could be a mechanism that Bacillus has evolved to protect cells from proton influx independently of well-known temperature effect.

Dialkyl-guanidines as a novel series of Rac1 GTPase inhibitors: an interdisciplinary approach

Wetzler DE[a], Ciarlantini M[b], Barquero A[a], Defelipe L[a], Dodes Traian MM[a], Bucci H[a], Turjanski A[a], Bayo Fina JM[c], Fiore E[c], Mazzolini Rizzo G[c], Ramírez J[d], Comin MJ[b]
a - Departamento de Química Biológica- FCEN- UBA/ IQUIBICEN-CONICET
b - Laboratorio de Síntesis Orgánica, Departamento de Ingredientes Activos y Bio-refinerías, INTI
c - Instituto De Investigaciones En Medicina Traslacional- UNIVERSIDAD AUSTRAL
d - Departamento de Química Orgánica- FCEN- UBA/ I UMYMFOR-CONICET

Rac1 is a GTPase which belongs to the Rho family of small GTP-binding proteins. These small GTPases regulate cytoskeletal and cell adhesion dynamics and thereby coordinate a wide range of cellular processes, including cell migration, cell polarity and cell cycle progression. As aberrant activation of Rac1 is associated with cell malignant transformation including tumorigenesis, angiogenesis, invasion, and metastasis, this protein is a promising target for drug development.

As in all GTPases, Rac1 activity is controlled by the GDP/GTP exchange and the GTP hydrolysis. These slow reactions are catalyzed in cells by two kinds of regulatory proteins. While the guanine exchange factors (GEFs) activate small GTPases catalyzing the exchange of bound GDP for the cellular GTP, GTPase activating proteins (GAPs) accelerate the intrinsic rate of GTP hydrolysis, leading to their inactivation. Thus, an interesting strategy is to inhibit Rac1 through its activation cycle, particularly by the disruption of the interaction between Rac1 and one specific GEF partner.

We conducted an interdisciplinary approach that comprised the rational design, synthesis, and evaluation of a new family of inhibitors. The work involved computational docking experiments, compounds synthesis, antiproliferative, apoptosis and cell migration assays, *in vitro* biophysical experiments with recombinant proteins and *in vivo* mice assays.

Based on structure/function information of the complex between Rac1 and its GEF, Tiam1, and employing as starting point a previously reported inhibitor we developed a novel series of Rac1 GTPase inhibitors.

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Structural and functional characterization of short versions of BlaR1 and MecR1 of *Staphylococcus aureus*

Mihovilcevic D[a], Llarrull L[a]
a - Instituto de Biología Molecular y Celular de Rosario, IBR-CONICET-UNR, Rosario, Argentina

The membrane proteins BlaR1 and MecR1 from the pathogen *Staphylococcus aureus* detect β-lactams and unleash a cascade of events that results in manifestation of resistance. Little is known about the intramolecular events that lead to activation of the cytoplasmic metallo-protease domain of these proteins. Full-length MecR1 and BlaR1 have not been crystallized so far, due to failed attempts to overexpress these two membrane proteins. Here we report the overexpression in *E. coli* and purification of two shorter versions of these sensor proteins. BlaR1JH1 is a shorter version of BlaR1 present in the β-lactam-resistant strain *S. aureus* JH1. BlaR1JH1 lacks the first three transmembrane helices and was overexpressed as a fusion to Mistic. MecR1-glz is the gluzincin domain of MecR1 and was expressed with a C-terminal His6x-tag. This domain contains the Zn(II) binding site of MecR1 with the mutation E205A (devoid of auto-proteolytic activity). Mistic-BlaR1JH1 presented an active sensor domain that was irreversibly acylated by the fluorescent penicillin Bocillin-FL. MecR1-glz inserted into the membrane of *E. coli*, in accordance with our proposal of the presence of a reentering loop that functions as a membrane anchor. Using circular dichroism, we confirmed that MecR1-glz was structured in ASB-14 micelles, with a secondary structure composition in agreement with our bioinformatic model of MecR1. MecR1-glz has a single Trp residue in the reentering loop and anchoring to the membrane would protect it from the solvent. However, fluorescence quenching assays with KI showed that this Trp was solvent accessible in ASB-14 micelles. In conclusion, we have succeeded in purifying two shorter versions of the BlaR1 and MecR1 proteins. Initial characterization of MecR1-glz in detergent micelles indicates incompatibilities with our model in a membrane environment. Hence, we are now setting up the conditions to purify them in nanodiscs, so we can study the proteins in their native environment.

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Water state/structure inside nanopores modulates hydrolytic enzymatic catalysis

Burgos I[a,b], Velasco M[c], Acosta R[c], Ochoa A[d], Perillo MA[a,d]
a - Instituto de Investigaciones Biológicas y Tecnológicas (IIByT-CONICET)
b - Universidad Nacional de Córdoba, Facultad de Ciencias Exactas Físicas y Naturales. Departamento de Química Industrial y Aplicada. Cátedra de Química Biológica.
c - IFEG-UNC CONICET. FaMAF-Universidad Nacional de Córdoba
d - Universidad Nacional de Córdoba. Facultad de Ciencias Exactas, Físicas y Naturales. Depto. de Química - Cátedra de Química Biológica

It is widely accepted that water molecules inside the cell compartments exhibit physical properties that differ of dilute solutions. Then, the microenvironment in which metabolic activity actually occurs in cells is not equivalent to the conditions used to determine the properties of enzymes in vitro. In this work the kinetic activity of β-Galactosidase confined in a nanopore of a silicate gel was studied and it was related to confined water dynamics. Encapsulation of proteins in silicate gels obtained by the sol-gel method has been of interest for the possibility of studying protein properties in conditions far from dilute solutions. The silicate gel obtained showed highly porous structure, quite narrow pore diameter distribution, around 40 nm, and high surface area. The results observed for the encapsulated β-Galactosidase ($E_{\beta\text{-Gal}}$), which catalyses a hydrolysis reaction, let us take into consideration the differences between bulk and confined water molecules inside the nanopores and the influence of these differences on the catalytic activity. The activity of $E_{\beta\text{-Gal}}$ was measured for two substrates: PNPG and ONPG. It is well known from mechanism studies that, for ONPG, the rate limiting step is the one in which a molecule of water takes part in the reaction, while for PNPG the rate limiting step is the previous one in which water is not relevant. The V_{max} value calculated, after fitting with Michaelis-Menten model, for $E_{\beta\text{-Gal}}$ was higher than the V_{max} obtained for the free soluble enzyme ($S_{\beta\text{-Gal}}$) when the substrate hydrolysed was ONPG, and was even higher for the enzyme encapsulated in aged gels. On the other hand, the V_{max} values for $E_{\beta\text{-Gal}}$ calculated from the measurements with PNPG did not evidence significant differences with $S_{\beta\text{-Gal}}$. V_{max} is proportional to the catalytic constant (k_c) which, in turn, measures the rate of the limiting step in the reaction mechanism. Strikingly, transversal relaxation times (T_2) from ^{1}H-NMR experiments of the hydrogens of water molecules inside the nanopores of the gel, evidenced three population groups and, in all cases, with lower diffusion rate than the bulk water molecules. Accordingly, to the results of V_{max} with ONPG, the diffusion of the three population of water lowered in aged gels. In view of these results, it is quite plausible to propose that some characteristic of confined water inside the nanopores influence the hydrolytic activity of $E_{\beta\text{-Gal}}$.

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A proposed reaction mechanism for maize monomeric glyoxalase I

Gonzalez JM[a], Agostini RB[b], Alvarez CE[b], Klinke S[c], Andreo CS[b], Campos-Bermudez VA[b]
a - INBIONATEC, CONICET, National University of Santiago del Estero. 4206, RN 9 Km 1125, Santiago del Estero, Argentina
b - CEFOBI-CONICET, Centro de Estudios Fotosintéticos y Bioquímicos
c - Fundación Instituto Leloir, Av Patricias Argentinas 435, (1405), Buenos Aires, Argentina

Detoxification of methylglyoxal, a toxic by-product of central sugar metabolism, is a major issue for all forms of life. The glyoxalase pathway evolved to effectively convert methylglyoxal into D-lactate *via* a glutathione hemithioacetal intermediate. Recently, we have shown that the monomeric glyoxalase I from maize exhibits a symmetric fold with two cavities, potentially harboring two active sites, in analogy with homodimeric enzyme surrogates. Here we confirm that only one of the two cavities exhibits glyoxalase I activity and show that it adopts a tunnel-shaped structure upon substrate binding. Such conformational change gives rise to independent binding sites for glutathione and methylglyoxal in the same active site, with important implications for the molecular reaction mechanism, which has been a matter of debate for several decades.

Reference

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Poster Presentations

Cell motility control at cell colony front and its influence on the propagation dynamics

Muzzio N[a], **Pasquale M**[b], Arvia A[b]

a - 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

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

The rational description of the growth dynamics of biological systems plays a key role in the understanding of many physiological and pathological processes as well as for designing improved strategies for disease treatments and tissue engineering applications. One approach has been the use of rather universal models commonly utilized for describing the front propagation of systems, irrespective of their nature. Cell trajectory, i.e., random or ballistic, have been demonstrated to affect the global cell colony dynamics and is useful to give physical meaning to the components of the proposed growth models. These models have been suggested to fall into the Kardar-Parisi-Zhang (KPZ) class.

In this contribution we report results from tumor cell colony spreading under radial and linear geometry. For the latter we also perform cultures on ridge-patterned substrates with different orientation respect to the initial quasi-linear colony front. For quasi-radial cell colonies (QRCs) at the first stages of growth, the spreading kinetics in the presence of dispersing agents, namely epidermal growth factor, exhibits a clear influence of the increase in cell motility. Thus, the commonly used growth models are modified to properly describe the results. In other set of experiments, the colony front displacement velocity of initially quasi-linear colonies (QLCs) on smooth substrates remains constant irrespective of the presence or absence of Mitomycin C, a compound that inhibits cell proliferation. Finally, the front displacement velocity of QLCs spreading on ridge-patterned substrates followed a quadratic relationship with the angle formed by the ridge direction and the normal to the colony front, in agreement with the KPZ standard equation. This behavior correlates with the enhanced cell motility at higher angles. To sum up, a bridge between the global dynamics and processes at the cellular level is possible through cell motility characteristics.

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Interactions and spreading dynamics of preosteoblast and breast tumor cell lines in co-culture experiments

Bibé S[a], **Pasquale M**[b]
a - Facultad de Ciencias Médicas, Cátedra de Patología B, Universidad Nacional de La Plata
b - Instituto de Investigaciones Fisicoquímicas Teóricas y Aplicadas (INIFTA), CCT- La Plata, CONICET. Facultad de Ciencias Exactas, Universidad Nacional de La Plata.

Co-culture systems are utilized for studying the interaction of cells with different phenotype or for testing new materials designed for selective cell adhesion. In this contribution we run co-culture experiments employing MC-3T3 preosteoblast and breast tumor T47D cells. Three different co-culture setups are employed: a) MC-3T3 and T47D cells are seeded together on an adherent surface and the growth and colony pattern formation is followed up; b) MC-3T3 cultures with T47D conditioned medium were performed. c) Quasi-linear colonies of each cell lines are generated on the same dish at the opposite sides, and their front propagation followed up. The latter setup consists of a Petri dish with a mobile partition of different width that is removed once the colony of each cell line becomes confluent. It allows studying the pattern evolution and the propagation dynamics of a cell colony front in the presence of another culture of either the same or different type of c ells. The setup a) and b) are also employed for the evaluation of alkaline phosphatase (ALP) expression of MC-3T3. The proliferation of MC-3T3 cells decreases in the presence of T47D cells or in conditioned medium. Furthermore, the average spreading area of MC-3T3 cells become smaller and the cell morphology changes into one with a larger number of filopodia. At relatively longer times, the co-culture morphology resembles that of T47D cell cultures. Results from the setup c) indicate a decrease in the MC-3T3 colony front displacement velocity with time, in the presence of T47D cells. Moreover, a distinct propagation pattern can be observed for each cell type before and after physical contact of both colony fronts. The overall results show that the changes in MC-3T3 characteristics are mainly due to soluble species coming from the conditioned medium that also produce a decrease in ALP expression.

The experimental setup and the results presented here may be useful to contribute to a better description of cell colonization processes.

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Regulation of plasma membrane calcium pump (PMCA) by the cytoskeleton

Vigil MA[a], Rey O[b], Rinaldi D[a], Picco ME[b], Espelt MV[a], Tezanos Pinto F[a], Mangialavori I[a], Rossi RC[a], Rossi JP[a], Ferreira-Gomes MS[a]
a - IQUIFIB-Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, UBA. Junin 956, 1113 Ciudad de Buenos Aires
b - Instituto de Inmunología, Genética y Metabolismo (INIGEM)

We have previously described a novel regulatory mechanism for PMCA activity that involves the actin cytoskeleton. In HEK293 cells, PMCA activity was regulated by the polymerization state of the cortical cytoskeleton: actin depolymerization with cytochalasin-D significantly increased PMCA activity while using jasplakinolide that stabilizes filamentous actin, decrease PMCA activity. Reports suggest that the increase in cytosolic Ca^{2+} concentration ($[Ca^{2+}]_{CYT}$) induced transient cortical actin depolymerization. We hypothesize that after an increase in $[Ca^{2+}]_{CYT}$, PMCA will be activated by short actin oligomers formed at the cell cortex. This may lead PMCA to actively restore $[Ca^{2+}]_{CYT}$ level. By lowering the $[Ca^{2+}]_{CYT}$, actin may re-polymerize into long filaments and the PMCA return to its inactive state. To test this hypothesis, we carried out experiments aimed at measuring $[Ca^{2+}]_{CYT}$ and actin dynamics in cells that overexpress different PMCA isoforms, both in normal and pathological cells.
The dynamics of $[Ca^{2+}]_{CYT}$ were studied in HEK293T cells using the fluorescent probe Fluo-4 and following $[Ca^{2+}]_{CYT}$ levels generated by its release from the endoplasmic reticulum (ER), and by the extracellular uptake through store-operated Ca^{2+} channels. Results show that fluorescence time courses measured in individual cells are different from those obtained from the whole population of cells, suggesting the presence of at least two different kinds of cell responses. Overexpression of isoforms 2 and 4 of human PMCA contributed to decrease the $[Ca^{2+}]_{CYT}$ arising from ER depletion and the extracellular medium. On the other hand, the use of different actin-markers and Life-act overexpression, that bio-mark actin, allowed us to measure different polymerization states of actin in the cells. The combination of these determinations will be used to establish the role of PMCA in what might be a versatile feedback mechanism that contributes to the interaction between Ca^{2+} and the cortical cytoskeleton.

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Adsorption of Cationic and Amphipathic Cell-penetrating Peptides to Porated Membranes

Ramirez PG[a], Del Pópolo M[b], Vila JA[a], Szleifer I[c], Longo G[d]
a - Grupo de Bioinformática Estructural-IMASL-CONICET-UNSL.
b - Facultad de Ciencias Exactas y Naturales (FCEN), UNCUYO, Mendoza, Argentina
c - Department of Biomedical Engineering, Northwestern University, Evanston, IL, USA.
d - 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

Cell-penetrating peptides (CPPs) are short peptide sequences that can translocate to the inside of most mammalian cells and transport molecular cargoes through lipid membranes. While initially most identified CPPs were of a cationic nature, recently, certain amphipathic sequences peptides have been developed that exhibit cell-penetrating properties. Since CPPs were first discovered, their translocation mechanism has been subject to intese debate. In this regard, computational methods can help us analyze a wide range of parameters, and extract precise data for each case, in order to better understand their translocation mechanism. In the present work, we develop a molecular theory that allows to investigate the adsorption and insertion of cationic and amphipathic CPPs on membranes bearing hydrophilic pores. This method accounts for size, shape, conformation, protonation state and charge distribution of the peptides, as well as the interactions between hydrophobic peptides and the membrane; this method also describes the state of protonation of acidic membrane lipids. We present a systematic investigation of the effect of different peptides and its concentration, membrane composition, pore size, salt concentration and pH on the extent of peptide adsorption and insertion into the pores.

Analysis of Laurdan fluorescence spectra in vesicles formed by esters and ethers lipid

Gonzalez Paz A[a], Gimenez RE[a], Frías MdlÁ[a], Disalvo EA[a], Pérez HA[a]
a - CIBAAL Centro de Investigación en Biofísica Aplicada y Alimentos CONICET UNSE

Many works study the behaviour of model membranes of varied characteristics using fluorescent probes sensitive to their environment. Among them Laurdan is specifically sensitive to the hydration of the membrane interphase. The membrane interphase has dynamic and dielectric properties different from those of the bulk aqueous phase and the hydrophobic region. With this purpose, the Generalized Polarization (GP) is calculated from the intensity of the emission and excitation wavelengths.

In this work, we report that using different emission maxima in gel phase and liquid crystalline phase different curves for GP of Laurdan vs temperature can be built. The results show that, for the same lipid vesicles, the curves of GP vs.Temperature differ when the wavelength chosen to perform the calculation is modified. This dependence of GP curves with wavelength makes it difficult to extract conclusions regarding the polarity of the membrane. Furthermore, since different lipids present different maxima, the choice of wavelengths becomes rather arbitrary and therefore the conclusions misleading. However, using Log-Normal deconvolution a parameter analogous to GP, independent of the wavelength, can be obtained. This parameter (ΔS) is obtained by calculating the difference between the relative area of the non-relaxable and the relaxable populations of solvent molecules (water in the interphase). The log-normal deconvolution of the emission spectra gives the contributions of relaxable and non-relaxable populations of Laurdan in large unilamellar vesicles (LUV) of ester and ether lipids, lipids that differ only on the kind of bond between the glicerol and the hydrophobic chain that gives different hydration states.

Using the ΔS parameters, we obtain more reliable data indicating that LUVs of ether lipids present a higher dipolar relaxation (relaxable population) than LUVs of ester lipids.

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A necessary connection: cholesterol and nicotinic receptors

Fabiani C[a], Peñalva DA[a], Corradi J[a], Antollini S[a]
a - Instituto de Investigaciones Bioquímicas de Bahía Blanca (INIBIBB). Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur (UNS- CONICET), Bahía Blanca, Argentina

It is known that the muscle nicotinic acetylcholine receptor (nAChR) is highly influenced by its lipid environment. It 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. Its transmembrane domain forms the ion channel pore and exhibits extensive contacts with the surrounding lipids. In this work we studied the close relationship between nAChR and cholesterol under different experimental conditions in order to enrich with, deplete of, redistribute between both hemilayers, and oxidate cholesterol molecules. These conditions were evaluated either in *T. californica* nAChR-rich membranes, in model membranes containing purified nAChR or in cells expressing nAChR. Cholesterol modifications were confirmed by lipid analysis using thin layer chromatography. Evaluation of a) membrane order perturbations, by Laurdan GP and fluorescence anisotropy, b) increase/decrease of Lo domains, by fluorescence microscopy, c) nAChR-Lo domains correlation, by detergent treatment and SDS-PAGE, and d) nAChR conformation and function, by fluorescence spectroscopy and electrophysiology showed that changes in the amount, distribution or oxidation of cholesterol impacts not only in the size, location and curvature-domain shape of Lo domains and in the nAChR preference for them, but also in nAChR functionality and nAChR structural conformation. A high correlation between the quantitative presence of cholesterol, its transmembrane and lateral asymmetry and nAChR conformation and functionality is postulated.

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Assesing the effect of Ursolic Acid on Lipid Droplet biogenesis using a Langmuir model system

Caruso B[a], Tohmé Chapini MJ[b], Wilke N[c], Delgui LR[d]

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 - IHEM, Universidad Nacional de Cuyo, CONICET, Facultad de Ciencias Médicas / Facultad de Farmacia y Bioquímica, Universidad Juan Agustín Maza. Mendoza, Argentina

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 - IHEM, Universidad Nacional de Cuyo, CONICET, Facultad de Ciencias Médicas / Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Cuyo. Mendoza, Argentina

According to the latest Lipid Droplet (LD) biogenesis models, interfacial tension plays a critical role on such process, determining the shape of the budding LD from the ER. We have previously presented triglyceride (TG) “lenses” at the air-water interface as a model system for studying LD biogenesis. The performance of Langmuir monolayers of mixed phosphatidylcholine (EPC)/TG in coexistence with TG microlenses (*i.e.* an excluded TG phase floating in the surface) and the characterization of their lenses´ thickness by Brewster Angle Microscopy (BAM) allow evaluating the proneness of TG lenses to form a LD. This is done by characterizing the associated thermodynamics and the resulting shape of the TG lenses. Here, we evaluated the modulation of TG lenses tendency to detach from the interface by the presence of a third component with interfacial activity. For this purpose, we choose Ursolic Acid (UA), a natural pentacyclic triterpenoid with many biological effects and a clear interfacial activity. We found that UA slightly diminished the surface pressure at which TG phase separation occurs (*i.e.*, lenses appearance) in composition dependent manner (both %UA and PC/TG relation). Such effect may be correlated with the dependency of UA interfacial partitioning on lipid composition. On the other hand, BAM analysis showed a lower reflectivity of lenses in the presence of UA. Assuming a negligible effect of UA on the refractive index of TG lenses, this implies a thinning of such structures. Additionally, the decreased radii of lenses observed allowed us concluding that the contact angle of the lenses is lower in the presence of UA. In conclusion, this experimental model allowed the detection of an interfacial active compound, like UA, affecting both the energy necessary to form the TG lenses and its shape, leading to structures of lower curvature and radii. These results are in accordance with observed effects of UA on LD in cells: a decrease in LD number and radii.

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Effect of growth temperature on the properties of *Listeria* membrane and on the interaction with the antimicrobial peptide enterocin CRL35

Dupuy F[a], Medina Amado C[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

The food borne pathogen *Listeria monocytogenes* is able to grow in a wide variety of environmental conditions, such as temperature. In this regard, the bacteria endure 4 °C and up to 37 °C as well. Membrane adaptation is an important biochemical process for maintaining steady bilayer elasticity, necessary for proper membrane protein functionality and membrane selective permeability. In this work, we studied the lipidic extract of *Listeria* cultures growth at 10, 25 and 37 °C by means of bilayer and monolayer model systems. The extracts obtained from cultures at 10 °C were more disordered than 25 and 37 °C, according to infrared spectroscopy and fluorescence anisotropy. Also, Langmuir monolayers assays indicated that lipids obtained from 10 °C cultures formed softer films than lipids from 25 and 37 °C. Results thus indicate that the cells adjust membrane properties according to homeoviscous adaptation theory by means of lipid composition. Changes in membrane properties affected enterocin CRL35 antimicrobial activity, which was probed in *Listeria* cells at different temperatures.

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Effects of "*de novo*" designed peptides on bacterial envelop of *Escherichia coli and Staphylococcus aureus*

Maturana P[a], Espeche JC[a], Martinez M[b], Maffia P[b], Hollmann A[a,b]
a - Laboratorio de Compuestos Bioactivos, Centro de Investigación en Biofisica Aplicada y Alimentos CIBAAL– CONICET, Universidad Nacional de Santiago del Estero
b - Laboratorio de Microbiología Molecular, IMBA, Departamento de Ciencia y Tecnología, UNQ, Argentina

The emergence of multidrug-resistant bacteria has a direct impact on global public health. In this context, antimicrobial peptides (AMPs) represent an alternative due to exhibit a broad spectrum of antimicrobial activity (bacteria, fungi, parasites, viruses) and do not easily induce resistance. In previous work, by using model membranes, we studied two related "the novo" design peptides, P6 that show no antimicrobial activity and P6.2 witch exhibited good antibacterial activity. However, in order to a proper characterization, effect on bacteria must be unraveled. In this context, we aimed to study the interaction of both peptides with whole bacteria using *Escherichia coli and Staphylococcus aureus* as a model.

Zeta Potential experiments confirming that both peptides were able to interact with the negative bacterial envelope of *E. coli* and *S. aureus*. However, the effects on P6.2 were much more noticeable in both bacteria. Then, the disrupt of the bacterial envelope was asses by dynamic light scattering following the polydispersion index. A significant increase in the dispersion of total particulates content was observed in both bacteria after being incubated only with P6.2, indicating disorganization of bacteria structure.

The ability of both peptides to disrupt the bacterial membrane was also evaluated. In the case of *E. coli*, the outer (OM) and inner (IM) membrane permeabilization were analyzed. Beside both peptides were able to induce damage in the OM, 6 times fold of P6 was needed in order to obtain comparable results than those obtained with P6.2. When IM disruption was evaluated, a similar trend was observed, P6.2 not only exhibits more product formation, implying a greater disruption of the membrane but a faster kinetic. In the case of *S. aureus,* both peptides were able to permeabilize the plasmatic membrane, but the effects were much more noticeable with the P6.2. When membrane permeabilization data were compared in both bacteria, *E. coli* showed faster kinetics and a lower amount of peptide was needed to obtain similar results.

All data together allows postulating, in a physiologic model, that the poorer affinity of P6 for bacterial envelope results in a lower concentration final of the peptide in the bacterial membrane, insufficient to cause enough membrane damage capable of triggering bacterial death.

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Effects of the insecticide Fipronil on membrane models. Experimental and computational studies

Felsztyna I[a,b], Sánchez-Borzone ME[a,b], Miguel V[a,b], García DA[a,b]
a - Instituto de Investigaciones Biológicas y Tecnológicas, IIByT (CONICET-UNC)
b - Universidad Nacional de Córdoba. Facultad de Ciencias Exactas, Físicas y Naturales. Cátedra de Química Biológica

Fipronil is a widely used phenylpyrazole insecticide with high activity against insect pests and low toxicity for mammals. It exerts its action by blocking the passage of chloride ions in the central nervous system GABA and GluCl receptors. Given that these are membrane intrinsic proteins and that Fipronil is a highly lipophilic compound, non-specific effects on the physical properties of the membrane cannot be discarded. In a previous work, we have demonstrated that Fipronil interacts with lipid membranes using DPPC Langmuir films as the membrane model. In this work, we complement those experimental results with studies in DPPC large unilamellar vesicles (LUVs). Moreover, we contrast these experimental results with molecular dynamics simulations (MDS) in mono- and bilayer systems. Free diffusion MDS of DPPC monolayers in three different molecular packing states and in a DPPC liquid-crystalline bilayer confirmed the strong tendency of Fipronil to partition in lipidic phases. The NH_2 group of Fipronil forms hydrogen bonds with the glycerol region of DPPC and, to a lesser extent, with the phosphate group. Bilayer MDS show that Fipronil is not able to pass from one hemilayer to the other or to cross the bilayer. We obtained spatially resolved free energy profiles of Fipronil partition into the DPPC bilayer based on umbrella sampling. These profiles allowed us to determine that the most favorable partition site of Fipronil would be located between the glycerol region and the acyl chain of DPPC. This result was confirmed by fluorescence anisotropy studies of DPPC-LUVs in the presence of Fipronil, where a stronger effect on anisotropy was observed for DPH probe compared to TMA-DPH, which locates closer to the phospholipid polar head groups. These experiments also showed that Fipronil has an ordering effect in the liquid-crystalline phase of the liposomes and a fluidizing effect in the gel phase. The same behavior was shown by the deuterium order parameter obtained from the MDS.

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Hydrogen bonds and water in steroid-containing phospholipidic membranes assessed by near infrared spectroscopy

Wenz JJ[a]

a - Instituto de Investigaciones Bioquímicas de Bahía Blanca (INIBIBB). Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur (UNS- CONICET), Bahía Blanca, Argentina

Near infrared spectroscopy (NIR) is useful to study the H-bonds network in water systems, which are sensitive to solutes. The less the number and/or the strength of H-bonds, the more the strength of the covalent O-H bonds of water. Hence, they vibrate and absorb radiation at higher frequencies. By taking advantage of this spectral shift water in DMPA membranes with and without one of two steroids, progesterone and 25-hydroxycholesterol, was assessed.

Spectra of vesicles of DMPA and DMPA/steroid in MilliQ water (500 µM; 80:20 molar ratio for the steroid-containing samples) between 1100 and 2300 nm were acquired at 10 temperatures in the range 13-58 °C, with a path length of 1 mm. For understanding the tangled spectra, principal component analysis was applied to disclose subtle changes mirroring the alterations in the vibrational energy of the O-H bonds, as an indirect measure of the H-bond network. The analysis encompassed the first overtone of water (centered at 1450/1455 nm) representing signals of overlapping water and O-H stretching bands, as well as a limited portion of 1100-1300 nm.

The shift to higher frequencies (i.e. higher energy) of the O-H vibration band with increasing temperatures or in the presence of 25-hydroxycholesterol is consistent with a strengthening of the O-H bonds caused by disruption/weakening of the H-bonds. The number of strongly H-bonded waters decreases whereas the number of weakly H-bonded waters increases. The observed shift agrees with reports about the distinct absorption of three states of water: waters having no -OH bonded, having one -OH group H-bonded and having two -OHs H-bonded. In contrast, DMPA and progesterone did not show significant effects on the H-bonds network. Findings concur with the lipid domain-promoting activity reported for 25-hydroxycholesterol (a cholesterol-like behavior) and with the slightly (or absent) lipid domain-disrupting activity of progesterone.

Interaction of the chlorogenic acid (CGA) with model lipid membranes and its influence in antiradicalary activity

Cejas JdP[a], Disalvo EA[a], Frías MdlÁ[a]
a - CIBAAL Centro de Investigación en Biofísica Aplicada y Alimentos CONICET UNSE

Free radicals production in living organisms has been associated with the oxidation of DNA, proteins and in particular membrane lipids. In the latter case, the alterations produced in its structure distort its functions, with consequent pathological changes in the organism under study. This is one of the reasons why there is a great interest in the study of natural antioxidants that, by eliminating free radicals in an effective way, can prevent many diseases directly associated with lipid peroxidation [1].

In this context, CGA is an important natural antioxidant composed by an ester of caffeic and quinic acid, that belongs to the group of polyphenols. Its strong antioxidant and antibacterial properties reduce the risk of diabetes, fungal infections and cancer, and also protects the liver and other organs. A high antioxidant activity with respect to the red blood cell membranes has also been reported but its mechanism of action is not yet well defined [2]. In this work, the interaction of CGA with model lipid membranes (monolayers with different lipid composition) was studied through changes in surface pressure. The results show that CGA interacts with DMPC membranes and in a lesser extent with those of DPPC generating structural changes at the interface. The analysis of the kinetics of interaction of the antioxidant with DMPC membranes, shows that it occurs with significant structural changes in the membrane. Also, preliminary results show that positively charged radical species such as $ABTS^{\bullet+}$ form an association with CGA in the immediate proximity of the membrane, preventing its propagation. These results allow us to discuss the possible mechanism of action of the CGA depending on the hydration state of the lipid interface preventing lipid oxidation in the biological membranes.

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Interaction of the Small-Molecule Kinase Inhibitors Tofacitinib and Lapatinib with Membranes

Alonso de Armiño D[a], Haralampiev I[b], Luck M[b], Abel T[c], Huster D[c], Scheidt HA[c], Di Lella S[d], Müller P[b]

a - Departamento de Química Inorgánica, Analítica y Química Física, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires / INQUIMAE-CONICET
b - Humboldt-Universität zu Berlin, Department of Biology,
c - Leipzig University, Institute for Medical Physics and Biophysics
d - Laboratorio de Fisiología de Proteínas, IQUIBICEN y Facultad de Ciencias Exactas y Naturales, UBA, Buenos Aires, Argentina

Lapanitib and tofacitinib are small-molecule kinase inhibitors approved for the treatment of advanced or metastatic breast cancer and rheumatoid arthritis, respectively. So far, the mechanisms which are responsible for their activities are not completely understood. Here, we focused on the interaction of these drug molecules with membranes, which has not been investigated so far in detail. Regarding their lipophilic characteristics, quantitatively reflected by large differences of the logP values, different membrane interactions of both molecules have to be expected. Applying experimental (nuclear magnetic resonance, fluorescence and ESR spectroscopy) and theoretical (MD simulations) approaches, we found that lapanitib and tofacitinib bind to lipid membranes and insert into the lipid-water interface of the membrane. For lapatinib a deeper embedding into the lipid bilayer was observed leading to a different impact of the molecules on the membrane. Whereas, for tofacitinib no influence was found, lapatinib causes a disturbance of membrane structure, as seen from an increased permeability of the membrane for polar molecules. These data may contribute to a better understanding of the effectiveness of these drugs in the treatment of respective deceases and their side effects.

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Study of the effect of antibacterial prenylated benzopyrans isolated from *Peperomia obtusifolia* (Piperaceae) on the Gram-positive bacterial surface

Ruiz Mostacero N[a], Castelli MV[a], Cutro A[b], **Hollmann A**[b,c], Lopez S[a]
a - Área Farmacognosia, Facultad de Ciencias Bioquímicas y Farmacéuticas, CONICET, Universidad Nacional de Rosario
b - Laboratorio de Compuestos Bioactivos, Centro de Investigación en Biofisica Aplicada y Alimentos CIBAAL– CONICET, Universidad Nacional de Santiago del Estero
c - Laboratorio de Microbiología Molecular, IMBA, Departamento de Ciencia y Tecnología, UNQ, Argentina

Peperomia obtusifolia is a herbaceous perennial plant native to the Americas used as a traditional medicine to treat snake bites and as a skin cleanser. The antimicrobial screening performed on organic extracts from its aerial parts showed that low polarity extracts were active on *Staphylococcus aureus* ATCC 25923. The bioassay-guided fractionation of hexane and dichloromethane extracts allowed the isolation of two benzopyran compounds already isolated from the species: peperobtusin A (C1) and 3,4-dihydro-5-hydroxy-2,7-dimethyl-8-(3”-methyl-2”butenyl)-2-(4’-methyl-1’, 3’pentadienyl)-2H-1benzopyran-6-carboxylic acid (C2). Compound C2 showed growth inhibitory activity on methicillin-resistant *S. aureus* isolates and other Gram-positive bacteria like *Staphylococcus epidermidis* and *Enterococcus faecalis* with Minimal Inhibitory Concentration values (MIC) of 4-8 µg/mL (10.8-21.6µM). The interaction of C2 induced changes in Zeta potential (ΔZ) of *S. aureus* and biomimetic bacterial membranes (DMPC:DMPG 5:1, liposomes). At concentrations in which it showed the antibacterial activity, C2 decreased ΔZ to more negative values, indicating that the compound is able of affect bacterial surface properties, and membrane damage was confirmed by fluorescent microscopy experiments. These findings indicate that C2 can establish a direct interaction with bacterial membranes possibly due to its amphipathic characteristic: while the isoprenoid chains could interact with the phospholipid membrane, the carboxylic acid moiety may expose its negative charge to the interface. Considering that surface acting agents have been found to display remarkable bactericidal effect with a lesser tendency to trigger resistance, the discovery of the mode of interaction on the bacterial surface for C2 is promising. However, additional research will be necessary to deep into the mechanism in which C2 acts as antibacterial, as well as the structural requirements to maintain and also to increase its activity

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Bacteria's inner and outer membrane permeabilization by "*de novo*" antimicrobial peptide P1 through spectroscopic methods

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

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

b - 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.

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 bacterial membranes as well as its ability to permeabilize the inner and outer membrane of Gram positive and Gram negative bacteria. P1 is a cationic peptide with 21 amino acids (WPKWWKWKRRWGRKKAKKRRG), designed identifying short putative active regions from AMP databases. Its antimicrobial activity was previously tested in *Staphylococcus aureus* and *Escherichia coli* strains having gotten a MIC value of 32 and 128 respectively.

Inicially, we evaluated the interaction of P1 with bacterial envelope by using Zeta Potential in suspensions containing the mentioned strains. In both cases, zeta potential became less negative after peptide incubation confirming the ability of the peptides to bound into the cell envelope. However, the effect becomes more noticeable in *S. aureus.* in a good agridemnt with MIC data

Next, in order to determine the ability of the peptide to permeabilize the outer membrane of these bacteria, fluorescence methods were used. Results have shown that P1 is able to disrupt both strains' outer membranes reaching higher values and faster kinetics in the case of *E. coli*. In the case of *S. aureus* kinetics are slightly slower.

Inner membrane permeabilization was assessed only for *E. coli* using absorbance. It was found that the kinetics for the disruption of this membrane is reached successfully by the peptide. In this case, it required longer time lapses for the permeabilization to happen.

From the results we got, we can conclude that the peptide is able to permeabilize both outer and inner membrane of *E. coli,* reaching the first effect in much shorter times (from seconds to a pair of minutes) than the second (1 hour approximately). This difference could be due to the fact that the peptide needs to permeabilize the outer membrane first in order to reach the inner. Differences observed in disruption speed for the membrane in *S. aureus* (Gram positive bacteria) could be explained since this bacteria have a thicker cell wall, that might hinder the reach of the peptide to the membrane. Furthermore, a relation between concentration of peptide and membrane damage was stablished for both membranes.

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Design and comparison of biomimetic membranes based on natural bovine and triatomine membranes

Colmano GN[a,b], Sanchez-Borzone M[a,b], Turina AdV[a,b]
a - Instituto de Investigaciones Biológicas y Tecnológicas, IIByT (CONICET-UNC)
b - Universidad Nacional de Córdoba. Facultad de Ciencias Exactas, Físicas y Naturales. Cátedra de Química Biológica

Phospholipid monolayers are often used to emulate biological membrane behavior and interactions with diverse compounds. Yet frequently, these monolayers consist of a single phospholipid or binary mixtures. These monolayers do not share many similarities with biological membranes, for this reason, the aim of this work is to construct Biomimetic Membranes (BM) and test whether they reproduce the behavior of Natural Membranes (NM) and their interaction with an insecticide of sanitary interest, Fipronil. The NMs were synaptosomal membranes of *Bos taurus* cerebral cortex (NM_{Bt}) and cerebral ganglia membranes of *Triatoma infestans* nymphs (NM_{Ti}). The BMs were assembled with the major lipid components of their respective MN (BM_{Bt} and BM_{Ti}). Biomimetic Membranes π-A isotherms do not reflect the same behavior of NMs, they are devoided of transition and exhibit a smoother outline. The collapse pressure (πc) of BM_{Bt} was quite similar to that of NM_{Bt}, yet πc of BM_{Ti} was higher than the one observed in NM_{Ti}. The compression modulus of both BMs at π =30 mN/m was higher than the one found in NMs, this means that BMs at that π, are more condensed than the natural ones. The presence of fipronil in the subphase, even at low concentrations, causes an increase of ΔV, more evident in NMs. π_{cutoff} essays showed that penetration of fipronil in NMs is only possible up to 34 mN/m, whereas in BMs this value increases to 50 mN/m in BM_{Bt} and 44 mN/m in BM_{Ti}, suggesting that the presence of proteins in NMs modifies the interaction of fipronil. Fluorescence anisotropy studies showed that BMs behaved similarly to NMs. Fipronil exerted no effect over DPH and TMA-DPH anisotropy values of NMs, but it did affect BMs. EFM images of transferred monolayers show that BMs allow a more homogeneous distribution of the DiIC18 fluorescent probe than NMs, but they both exhibit non-defined condensed domains at π above 30 mN/m. Fipronil exerts changes in the distribution of the fluorescent probe in NMs and BMs and this effect was more noticeable at high π values (30 mN/m or higher). In Summary, even though BMs did not emulate their respective NMs perfectly, many parameters were successfully reproduced, and BM_{Ti} reproduced its natural counterpart more accurately.

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Determining the fluid ordered and disordered phases in pulmonary surfactant by two different ESR methods

Cimato A[a], Facorro G[a], Martinez Sarrasague M[a]
a - Cátedra de Física, Facultad de Farmacia y Bioquímica. UBA

Pulmonary surfactant (PS) main function is to reduce surface tension at alveolar interface. Two lipids domains coexist in PS membranes: a liquid-ordered (Lo) and a liquid-disordered (Ld) phases. This coexistence of phases would be crucial for the surfactant activity. Until now, the proportion of phases was determined qualitatively by ESR with the empirical S/W ratio obtained from the 5DE spectrum. This ratio was questioned in the literature as it can be affected by other variables such as probe solubility and membrane fluidity. We had already developed an ESR technique using TEMPO as spin label to quantify the % of Ld phase. This method is laborious and requires the quantification of PL. The aim of this study was to validate the S/W ratio as a phase proportion indicator, contrasting it with the value of A/B obtained by the TEMPO technique.

Methods: Samples labeled with TEMPO were used to determine the lipid fraction in Ld phase. PS with or without extra Albumin, Fluticasone, VLDL or Cho was labeled with 5DE to estimate S/W ratio. Spectra of EPS labeled with TEMPO and 5DE were obtained at different temperatures. PL concentration were measured by Stewart method. Pearson correlation coefficients between S/W and A/B or % of Ld were determinate in all the cases. A unique equation was established for the calculation of PL in Ld phase.

Results: The A/B and S/W parameters correlated inversely with R2 0.86 for the same sample at different temperatures.PS with different amount of Cho showed a Pearson coefficient of 0.944 between the same parameters. Also, S/W ratio from different samples of PS added with substances that modify the proportion of Ld/Lo phases decreased proportionally with the increase in the % Ld (R2 = 0.949).

Conclusions: The results obtained validate the S/W parameter as an estimator of the proportion of Lo/Ld phases. This represents an advantage since with the same spin label you can determine the fluidity of the membrane and quantify the proportion of phases.

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Fibril like structures induced in lipid/β-amyloid peptide Langmuir monolayers can be broken in the presence of gangliosides

Bolaño Álvarez A[a], Caruso B[b], Rodriguez P[c], Fidelio GD[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.
b - Instituto de Investigaciones Biológicas y Tecnológicas, IIByT (CONICET-UNC)
c - Ministerio de Ciencia y Tecnología de la Provincia de Córdoba

Langmuir monolayers at the air-water interface is a proper technique to study the interfacial properties of film forming peptides mixed with lipids. It allows to manipulate the lipid/peptide mole ratio, the amount of the **P**eptide **C**overed **A**rea (**PCA**), the physical state of the lipid and the degree of lateral compactness in a confined environment, mimicking a biological membrane interface. Using lipid/peptide monolayers we studied the surface properties of Aβ(1-40) **A**myloid **P**eptide (AP) mixed with POPC lipid and gangliosides.

At low peptide proportion, AP forms fibril-like structure in monolayers when mixed with POPC phospholipid with reproducible π-Area isotherms. The maximal stability acquired by the mixed films is within 35-40 $mN.m^{-1}$ compatible with the equivalent surface pressure postulated for natural biomembranes.

Pure AP forms insoluble monolayers with high lateral stability without forming fibril-like structures. In a liquid-expanded lipid environment (POPC:AP mixture), we observed that both miscibility and stability of the film depend on PCA. In the range of 2.5 % to 10 % of PCA we observed fibril like structures. These fibril-like structures are clearly observed with both BAM and AFM techniques. In this experimental condition, we found that GM1 and total brain gangliosides break these fibril-like structures indicating a lateral fibril-segregation imposed by the presence of gangliosides at the mixed interface. This effect is observed at 5-20 mole % of ganglioside with respect to POPC lipid. According to the literature the effect of gangliosides on AP aggregation is controversial. In our experimental condition gangliosides have a disaggregating effect.

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Interaction of green silver nanoparticles, determined by surface characterization techniques, with *Staphylococcus aureus* and *Escherichia coli*.

Ferreyra Maillard A[a], Gonçalves S[b], Santos N[b], Lopez de Mishima B[c], Dalmasso P[d], **Hollmann A**[e,a,f]

a - Laboratorio de Compuestos Bioactivos, Centro de Investigación en Biofisica Aplicada y Alimentos CIBAAL– CONICET, Universidad Nacional de Santiago del Estero
b - Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal
c - INBIONATEC, CONICET, Universidad Nacional de Santiago del Estero
d - CIQA, CONICET, Departamento de Ingeniería Química, Facultad Regional Córdoba, Universidad Tecnológica Nacional
e - Facultad de Agronomía y Agroindustrias, Universidad Nacional de Santiago del Estero
f - Laboratorio de Microbiología Molecular, IMBA, Departamento de Ciencia y Tecnología, UNQ

The nanoparticles have unique physical, chemical and biological properties and different from the bulk material they come from. In particular, silver nanoparticles (AgNPs) has proven to be an alternative for the development of new antibacterial agents. The possibility to generate AgNPs coated with novel capping agents, such as phytomolecules obtained *via* a green synthesis (GAgNPs), is attracting great attention in scientific research.

Recently, we showed that membrane interactions seem to be involved in the antibacterial activity of AgNPs obtained via a green chemical synthesis using the aqueous leaf extract of chicory (*Cichorium intybus L.*). Furthermore, we observed that these G-AgNPs exhibited higher antibacterial activity than those obtained by chemical synthesis.

Although the antibacterial activity of AgNPs in gramnegative and grampositive bacteria has been evidenced, its mechanism of action is not fully elucidated. The objective of this work was to study the antibacterial activity of green AgNPs against *Escherichia coli* and *Staphylococcus aureus* to characterize its mode of action and determine its cellular target.

The effect of the G-AgNPs on the bacterial surface was first evaluated by zeta potential measurements and correlated with direct plate count agar method. Zeta potential of both tested bacteria become more negative after being incubated with increasing concentrations of G-AgNPs, but this effect becomes more noticeable in *E. coli*. This behavior matches with higher diminution of CFU obtained in *E. coli*. These results allow confirming a strong correlation of zeta potential variation with bacterial damage.

Afterward, atomic force microscopy was applied to directly unravel the effects of these G-AgNPs on bacterial envelopes observing several damages in both bacteria.

Overall, the data obtained in this study seems correlated with a multi-step mechanism by which electrostatic interactions is the first step prior to membrane disruption, resulting in antibacterial activity.

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Langmuir films at the oil/water interface revisited

Mottola M[a,b], Caruso B[a,b], Perillo MA[a,b]
a - 1 Universidad Nacional de Córdoba. Facultad de Ciencias Exactas, Físicas y Naturales. Depto. de Química - Cátedra de Química Biológica. Córdoba, Argentina.
b - 2 CONICET, Instituto de Investigaciones Biológicas y Tecnológicas (IIBYT). Córdoba, Argentina.

Lipid miniemulsions (ME) are oil in water dispersions stabilized by an interfacial layer of a surfactant are systems commonly used to encapsulate, maintain and release molecules of pharmacological interest. 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.

We studied monomolecular layers at the oil/water interface (O/W_{int}) in a Langmuir interfacial trough using egg-yolk phosphatidylcholine (EPC) (the model phospholipid) and Vaseline (VAS) as oil phase. The temporal dynamics in the surface pressure (π) evolution depended on the method (spreading/adsorption) used for monolayers preparation and reflected the different distribution of EPC between all the system compartments (bulk phases and interfaces). We distinguished between EPC located either stable at the interface or hopping between the interface and bulk phases. The size order of the apparent mean molecular area, at constant π, of EPC at different interfaces ($EPC_{O/W}$ > $EPC/VAS_{0.02;A/W}$ > $EPC_{A/W}$), suggested that VAS molecules intercalated between the hydrocarbon chains of $EPC_{O/W}$, at a molar fraction x_{VAS} > 0.02. However, $EPC/VAS_{0.02;A/W}$ showed the highest compressional free energy. This leaded us to study the $EPC/VAS_{0.02}$ mixture at A/W by Brewster Angle Microscopy (BAM), finding that upon compression VAS segregated over the monolayer, forming non-coalescent lenses (as predicted by the spreading coefficient S = −13 mN/m) that remained after decompression and whose height changed (increase/decrease) accompanied by the compression/decompression cycle. At the O/W_{int}, while some VAS molecules remained at the interface up to the collapse, others squeezed out towards the VAS bulk phase with an energy requirement lower than towards the air.

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Permeability of lipid membranes to hydrogen peroxide

López AC[a,b,c], Thomson L[a,b], Möller M[a,c]
a - Centro de Investigaciones Biomédicas, Universidad de la República
b - Laboratorio de Enzimología, Instituto de Química Biológica, Facultad de Ciencias, Universidad de la República
c - Laboratorio de Fisicoquímica Biológica, Instituto de Química Biológica, Facultad de Ciencias, Universidad de la República

Hydrogen peroxide (H2O2) is an oxidizing species produced by several enzymatic mechanisms. It has many physiological functions including defense against pathogens, vasodilatation and regulation of cell growth, migration and proliferation. It has been shown that cell membrane permeability to H2O2 depends on the presence of specific aquaporins, but there is no data on simple diffusion through purely lipid membranes.

In this work we set out to determine the permeability coefficient (Pm) of liposome membranes to H2O2 through an enzyme latency method, using liposomes with trapped catalase. Preliminary results indicate that the permeability of membranes composed of DMPC, DOPC, DPPG, and cholesterol in different proportions varies between 4.0 x 10-5 cm/s and 5.0 x 10-4 cm/s.

The partition coefficient (Kp) between organic solvents (octanol, hexadecane and olive oil) and water was also determined, as well as the thermodynamics of the process. This gives us an idea of the solubility profile of H2O2 at different membrane depths. The Kp was found to be 0.07 in octanol vs water, and it decreased 4 orders of magnitude in hexadecane vs water. The distribution in organic solvents is thermodynamically unfavorable, mainly because of the entropic component, consistent with a hydrophobic effect on H2O2. Therefore, the main barrier to the transport of H2O2 through the membrane is thermodynamic and is because of the low solubility of H2O2 in the hydrophobic fraction of the bilayer.

These results will be useful for understanding the permeability of different cellular membranes to H2O2 and distinguish the relative importance of the lipid fraction and the protein channels in H2O2 diffusion.

Regulation of the action of phospholipases on lipid membranes by HePC

Zulueta Díaz Y[a], Fanani ML[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.

Miltefosine (HePC) is an amphiphile that is currently used as a cytotoxic for anti-tumor treatments or against infection diseases such as Leishmaniasis. It has been reported that the action of enzymes related to lipid metabolism, such as phospholipases, are very susceptible to changes in the physical environment of the membrane. Based on these reports, we hypothesize that HePC could exert its pharmacological function by affecting the action of phospholipases (Sphingomyelinase: SMase and Phospholipase C: PLC) indirectly by modulating these membrane properties. Therefore, we study the effect of HePC on changes either at the level of enzymatic activity or on the changes that the formation of products has on the physical properties of those membranes.

In the present study, we used large unilamellar vesicles (LUVs) composed of DOPC (PLC substrate): DOPE or SM16 (SMase substrate): DOPE in the absence and presence of HePC. In these systems, the activity of both enzymes was determined by thin plate chromatography (TLC). Simultaneously, we studied the change in turbidity of the LUV suspensions by spectrophotometry and the changes induced in the particle size distribution by Dynamic Light Dispersion (DLS).Our results indicated that the presence of HePC did not affect the initial hydrolysis rate of DOPC or SM16 (or the production of DAG or Cer) at short times. However, in the case of PLC, the addition of 20 mol % of HePC inhibited the restructuring of membranes, characteristic of the DAG enzymatic formation process. A drastic decrease in vesicle aggregation, DAG formation and generation of smaller of LUVs populations compared to the control was observed at long times (40 min). These results show for the first time that HePC is able to inhibit the enzymatic action and therefore the aggregation induced by the PLC in a remarkable way. In the case of SMase, a modest effect on vesicle restructuring was observed.

In general, results obtained appear not to be related to an enzyme inhibition or interaction of HePC with the substrates or products of the reaction but with a non-specific geometric compensation.Therefore, these results gain relevance in the understanding of the mechanism of action by which the HePC regulates the lipid metabolism and signal transduction pathways in which these enzymes are involve.

Abbreviations: DOPC: dioleoylphosphatidylcholine; DOPE: dioleoylphosphatidylethanolamine; DAG: diacyl glycerol; Cer: ceramide; Hexadecylphosphocholine: HePC.

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Relationship between different hydrolysis rates of phospholipase A_2 and water activity in lipid interfaces in connection to Laurdan fluorescence properties

Fidelio GD[a], Bagatolli LA[b]

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.
b - Instituto de Investigación Médica Mercedes y Martín Ferreyra, INIMEC (CONICET), UNC, and Departamento de Química Biológica Ranwel Caputto, Facultad de Ciencias Químicas, UNC, Argentina

A way of analyzing the decrease in surface tension upon adsorption of surfactant molecules to the interface is through the Gibbs' adsorption equation. This theory considers the dividing surface area in which only the excess of the surfactant is taken into account. However, the thickness of the interfacial region between water and air is estimated to be between 6-8 Å thick, where the water density ranges from 0 (air phase) to 1 gr/cm^3 (pure water surface). According to Damodaran's approximation (1), water activity in this interfacial region (where the density of water is around 0.17 gr/cm^3) plays a fundamental role on the emergence of the variable surface pressure. Surface pressure can then be expressed ***as a function of surface water activity***. Interestingly, the dependence of surface pressure vs water activity of this region is quite similar to the liquid-expanded phospholipid monolayer π vs A isotherm (1). LAURDAN fluorescence parameters has been shown to be sensitive to the extent of water dipolar relaxation in lipid interfaces displaying different phases (2). In particular, the steady-state fluorescence parameter known as generalized polarization (GP) function has been characterized and applied to study phase transitions in biomembranes (2). GP linearly correlates with the lipid order parameter measured by NMR (3) and in a liquid-expanded lipid interface the ***GP function depends on the lipid packing (surface pressure)*** (4). Since both water activity and GP depend on surface pressure, we propose ***to combine these variables and correlate water activity with Laurdan GP***. As the activity of PLA2s depends on lateral packing (5), our correlation allows us ***to quantitatively correlate enzymatic activity with water activity in the surface***. As complex glycosphingolipids impose greater hydration (6) (lower GP) and inhibit the activity of both PLA_2 and PLC (7) in isobaric form, we propose that ***this inhibition is regulated by changes in the interfacial water activity***.

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Thermodynamic study of glyphosate insertion into pure and binary lipid bilayers

Frigini EN[a], Porasso RD[a]
a - Instituto de Matemática Aplicada San Luis (IMASL), UNSL-CONICET, San Luis, Argentina.

Glyphosate is the most used herbicide for the control of weed growth, allowing to reduce the use of fertilizers. In this work, simulations of molecular dynamics (MD) were carried out to study the influence of glyphosate
(in its charged state, $GLYP^{2-}$) on different models of pure (1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and 1,2-dipalmitoyl-sn-glycero-3-phosphoserine (DPPS)) and binary (DPPC/DPPS) lipid bilayers completely hydrated. In this sense, the profiles of free energy, entropy and enthalpy were obtained by means of umbrella sampling calculations at three different temperatures (350, 355, and 360 K). For the systems (i) DPPC/DPPS for the temperatures of 350 and 355 K, and (ii) DPPS for the three temperatures, water defect and Na^{+} ions were observed together with the $GLYP^{2-}$ inside the lipid bilayers. In addition, different properties of the different lipid bilayer model at different temperatures were analyzed. Both the mean area per lipid, the thickness of the lipid bilayer and the orientation of the lipid head groups were not observed noticeable changes (less than 5 %) when comparing the system in presence and absence of $GLYP^{2-}$. Translational diffusion coefficient value for the systems in presence and in absence of the $GLYP^{2-}$ was obtained a considerable change (greater than 6 % until 59.6 %). In the process of $GLYP^{2-}$ insertion into the membrane for the DPPC and DPPS system, they are driven by a favorable enthalpic change. For binary lipid bilayer systems it is driven by favorable entropic change.

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Biophysical characterization of *Medicago truncatula* PIP aquaporins

Canessa Fortuna A[a], Vitali V[a,b], Giorgetto F[a], Frare R[c], Scochera F[a,b], Bredeston L[b], Ayub ND[c], Soto G[c], Alleva K[a,b]

a - Cátedra de Física, Facultad de Farmacia y Bioquímica. UBA

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

c - Instituto de Genética, INTA-Castelar-CONICET

Legumes are important cultivated species in part due to symbiotic nitrogen fixation by rhizobia. *Medicago truncatula* is the closest plant model to alfalfa. The colonization of legumes roots triggers a development program that forms nitrogen-fixing nodules. Among all the transmembrane proteins of the legume nodules so far described, the recurrence of members of the aquaporin family is striking. In this work we present the progress achieved in the biophysical characterization *Medicago truncatula* PIP aquaporins. First, by means of cloning, heterologous expression in *Xenopus laevis* oocytes and water transport assays, we characterized the functional consequences of the interaction among two PIP2 (here named MtPIP2A and MtPIP2B) and two PIP1 (here named MtPIP1A and MtPIP1B). We found that not all pairs PIP2-PIP1 present the same pH gating profile, showing the amplification of the biological response that can be obtained by these kind of interactions in a cell. Then, we explored the expression profile of each of these four PIP in whole plant by analysis of available transcriptomic data to know if the interactions found are plausible in vivo. Finally, we present the first steps in the GFP-based optimization scheme followed to achieve the overexpression and purification of these channels in *S. cerevisiae*. These biophysical studies not only contribute to the understanding of PIP biological function in legumes but also are an opportunity to explore the complex mechanism of hetero-oligomerization and pH gating showed by the PIP aquaporin family of channels.

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FaPIP2-FaPIP1 heterotetramers and FaPIP2 homotetramers show different sensitivity to mechanical regulation

Guastaferri F[a], Galizia L[b], Goldman R[c], Sutka M[a], Ozu M[a]
a - Instituto de Biodiversidad y Biología Experimental y Aplicada (IBBEA) - UBA-CONICET
b - Instituto de Investigaciones Médicas Alfredo Lanari, IDIM UBA-CONICET
c - Departamento de Ciencias Fisiológicas, Facultad de Medicina, UBA

FaPIP2;1 is a high P_f strawberry water channel that forms heterotetramers with FaPIP1;1, and can be regulated by pH, by means of a cooperative mechanism. In our previous work we studied the transport properties of FaPIP2;1 and found that this water channel responds just like a mechanosensitive aquaporin. It is hypothesized that this mechanism would also be cooperative involving the four monomers. The study of pH sensitivity with FaPIP2;1-FaPIP1;1 heterotetramers demonstrated that the interactions between different subunits modify the cooperativity among monomers. We consider FaPIP2;1-FaPIP1;1 heterotetramers would be a good model to study the cooperativity in mechanical regulation of aquaporins. Our hypothesis is that FaPIP2;1-FaPIP1;1 heterotetramers show different sensitivity to mechanical regulation than FaPIP2;1 homotetramers. By means of the same methods followed in previous works, we determined the water flux (J_w) and osmotic permeability coefficient (P_f) of xenopus oocytes injected with a mix of cRNA of FaPIP2;1 and FaPIP1;1 (1:3). Each oocyte was injected with 5 ng of cRNA and subjected to different osmotic gradients (Δosm) created with mannitol. On our most recent results the J_w ($nl.s^{-1}$) measured in oocytes subjected to 40, 90 and 140 $mOmol.Kg_w^{-1}$ was 0.30 ± 0.10 (n=6), 0.59 ± 0.10 (n=8) and 1.13 ± 0.07 (n=8), respectively. These results show a linear J_w-Δosm relationship (R^2>0.98), and the same P_f value, of about $2.0x10^{-2}$ $cm.s^{-1}$, in all tested conditions. In comparison, FaPIP2;1 homotetramers show deviations from linearity for said relationships, a typical behavior of mechanosensitive aquaporins. That being so, it can be said that FaPIP2;1-FaPIP1;1 heterotetramers appear to be at least less sensitive to membrane tension changes than FaPIP2;1 homotetramers.

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Flexible N and C terminal domains in aquaporin gating

Zerbetto De Palma G[a,b,c], Scochera F[a,c], Toriano R[d], Soto G[e], Zeida A[f], Alleva K[a]
a - Departamento de Química Biológica e IQUIFIB (UBA-CONICET), Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires
b - Instituto de Biotecnología, Universidad Nacional de Hurlingham
c - Universidad de Buenos Aires, Departamento de Fisicomatemática, Cátedra de Física.
d - Laboratorio de Biomembranas, IFIBIO-Houssay (UBA-CONICET), Facultad de Medicina, UBA.
e - Instituto de Genética, INTA-Castelar-CONICET
f - Departamento de Bioquímica and Center for Free Radical and Biomedical Research, Facultad de Medicina, Universidad de la República

Plant plasma membrane intrinsic proteins (PIP) have a very conserved 3D structure but many questions remain open regarding the structure-function relationship involved in gating. These are tetrameric channels that are largely composed by transmembrane alpha helices (approximately 60%) and flexible elements, such as loops and both, N and C termini (NT and CT). It is known that NT and CT can have important roles in channel functionality and some reports suggested that they may be involved in PIP gating. NT and CT are quite flexible elements and are not present in the available structures. PIP have longer NT (30-46 residues) than CT (10-17 residues), being NT negatively charged and CT positively charged. Despite these ends are the least conserved elements in PIP subfamily, some residues are highly conserved: in particular a PPP motif in NT, Glu and Asp residues in NT[1,2] and Ser and Arg residues in CT. Previous studies confirmed that NT acidic residues are important in PIP gating and controversial results were reported for CT's serine[3,4]. In this work, we study the participation of both CT and NT in pH gating of Beta vulgaris PIP. We constructed BvPIP mutants altering conserved residues, performed functional experiments and molecular dynamics simulations. Our results show that the PPP motif and R285 are involved in the PIP open-close transition triggered by cytosolic acidification, while S283 is not. These experimental and in silico approaches will let us unveil the role of NT and CT in PIP gating mechanism.

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Response alteration of a protein kinase by punctual mutations in its transmembrane domain.

Almada JC[a], Bortolotti A[a], Larisa C[a]
a - Departamento de microbiología. Facultad de Ciencias Bioquímicas y Farmacéuticas. Universidad Nacional de Rosario. CONICET.

Organisms have to transmit environmental signals across cell membranes to survive. An example of a conserved mechanism in *Bacillus subtilis* to maintain homeostasis in adverse conditions is DesK, a transmembrane histidine kinase. Its physiological regulation shows that the lower temperature, the higher activity. In contrast, a truncated DesK variant lacking the transmembrane region but retaining the cytoplasmic catalytic domain activates the circuit regardless of temperature, with higher activity at 37°C than 25°C. Through reverse engineering, punctual mutations and a biophysical approach, we discovered that inversion of the signal dependence requires that the catalytic domain be fused to a transmembrane segment containing a hydrogen-bond zipper at a particular face of the helix. That module allows catalytic domain's enzymatic activity to be inhibited at optimal growth temperatures and increased in suboptimal conditions. Furthermore, changing the pattern of dimerization-promoting hydrogen bonds allowed us to tune the activity of the sensor, including reverting back the regulation (high activity at high temperature). Thus, we showed that the hydrogen-bond zipper is a design used by mesophilic bacteria to increase enzymatic activity at low temperature, decoupling the natural thermo-dependency of the catalytic domain of the enzyme.

Spf1 P5-ATPase remains functional after proteolytic cleavage at the C-terminal region

Petrovich GD[a], Corradi GR[a], Adamo HP[a]
a - Departamento de Química Biológica, IQUIFIB, Facultad de Farmacia y Bioquímica, UBA

P-ATPases are active transporters essential for cellular homeostasis. Loss of function of the P-ATPases of group 5 or P5-ATPases in humans has been associated with early-onset Parkinsonism (Kufor-Rakeb syndrome) and other neurodegenerative diseases. P5-ATPases are still poorly characterized. We aimed to learn about the structure and function of the Spf1 P5-ATPase from yeast by partial proteolysis. Recombinant Spf1 was purified in mixed micelles of $C_{12}E_{10}$ detergent. In this condition, low amounts of chymotrypsin rapidly decreased Spf1 ATPase activity. In contrast, when the purified protein was supplemented with soybean asolectine the ATPase activity was only marginally affected by the protease despite the fact that SDS-PAGE and mass spectrometry of the proteolyzed sample indicated that the Spf1 (Mr 135 kDa) was being cut at a QT1 site located in the C-terminal portion of the molecule producing a fragment of about 110 kDa (Petrovich et al. SAB2018). After QT1 cleavage the mayor Spf1 fragment exhibited normal phosphoenzyme formation and turnover. Current homology models of the Spf1 structure place the QT1 site near the M5 transmembrane segment, a critical region for the function of the protein. Thus our observations light up unanticipated features of the P5-ATPases.

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Characterization of endogenous volume-sensitive ion currents in *Xenopus laevis* oocytes

Galizia L[a], Bazzi Z[b], Guastaferri F[c], Ozu M[c], Schwarzbaum P[b]
a - Laboratorio de Canales Iónicos, Instituto de Investigaciones Médicas Alfredo Lanari, Universidad de Buenos Aires, IDIM-CONICET, Buenos Aires, Argentina.
b - Departamento de Química Biológica e IQUIFIB (UBA-CONICET), Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires
c - Departamento de Biodiversidad y Biología Experimental, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires

The *Xenopus laevis* oocyte is a well-known heterologous expression system. Several endogenous ion channels and transporters of *Xenopus laevis* oocytes have been well characterized, but the identification of volume-sensitive endogenous channels is still a matter of debate. In previous communications we showed that, following shrinkage, oocytes trigger an endogenous ion conductance denoted as G_{shrink} with simultaneous ATP release. Since shrinkage depends on particular features of aquaporins mediating transmembrane water transport, and these may modulate ion transport by physical interactions, we now evaluated G_{shrink} using two electrode voltage clamp (TEVC) when either aquaporin-1 (AQP1) or -4 (AQP4) were expressed. In addition, we studied the possible role of the plasma membrane voltage dependent anion channel-1 (pl-VDAC-1) mediating G_{shrink}. For this purpose, we tested the effect of a hypertonic stimulus G_{shrink} using oocytes injected with an antisense oligonucleotide of pl-VDAC-1, a channel reported to act as a volume-sensitive channel, permeable to Cl^- and other anions such as ATP. Expression pl-VDAC was reported for *Xenopus laevis* oocytes, but the associated currents were not functionally characterized. Our results indicate that G_{shrink} is increased in both AQP1 and AQP4 injected oocytes, suggesting the effect of AQPs is not specific. Interestingly, injection of pl-VDAC-1 oligonucleotide antisense reduced G_{shrink} from 3.7 ± 0.4 to 1.2 ± 0.2 µS ($p < 0.05$, n=6) in the absence of any aquaporin expression, suggesting a possible role of this channel as direct or indirect mediator G_{shrink}.

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Characterization of the effects of reactive oxygen species on $GABA_{A\alpha1\beta2}$ receptors

Lopez Pazos MI[a], del Vas M[b], Beltrán González A[a], Calvo DJ[a]
a - IFIByNE - UBA - CONICET
b - INTA

Endogenous reactive oxygen species (ROS) were involved in neuronal signalling and plasticity, in normal physiology, aging and neurodegenerative disorders. Besides, GABAergic neurotransmission was shown to be sensitive to redox agents. We previously demonstrated that tonic responses mediated by homomeric $GABA_{A\rho1}$ receptors can be modulated by hydrogen peroxide (H_2O_2), ascorbic acid, glutathione and nitric oxide, through thiol modification of cysteines. We also identified endogenous redox agents that modulate $GABA_A$ receptors involved in fast inhibitory neurotransmission in the retina and hippocampus, but the molecular mechanisms of action remain elusive. We found earlier that H_2O_2 modulates $GABA_{A\alpha1\beta2}$ receptors by using heterologous expression of these GABA-gated Cl^- channels in *Xenopus laevis* oocytes, followed by two-electrode voltage-clamp recordings of the GABA-evoked ionic currents. Now, we further characterized H_2O_2 effects on $GABA_{A\alpha1\beta2}$ responses. H_2O_2 induced dose-dependent, reversible and voltage-*insensitive* potentiating effects, *which were* partially prevented by irreversible alkylation of sulphydryl groups with NEM. Concentration-response curves in the presence of H_2O_2, compared to control values, showed a leftward shift and an increase in the maximal response ($EC_{50\ GABA}$= 2.7(2.4 to 3.0) µM, nH=1.46±0.12; $EC_{50\ GABA+H2O2}$=1.9(1.5 to 2.4)µM, nH=1.85±0.38). As observed for many redox agents acting on $GABA_{A\rho1}$ receptors, the degree of potentiation exerted by H_2O_2 on $GABA_{A\alpha1\beta2}$ responses decreased as GABA concentration increased. Additional experiments are being performed to elucidate the mechanisms of action underlying the effects of H_2O_2 on $GABA_{A\alpha1\beta2}$ receptors.

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Design and Synthesis of a Novel Series of Phosphonate-functionalized 1,2,3-triazoles as Positive Allosteric Modulators of α7 Nicotinic Acetylcholine Receptors

Nielsen BE[a], Stabile S[b], Vitale C[b], Bouzat C[a]
a - Instituto de Investigaciones Bioquímicas de Bahía Blanca (INIBIBB). Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur (UNS- CONICET), Bahía Blanca, Argentina
b - INQUISUR (CONICET-UNS)-Instituto de Química del Sur

α7 nicotinic acetylcholine receptors are pentameric ligand gated ion channels widely distributed throughout the central nervous system, mainly in hippocampus and cortex. As α7 plays an important role in memory and cognition, the enhancement of its activity by positive allosteric modulators (PAMs) is a promising therapeutic strategy for cognitive deficits and neurodegenerative disorders. We designed and synthesized a novel series of phosphonate-functionalized 1,4-disubstituted 1,2,3-triazoles using supported cooper nanoparticles as cycloaddition reaction catalyst, and evaluated their activity on α7 receptors by electrophysiological techniques. We identified several compounds that displayed a type I PAM activity, which was evidenced by the increase of the peak current elicited by acetylcholine with minimal effects on desensitization. At the single-channel level, the active compounds did not affect channel amplitude and increased the duration of openings and activation episodes as observed for type I PAMs. We applied structure activity relationship (SAR) strategies on the most effective compounds to obtain derivatives with higher effect by modifying the chain length, inverting triazol geometry and varying the aromatic nucleus. Our findings revealed that the phosphonate-functionalized 1,4-disubstituted 1,2,3-triazole is a key pharmacophore for the development of potential therapeutic agents.

Expression, purification and functional characterization of SLC35B1, the human ADP/ATP exchanger from the endoplasmic reticulum

Schwarzbaum P[a], **Bredeston L**[a]
a - IQUIFIB-Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, UBA. Junin 956, 1113 Ciudad de Buenos Aires

In most eukaryotic cells the flow of ATP towards the lumen of the endoplasmic reticulum (ER) from the cytoplasm is critical for the functioning of various chaperones. It was recently demonstrated that the human SLC35B1 protein (hSLC35B1) is responsible for exchanging ATP/ADP in ER. However, the underlying molecular mechanism is not completely known. In the present work, hSLC35B1 was expressed in yeast, purified and reconstituted in phosphatidylcholine liposomes. Functional characterization was performed by loading proteoliposomes with 10 mM of ADP or ATP, and incubating them with [ɣ^{32}P]ATP. Results showed that hSLC35B1 was capable of exchanging [ɣ^{32}P]ATP/ADP or [ɣ^{32}P] ATP/ATP. Notably, studies using liposomes loaded with various nucleotides and incubated with [ɣ32P] ATP indicated that [ɣ^{32}P]ATP/UDP and [ɣ32P]ATP/UTP exchange occurred at a rate similar to [ɣ^{32}P]ATP/ADP exchange, while using other di- or triphosphate nucleotides (GDP, GTP or CTP), [ɣ32P]ATP uptake was reduced by 80-90%. Finally, proteoliposomes loaded with nucleoside monophosphates (GMP, AMP, UMP) were not able to exchange with external [ɣ^{32}P]ATP. Results suggest that at least two phosphate groups need to be present in the nucleotide during the initial recognition of the transporter binding site and / or that these groups are relevant during the transport process. Nevertheless, the role of the nitrogen base is difficult to assess, since in the presence of derived di- or triphosphates nucleotides adenine or uridine (purine or pyrimidine, respectively), no significant differences of [ɣ32P]ATP uptake, by hSLC35B1, were observed. On the contrary, the guanine or cytidine nucleotides (purine or pyrimidine, respectively) are much less effective in exchange. The study of the kinetic properties of hSLC35B1, in liposomes loaded with 10mM ADP and incubated with different [ɣ 32P] ATP concentrations (1-150µM), showed that the uptake of [ɣ32P]ATP followed a hyperbolic -like behavior, with an apparent affinity value ($K_{0.5}$ external ATP) of 62µM. On the other hand, when liposomes loaded with varying concentrations of ADP (0-10mM) were incubated in the presence of 50 µM [ɣ32P]ATP, uptake showed a hyperbolic behavior with an apparent affinity ($K_{0.5}$ internal ADP) of 5mM. These results are compatible with an asymmetry in the affinity of the external and internal binding sites of hSLC35B1. Alanine substitution of charged residues K117, K120 (on TM4) or R276, K277 (on TM9) abolished [ɣ32P]ATP/ADP exchange.

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Glutathione effects on $GABA_A$ receptors

Beltrán González A[a], López Pazos MI[a], Parlato ML[a], Di Martino T[a], del Vas M[b], Calvo DJ[a]
a - IFIByNE - UBA - CONICET
b - INTA

Glutathione (GSH) is the major antioxidant and redox buffer in the brain. GSH exerts a protective role against oxidative stress, in the detoxification of H_2O_2 and lipid peroxides, scavenging of $OH^{\bullet}$ and regeneration of the most important antioxidants (Vit C and E). GSH is also involved in the regulation of many synaptic functions and in neuronal plasticity. Furthermore, GSH deficiency has been implicated in neurodegenerative diseases. Previous reports demonstrated that GSH modulates GABAergic neurotransmission, although experimental evidence is limited.

We have previously described that spontaneous responses exhibited by homomeric $GABA_A\beta3$ receptor increased in the presence of GSH and that effects were insensitive to irreversible alkylation of sulphydryl groups within the β3 subunits by N-ethylmaleimide, discarding a redox-dependent mechanism mediated by cysteine. In this study we evaluated the effect of GSH in different phasic and tonic $GABA_A$ receptor subtypes. Human $GABA_A$ receptors were expressed in *Xenopus laevis* oocytes and GABA-evoked Cl^- currents were recorded by two-electrode voltage-clamp. GSH (1mM) potentiated $GABA_A\alpha4\beta3$, $GABA_A\alpha5\beta3$ and $GABA_A\alpha1\beta2$ receptor responses elicited by GABA (~EC_{10}). In contrast, GSH had no effect on $GABA_A\alpha4\beta3\delta$, GABAα4β3γ2 and $GABA_A\alpha1\beta2\gamma2$ receptors. Previous results showed that GSH prevented inhibition of $GABA_A\beta3$ responses by Zn^{2+}, the second most abundant trace metal in neurons. Considering the different sensitivity that $GABA_A\alpha\beta$ and GABAαβγ/δ receptors exhibit to Zn^{2+}, we performed experiments in the presence of Tricine, a Zn^{2+} chelator. Tricine (10mM) prevented GSH potentiation of $GABA_A\alpha1\beta2$ responses, suggesting that GSH may exert its action indirectly, via chelation of Zn^{2+} traces contained in the buffer medium. Given the importance of Zn^{2+} as an allosteric modulator of $GABA_A$ receptors and the role of GSH as a neuronal antioxidant, this might represent a relevant mechanism of control of the GABAergic neurotransmission.

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Inhibition of plasma membrane calcium pump by aurintricarboxylic acid

Bruno C[a], Souto Guevara C[a], Saffioti N[a], Ferreira-Gomes MS[a], Rossi JP[a], Mangialavori I[a]
a - Instituto de Química y Fisicoquímica Biológicas y Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires-CONICET

Plasma membrane calcium ATPases (PMCAs) are high-affinity calcium pumps that extrude calcium from the cytosol to the extracellular medium. In humans, PMCAs are encoded by 4 independent genes termed PMCA1 to 4. PMCA1 and 4 are expressed ubiquitously, whereas the expression of PMCA2 and 3 is restricted to specific cells and tissues. PMCA4 regulates calcium concentration within its vicinity and modulates the activity of its interacting partners.

PMCA4 is the isoform more abundant in hematopoietic tissue. PMCA4 has emerged as an important negative regulator of several pathophysiological processes that involve angiogenesis, increasing the interest on its pharmacological blockade to potentiate the efficiency of therapeutic strategies. Mohamed et al (2013)[1] showed that low concentrations of aurintricarboxylic acid (ATA) strongly inhibit the calcium extrusion activity of PMCA4 in HEK cells without affecting the activity of PMCA1 or other major pumps. Recently, Kurasamy et al (2017)[2] have showed that ATA inhibits PMCA4 in endothelial cells triggering a marked increase in cell motility and blood vessel formation. However, the PMCA inhibition mechanism by ATA remains unknown.

The aim of this study was to characterize the effect of ATA on PMCA from human erythrocytes (ePMCA), where about 80% is PMCA4 and 20% PMCA1. Our current results show that: (1) ATA inhibited Ca^{2+}-ATPase activity of purified ePMCA with an apparent K_i = 100 nM; (2) The value of K_i depended on Mg^{2+} concentration; (3) The value of K_i did not modify by ATP concentration; (4) When ePMCA is in its native membrane environment, the value of K_i increased more than twenty times.

Our results suggest that ATA does not bind to the ATP binding site of ePMCA and Mg^{2+} is necessary to the total inhibition of Ca^{2+}-ATPase activity.

Bruno C and Souto Guevara C contributed equally to this work

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Rb^+ occlusion in dephosphorylation intermediates of the gastric H,K-ATPase catalytic cycle

Valsecchi WM[a], Faraj SE[a], Cerf NT[a], Rossi RC[a], Montes M[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 an integral membrane protein responsible for gastric acid secretion. The enzyme catalyzes H^+ transport at the expense of ATP hydrolysis through a mechanism traditionally described by the E1-E2 reaction cycle. In the E1 conformation, H^+ are bound to the cytoplasmic side of the pump, leading to phosphorylation and the formation of the E1P intermediate, which is then converted to E2P, releasing H^+ at the opposite side of the membrane. The counter-transport ion, K^+, binds from the lumen access and triggers E2P dephosphorylation leading to the occluded form, in which K^+ is trapped within the membrane domain. The cycle completes with the transition to E1 and the release of K^+ into the cell. The physiological dephosphorylation sequence proposed for this and other ATPases may be summarized as:

E2-P (ground state) → E2·P (transition state) → E2Pi (product state) → E2 + Pi

where each species should present different capacity to bind and occlude K^+. Despite the great similarities between Na,K-ATPase and H,K-ATPase, in the gastric enzyme the isolation of K^+-occluded states has been challenging. It was shown that vanadate promotes the occlusion of Rb^+, a K^+ congener (*Rabon et al., 1982*; *Montes et al., 2011*), and that vanadate and metal fluoride complexes induce structures that represent the intermediates during the E2-P dephosphorylation sequence (*Abe et al., 2010*). To understand the coupling between the structures of the phosphorylated domain and the movement of the transmembrane segments, we analyzed the ability of the E2P intermediates to bind and occlude Rb^+. Results show that the level of Rb^+ occlusion increases from the E2P ground state (the species that first contacts with luminal K^+) to the E2P product state. These observations are in accordance with the shift from an open-luminal to a close-luminal conformation during the transition from E2P to E2.

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Structural analysis of Trypanosoma cruzi´s aquaporins

Tesan FC[a,b], Armentia L[a], Zerbetto de Palma GG[a,b], Parodi L[a], Fox R[c], Penas FN[d], Goren NB[d], Alleva K[a,b]

a - Cátedra de Física, Facultad de Farmacia y Bioquímica. UBA
b - IQUIFIB-Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, UBA. Junin 956, 1113 Ciudad de Buenos Aires
c - Centro de Investigación Veterinaria de Tandil (CIVETAN)
d - Instituto de Investigaciones Biomédicas en Retrovirus y SIDA (INBIRS)

Aquaporins (AQP) constitute a well distributed and diversified protein family of channels whose structure remain highly conserved throughout species. These channels transport mainly water but some of them also transport solutes such as glycerol, hydrogen peroxide, etc. Trypanosoma cruzi (Tc) would have different AQP variants in its genome but there are only partial functional results for one of them. Thus, structural characterization of TcAQP is key to evaluate the physiological relevance of these channels on the parasite and to get progress in the knowledge of structure-function particularities of this protein family. In this work, AQP compatible sequences were searched for within Trytripdb database for all T cruzi strains. DNA was extracted from trypomastigotes (RA strain) cultured in Vero cell line. Each AQP sequence was elucidated after cloning in T7TS vector, bacteria transformation (E coli, DH5α), plasmid purification and sequencing (Macrogen). Multiple sequence alignments of AQPs was performed using all data base Tc strains and RA strain and a Phylogenetic analysis of all Kinetoplastid AQPs was also performed. 4 TcAQP were found (α to δ) for 13 Tc strains. Protein sizes are around 230 amino acids with the exception of β (450) and key amino acids and specific transport related motifs were compatible with water specific transport (Frogger's positions, Ar/R, NPA1 and NPA2). 3D models were built by ab initio methods (Rosetta, iTasser) and pores were characterized based on molecular dynamics results (AMBER14SB and LIPID14 force field; Hole). Conserved AQP like tetrameric structures were shown by all constructed 3D models. This work is the first step in the full characterization of all TcAQP, further functional analysis of the transport of each TcAQP in vitro is still needed.

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The magnitude of transmembrane electric fields can modulate the water flow through aquaporins

Ozu M[a,b], Fernández M[c], Carmona E[c], Gárate JA[c], Latorre R[c], González C[c], Amodeo G[a,b]
a - Departamento de Biodiversidad y Biología Experimental, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires
b - Instituto de Biodiversidad y Biología Experimental y Aplicada (IBBEA) - UBA-CONICET
c - Centro Interdisciplinario de Neurociencias de Valparaíso (CINV), Universidad de Valparaíso, Chile

Aquaporins (AQPs) are transmembrane tetrameric proteins that transport water and solutes. Each subunit has its own permeable pathway. Molecular dynamic (MD) simulations predict how the water molecule move through the permeable pathway. However, experimental records are lacking. AQP4 is an animal aquaporin regulated by membrane tension changes and possibly by transmembrane electric fields too. Our recent results indicate that FaPIP2;1 from strawberry seems to be a mechanosensitive aquaporin that could also be regulated by electric fields. To test the combined effects of both electric fields and membrane tension on the water transport rate we performed molecular dynamic simulations on homotetramers of FaPIP2;1 and AQP4. Molecular dynamic simulations were performed with NAMD v.2.7 and the CHARMM27 force field, using an homology model of FaPIP2;1 developed with the crystal of SoPIP2;1 (PDB 2B5F) and the structural data of human AQP4 (PDB 3GD8). In addition, we used the heterologous xenopus oocytes system to study the mechanosensitivity of the plant FaPIP2;1 and the animal AQP4 channels. Functional parameters were obtained from the kinetics of cell volume changes with different osmotic gradients. Molecular dynamic simulations predict that the water transport rate through FaPIP2;1 and AQP4 can change with the applied electric field. These results suggest that changes in the electrical field perturbates the movement of water flow through aquaporin channels.

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The Parkinson's associated P5-ATPase Spf1 is inactivated by Zn^{2+}

Petrovich GD[a], Corradi GR[a], Adamo HP[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

P-type ATPases (P1 to P5) are integral membrane proteins that are responsible for the active transport of different ligands. P5-ATPases are the least studied members of the whole family of P-ATPases and its transported substrate remains unknown. In humans, mutations in the P5-ATPases genes are implicated in neurological disorders, as the Kufor-Rakeb syndrome, a Parkinsonism with dementia, hereditary spastic paraplegia, neuronal ceroid lipofuscinosis, autism and intellectual disability. While the mechanism behind these pathologies is not well understood recent studies suggest a complex interaction between genetic predisposition and environmental factors. In this line, several Parkinson's associated genes have been shown to cause loss of metal ion homeostasis. We have measured the effect of metals on the ATPase activity of the micellar preparation of purified recombinant Spf1 P5A-ATPase from the simple eukaryote Saccharomyces cerevisiae. The addition to the reaction media of Ca^{2+}, Mn^{2+} and Co^{2+} reversible inhibited the enzyme with Ki >100 mM. In contrast, preincubation of the protein with low concentrations of Cd^{2+} or Zn^{2+} led to the loss of ATPase activity that could not be reverted by a subsequent treatment with EGTA or DTT. The inactivation by Zn^{2+} was not altered by Mg^{2+}. In contrast, catalytic concentrations of ATP during preincubation protected from Zn^{2+} inactivation. Similar protection was attained by adding ADP instead of ATP, suggesting that protection was due to the binding of the nucleotide to Spf1. Kinetic analysis indicates that the the modulation of the catalytic efficiency by high concentrations of ATP was the most affected parameter in the Zn^{2+}-treated enzyme. These results are consistent with the hypothesis that the modification of a target residue at the catalytic nucleotide binding domain of the enzyme prevents the acceleration by ATP of the conformational transition of the enzyme from E2 to E1.

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Two different mechanisms of PMCA inhibition by flavonoids

Ontiveros M[a], Rinaldi D[a], Pantano S[b], Marder M[a], Mangialavori I[a], Rossi R[a], Rossi JP[a], Ferreira-Gomes MS[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

Research on flavonoids from plant sources has recently sparked increasing interest because of their beneficial health properties. Different studies have shown that flavonoids change the intracellular Ca^{2+} homeostasis linked to alterations in the function of mitochondria, Ca^{2+} channels and Ca^{2+} pumps. These findings hint at plasma membrane Ca^{2+}-ATPase (PMCA) involvement, as it transports Ca^{2+} actively to the extracellular medium coupled to ATP hydrolysis, maintaining the cellular homeostasis. The aim of this study was to investigate the effect on purified preparations of PMCA of quercetin and gossypin, two flavonoids that are very effective to inhibit PMCA activity.

Results showed that quercetin and gossypin inhibit PMCA activity with Ki of 0.3 and 4.1 mM, respectively. The inhibition of PMCA was dependent on the Mg^{2+} concentration and pH in a way that suggests that the main inhibitory species are Mg^{2+}-flavonoid complexes, which show dissociation constants of around 1 mM at pH = 7.4. The measurement of partial reactions of phosphorylation and dephosphorylation of PMCA under conditions that favor the formation of Mg^{2+}-flavonoids complexes shows an increase in the concentration of phosphoenzymes, *E*P, particularly of that sensitive to ADP. These results suggest that both, the Mg^{2+}-quercetin and Mg^{2+}-gossypin complexes prevent the conformational change between *E*1P → *E*2P. Under experimental conditions not favoring the formation of the Mg^{2+}-flavonoid complex, PMCA activity was inhibited but the phosphorylated intermediate decreased, suggesting that flavonoids compete for the ATP-binding site. For a better comprehension of our results, we performed docking assays of Mg^{2+}-flavonoid complexes and PMCA. Structures of PMCA were obtained by homology modeling on Na,K-ATPase crystallographic structure. Based on these simulations, we propose an interaction model between the Mg^{2+}-flavonoid complexes and the ATP binding domain in the *E*1P intermediate.

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The role of the lipid bilayer on the inhibition of the Plasma membrane calcium ATPase (PMCA) by aluminium

de Sautu M[a], **Bianco B**[a], Ferreira-Gomes MS[a], Rossi JP[a], Mangialavori I[a]
a - Instituto de Química y Fisicoquímica Biológicas y Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires-CONICET

PMCA is a P-ATPase involved in the regulation of the cell calcium homeostasis transporting Ca^{2+} from cytoplasm towards the extracellular medium. PMCA like other integral membrane proteins operates surrounded by a complex and dynamic lipid bilayer, and its activity largely depends on the type of lipids [1]. Aluminium (**Al^{3+} and other soluble species**) is environmentally ubiquitous, providing human exposure and neurotoxic effects in humans and animals. The mechanisms proposed to explain aluminium toxicity are linked to changes in the cellular calcium homeostasis. In previous works, we showed that $AlCl_3$ inhibits calcium efflux mediated by PMCA in HEK293T cells. Also, we demonstrated that aluminium inhibits PMCA activity irreversibly by preventing the dephosphorylation of the pump [1].

The aim of this work is to understand the effect of aluminium on the lipidic environment of PMCA. Aluminium would have distinct effect depending on the lipid composition of the cell membrane where the PMCA is located. To characterize this effect, mixed micelles of phospholipids and detergent ($C_{12}E_{10}$) were formed at different molar fractions and we measured how PMCA activity varied with or without the presence of aluminium. Further, we characterize the interaction of aluminium in the same micelles with an aluminum-specific fluorescent probe (Lumogallion).

Our results indicate that the inhibition of the pump by aluminium depends largely on the composition and concentration of phospholipids surrounding PMCA. Moreover, we show how aluminium interacts with the micelles, in agreement with lumogallion fluorescence changes.

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Physico-chemical study of nanostructures of L-ascorbic acid alkyl esters that allow Amphotericin B to be solubilized in its monomeric and bioactive form

Nocelli N[a], Zulueta Diaz YdlM[a], Millot M[b], Colazo ML[b], Vico R[c], Fanani ML[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.
b - Departamento de Química Biológica Ranwel Caputto, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba
c - Departamento de Química Orgánica, Facultad de Cs Químicas, UNC

The L-ascorbic acid alkyl esters (ASCn) are lipophilic forms of vitamin C, with pharmacological interest due to their antioxidant properties and amphiphilic behavior,[1,2] have the ability to self-organize into micelles and form coagels when dispersed in water[1]. $ASC_{12,14,16}$ have been proposed as enhancers of dermal permeation, so they would act by increasing drug permeation through skin. Amphotericin B (AmB) is a potent antimicrobial widely used in the treatment of different diseases[3], such as Leishmania[4] infestation. This drug is highly effective, but there are several important clinical limitations that lead to severe hepatic nephrotoxicity, which can cause renal insufficiency. In this work, we study the ability of the nanostructures formed by $ASC_{12,14,16}$ to solubilize AmB in its monomeric and bioactive form, and address a formulation for the topical treatment of AmB. Here, a physicochemical study is exposed using particle analysis, differential scanning calorimetry and Langmuir monolayer characterization. The results obtained with ASC_{16} compared to other ASCn evaluated were superior and allow this nanostructured system to be attractive for additional tests for the treatment of fungal and parasitic skin infections. Our results contribute to the understanding of the physicochemical bases that make ASCn coagels a good environment for monomeric AmB, based on their electrostatic and thermodynamic properties as well as the in-plane structure of the ASCn supramolecular arrangements.

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Rational design and characterization of a protein cage for fluorescent dyes

Sosa S[a,b], Szalai A[a], Bonomi HR[b], Stefani FD[a], Goldbaum FA[b]
a - Centro de investigaciones en Bionanociencia (CIBION), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)
b - Fundación Instituto Leloir

The rational design of nano-structures based on the engineering of biological molecules is a promising and challenging field. In recent years there has been a growing number of work using DNA origamis as fluorophores scaffold systems both for the study of their photophysical properties and for the development of nanotechnological applications. Due to the difficulty of re-designing protein structures, there are few works using them as building blocks in the nanoscale. However, they are a promising tool, as they present a high versatility in functions and structures.

In this work we present the development of a protein cage based on the engineering of a non-viral multimeric protein. BLS (*Brucella* Lumazine Synthase) is a highly stable homodecamer that can be regarded as a dimer of head-to-head oriented homopentamers. It's cavity is relatively small (between 2 and 3 nm diameter) compared to most of the protein cages known so far, making it an interesting candidate for the confinement of fluorescent molecules. This can be used to transport them and improve their photophysical properties.

Through bioinformatic studies we have located a position in which it was possible to insert a reactive cysteine without affecting the stability of folding or self-assembly. After cloning, expressing and purifying this mutant we have been able to covalently bind different dyes (Cy5, Cy3 and fluorescein). We have studied their properties inside the protein cage both when it was open or closed. We have found that lifetime and fluorescence intensity have been affected in different ways, depending on each dye. In some cases, photophysical properties were improved, making it a promising tool for the development of brighter and more stable fluorescent particles. We are now doing single molecule TIRF microscopy studies in order to make a more detailed characterization of this system.

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The C-terminus to N-terminus orientation of tyrosine rich amphiphilic peptides on Langmuir monolayers facilitates the electron flow

Bolaño Álvarez A[a], Pino M[a], Petersen S[b], Fidelio GD[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.
b - Department of Health Science and Technology, Faculty of Medicine, Aalborg University.

We have studied the conductivity properties of two reversing sequence amphiphilic synthetic peptides which have a lipid-like behavior at air/water interface with an enriched tyrosine domain that undergoes di-Tyr formation upon surface illumination.

We have measure lateral conductivity in the monomolecular layer in absent or presence of a negative plaque of polarization beneath of the peptide array. We have also designed a second circuit able to measure the semiconductor-diode behavior of the confined peptide in a close packed two dimensional array. Both peptides are retro isomers with the same amphiphilicity but differing in the asymmetry of peptide bond direction named as PF3 Ac-KKGALLLLLGYYY-NH_2 and PF4 peptide Ac-YYYGLLLLLAGKK-NH_2. Both peptide monolayers undergo di-Tyr formation when they are illuminated directly to the surface detected by both fluorescence and RAMAN spectroscopy. The di-Tyr formation induces surface heterogeneity detected by BAM microscopy.

Both peptides are able to laterally conduct electrons almost 8 times higher than DMPC phospholipid. The PF4 conductivity was higher than PF3 and can be attributed to the C®N orientation of PF4. Therefore, we observed an asymmetry in conductivity due to peptide bond orientation even when the surface properties of both peptides are almost identical. The di-Tyr formation increases the conductivity properties of both peptides, suggesting that the laterally formation of Tyr cross-linking enhance lateral conductance.

When we measure the current against voltage for each peptide under appropriate condition evaluating that both have semiconductor-diode properties. The electron flow is facilitated from the C-terminus to the N-terminus coinciding with the –N–(C=O)– peptide bond orientation when compared with the reversal flow from N-terminus to C-terminus. Assimilating the peptide backbone as a diode system connected in series where the **N material part** is represented by the **oxygen** whereas nitrogen would correspond to the **P material part** of the semiconductor. We propose that this conductivity asymmetry may be of biological importance in polytopic transmembrane proteins.

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The kinetics of calcium alginate bead formation connected through light microscopy imaging, microstructure by SAXS and rheological analysis

Posbeyikian A[a], Tubert E[a], Bacigalupe A[b], Santagapita P[c], Perullini M[d], Amodeo G[a]
a - Instituto de Biodiversidad y Biología Experimental y Aplicada (IBBEA) - UBA-CONICET
b - Instituto Nacional de Tecnología Industrial (INTI), Buenos Aires, Argentina.
c - Departamento de Industrias y Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina and Instituto de Tecnología de Alimentos y Procesos Químicos (ITAPROQ) Universidad de Buenos Aires y Consejo de Investigaciones Científicas y Técnicas, Buenos Aires, Argentina
d - Universidad de Buenos Aires-CONICET, DQIAQF-INQUIMAE, Facultad de Ciencias Exactas y Naturales

Alginate encapsulation systems have grown in use across many fields, from agriculture to the food industry, medicine, and 3D tissue culture. Basically, alginate consists of linear blocks of alternated mannuronate and guluronate residues which can experience ionotropic gelation when they cross-link in presence of cations (e.g. Ca^{2+}). During this process, the Ca(II)-alginate hydrogel (Ca-Alg) shrinks due to the expulsion of water from aqueous pores reducing the bead final volume. Our goal was to perform a characterization of the Ca-Alg bead formation by means of complementary tools. First, we employed a video-microscopy set-up to record changes in bead cross-sectional area over time right after bead generation. The analysis of the digitized images was automatized using free distribution software (ImageJ & plug-ins). To improve the bead image contrast we tested fluorophores as well as a clay mineral, kaolinite. Results were compared to manual measurements performed with a caliper. Alginate systems ranging 1.5-3.0 % (w/v) were also tested. Methylene blue dye was used to stain the beads and a method was developed to track the layer of the 'gel front', that forms concentrically from the exterior of the alginate drop towards its interior. To determine the impact of the kaolinite and other dyes on the spatial arrangement of the alginate chains, we studied the microstructure of the systems through small-angle X-ray scattering. Kaolinite caused a slight increase in the interconnectivity of the alginate rods at all alginate concentrations, but had no effect on the distance between alginate dimers. Rheological studies showed a correlation between the gelation kinetics and the stress resistance of the encapsulation systems. The rapid and convenient experimental design we here propose makes it possible to test a larger range of variables that affect the formation kinetics. Moreover, the characterization of the movement of this 'gel front' is proposed as a new attribute to describe alginate systems, and may reveal features regarding the dynamics of ionotropic gelation. Collectively, the described methodology facilitates easier characterization of alginate bead formation dynamics, which provides useful information both for industrial and scientific analysis of these systems.

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Halogen bond induces an activity cliff in Cruzain inhibitors

Angelina E[a], Bogado ML[a], Villafañe RN[a], Luchi AM[a], Peruchenia NM[a]
a - Laboratorio de Estructura Molecular y Propiedades, Área de Química Física, Departamento de Química, Facultad de Ciencias Exactas y Naturales y Agrimensura, Universidad Nacional del Nordeste. Av. Libertad 5470, Corrientes (3400), Argentina

A survey on public databases with activity annotations revealed that several Cz inhibitors containing a halogen atom (X = Cl, Br, I) were up to thousands of times more active than their non-halogenated analogs giving rise to an activity cliff in the Cz/inhibitor interaction landscape. On the other hand, structural evidence indicates that halogenated compounds form an X···S halogen bond (XB) with Methionine 68 (PDB ID 3KKU). These findings taked together suggest that XB formation could induce disruptive changes within the enzyme protein structure.

To understand the origin of activity cliff caused by XB formation in Cz/Inhibitor complexes, a comparative study of pairs of Cz inhibitors (Inh) was performed, the only structural difference consisted in the presence or not of an X atom in its structure and where the activity of the halogenated inhibitors was 100 times or more higher than the non halogenated counterpart. Five pairs of inhibitors from the thiosemicarbazone family that met these characteristics were compiled and subjected to Docking calculations followed by Molecular Dynamics (MD) simulations. An extra-point mimicking the halogen positive sigma-hole region was parametrized to account for the XB quantum effects during simulations.

XB effects on Cz structure were analyzed locally at the enzyme binding cleft by performing a topological analysis of electron charge density (QTAIM) on reduced model systems. Non-local effects of XB over remote regions of the protein were also analyzed through the Dynamic Cross Correlation Matrix (DCCM) and derived studies such as Principal Component Analysis (PCA) and Network Analysis.

XB formation with Met68 at the bottom of the enzyme S2 sub-pocket was verified for all halogenated inhibitors, in agreement with available structural evidence. On the other hand, the non-halogenated counterparts could not reach the bottom of S2 and so they were washed away from that sub-pocket thus partially explaining the observed activity differences. Through the Network Analysis, it was found that the integrity of the network associated with the halogenated complexes is particularly affected by the loss of communication between two specific regions of the Cz, which induces conformational changes that could enhance complex stability.

These results provide some clues about how XBs can be exploited to design more potent Cz inhibitors.

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Interaction of the HIV-1 matrix domain with lipid membranes: driving forces and bilayer properties governing the process

Pérez Socas LB[a], Ambroggio E[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.

In the second part of the HIV-1 lifecycle, the GAG polyprotein act as the main coordinator of the virus assembly process at the plasmatic membrane (PM). The direction of the process toward the lipid bilayer is mediated by its N-terminal matrix domain (MA), which is myristoylated and possess a highly basic region (HBR) responsible for the interaction with the negative lipids of the PM, especially with PIP_2. In addition, MA is able to bind RNA molecules that can compete with non-specific membranes as a regulatory step of the assembly process, allowing the protein to interact only with PIP_2-containing membranes (i.e. the PM). The N-terminal of the MA domain, which contains the myristoyl group and the HBR, appears to be the main functional part of the protein. Therefore, in this work we study the interaction of a synthetic peptide (first N-terminal 21 amino acids of MA), and LUVs with several characteristics. For this, we had two different approaches: fluorescence properties of the peptide single Trp were analyzed under LUVs titration and FRET between the Trp and NBD-labeled vesicles was measured under peptide titration. The FRET technique was also used for studding the reversibility of the peptide partition toward membranes. In this sense, partition coefficients was obtained for both myristoylated and unmyristoylated peptides toward vesicles with different amount of a negatively charged lipid (PS). As expected for a highly positive peptide, partition was preferred toward highly negative membranes. We also studied the effect of a small amount (2%) of PIP_2, as well as the presence of cholesterol in the membrane. Additionally, the influence of nucleic acids in the peptide-membrane interaction was studied by pre-incubation the peptides with 5-fold amount of a single strand DNA (ssDNASel25), which is known to have a strong interaction with the protein. Partition coefficients were obtained for different ionic strength (0 - 250 mM NaCl) in order to differentiate the electrostatic component of the interaction and, therefore, infer about the specific, non-electrostatic, possible contributions in the partition process. Lipid composition and interaction with ssDNASel25 influence the irreversibility of the partition of the peptide toward membranes. The results obtained in our study, analyzed together with the current available literature on the subject, represents one more step that could lead to a better molecular understanding of HIV-1 MA biological role.

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Tryptophan-Laurdan interaction in ester and ether PC membrane interfaces

Pérez HA[a], Gonzalez Paz A[a], Disalvo EA[a], Frías MdlÁ[a]
a - CIBAAL Centro de Investigación en Biofísica Aplicada y Alimentos CONICET UNSE

Numerous investigations have been carried out to interpret the role of tryptophan (Trp) in the peptides and proteins (such as melittin, annexins, among other molecules of biological importance) interaction with lipid membranes through various techniques, such as NMR, FTIR, X ray diffraction, among others. In particular, studies using Laurdan as a fluorescent probe has been extensively done to have an insight on the hydration states of the interface. In this regard, the presence or absence of hydration centers such as carbonyl groups can be decisive in the interaction of Trp with membranes.

In this work, the interaction of Trp with lipid membranes models composed of ester and ether lipids of different chain lengths in the presence of the Laurdan fluorescent probe is studied.

The results show that there is an interaction between Trp and Laurdan, regardless of the phase state in 1,2-dimyristoyl-sn-glycero-3-phosphocholine multilamellar vesicles (14: 0 PC-DMPC) and 1,2-di-O-tetradecyl-sn-glycero-3-phosphocholine (14: 0-DTPC). The values of the Stern Volmer constant (K_{SV}) are approximately of 75 M^{-1}, in both cases. In contrats, in vesicles of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (16: 0-DPPC) and 1,2-di-O-hexadecyl-sn-glycero-3-phosphocholine (16: 0-DHPC), the phase state affects Laurdan quenching by Trp, obtaining values of $K_{SV} \approx$ 55 M^{-1} for the gel phase and $K_{SV} \approx$ 80 M^{-1} for crystalline liquid. These results indicate that the presence or absence of the carbonyl group for lipids having the same chain length does not influence the penetration of Trp at the interface. However, the chain length produces a lower efficiency of gel phase quenching when the lipid chain length increases. The latter suggests that vesicles formed by DPPC and DHPC in gel phase have a higher degree of interface ordering compared to DMPC and DTPC, decreasing the entrance of Trp in the interface.

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Antagonistic antimicrobial effect observed in silver nanoparticles stabilized with lysozyme.

Rey V[a], Espeche Turbay MB[a], Sosa M[a], Dorado RD[a], Borsarelli CD[a]
a - INBIONATEC, CONICET, National University of Santiago del Estero. 4206, RN 9 Km 1125, Santiago del Estero, Argentina

Silver nanoparticles (AgNPs) are well recognized new generation of antimicrobials, as well as lysozyme (14 kDa) is a protein present in body fluids with membrane lytic activity, acting as a natural antimicrobial. Therefore, could be expected that combination of both types of antimicrobial can result in a synergistic effect, particularly for the treatment of multi-strains or pan-resistant strains. Recently, we characterized the photoreductive synthesis of silver nanoparticles (NPs) stabilized with lysozyme (AgNP@LZ). Surprisingly, AgNP@LZ lacks antimicrobial activity on grampositive and gramnegative strains, being a biological example of *"antagonistic effect"*. In order to understand the interactions that control the functionality of this bionanocomposite, we have studied the structural modifications of the stabilizing protein, the type of specific interactions between the metal surface and the peptide residues, and also by comparing the antimicrobial activity of others AgNPs stabilized with ion citrate (CIT) or serum bovine albumin (BSA) by the effect of adding native LZ. Results show that in the AgNP@LZ the protein strongly interacts with the metal surface, forming a hard crown on the nanoparticle surface. As a result, this hard corona efficiently binds the silver cation Ag^+ (which believed the antimicrobial agent) hindering its diffusion into the medium avoiding microbicide activity.

Hence, this study remarks the relevance of a complete knowledge of the specific interactions between components in a nanocomposite system for the design of nanobiomaterials with suitable antimicrobial activity.

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Characterization of the surface properties of sucroglycerides by means of potential z measurements.

Soria NAdJ[a], Frías MdlÁ[a], Disalvo EA[a]
a - CIBAAL Centro de Investigación en Biofísica Aplicada y Alimentos CONICET UNSE

Sucroglycerides are mainly composed of mono-, di- and tri-esters of sucrose and fatty acids mixed with residual mono-, di- and triglycerides of fats or oils. These products are used primarily in the agricultural, food and cosmetic industry, as an emulsifier and as a surfactant. The critical aggregation concentration (CAC) is $9x10^{-3}$% P/V in water. Under these conditions, the sucroglyceride aggregates show a spectrum of MC540 with two peaks: one at 500nm and another at 530nm in water but the peak at 570nm, characteristic of hydrophobic areas, is absent, indicating that the surface of the aggregates is essentially hydrophilic. On the other hand, at a concentration above CAC the zeta potential -electrostatic potential between the particle and the aqueous medium- is equal to -106.2 millivolts. As the medium is acidified with HCl, the z potential changes to less negative values, but is not altered with NaOH. At high concentrations of sucroglycerides the titration with acid does not produce significant changes in the z potential up to a $[H^+]$ equal to 0.08 mM, after which it runs at less negative values. It is also observed that in a range of pH 8.5-6.5 the zeta potential does not present significant changes, which could be due to the fact that the aggregates are saturated with H^+. With these results it is concluded that sucroglyceride aggregates are negative, that they are capable of adsorbing positive charges and would exhibit pH buffering properties. The regulatory properties of the aggregates are compared with those of sucroglycerides in solution before the critical aggregation concentration.

Conformational and electronic intricacies of dopamine interacting with the D2 Dopamine Receptor. A comprehensive theoretical study

Goicoechea Moro L[a], Tosso R[a], Parravicini O[a], **Zarycz MNC**[a], Angelina E[b], Vettorazzi M[a], Andujar S[a], Enriz R[a]

a - Facultad de Química Bioquímica y Farmacia, Universidad Nacional de San Luis, Chacabuco 915, 5700 San Luis, Argentina. IMIBIO-SL (CONICET), Chacabuco 915, 5700 San Luis, Argentina

b - Laboratorio de Estructura Molecular y Propiedades, Área de Química Física, Departamento de Química, Facultad de Ciencias Exactas y Naturales y Agrimensura, Universidad Nacional del Nordeste. Av. Libertad 5470, Corrientes (3400), Argentina

Understanding of the biological behavior of different L-R complexes requires determining the conformational and electronic aspects of both the small ligand as well as the binding site of its receptor. Here we report the conformational and electronic behavior of dopamine (DO) interacting at the active site of the D2 dopamine receptor (D2DR). The selection of this molecular target is due to two main reasons: it is a molecular target of great importance for medicinal chemistry and very useful structural information has been recently reported due to the D2DR has been crystallized. Different computational techniques have been used in combination in this study. In this way, docking calculations, molecular dynamics simulations and quantum mechanical calculations have been performed. Moreover, the different molecular interactions of the complexes were evaluated in detail using two techniques: QTAIM (Quantum Theory of Atoms in Molecules) and NMR nuclear magnetic shielding constants calculations.

Our study goes much further than any previously done, since for the first time we have been able to obtain and report the complete conformational potential energy surface (PES) for DO in its binding pocket. Analysis of the complete PES is the most comprehensive way to understand the conformational behavior of a ligand such as DO, which possesses two rotatable bonds, since it is possible to locate all critical points on the surface and even see its different conformational inter-conversion paths.

Our study indicates that seven different conformations of DO are the most relevant. From these seven ones, two are those that could be considered as the biologically relevant conformations of DO. On the other hand, the most important molecular interactions that stabilize these molecular complexes are those with Asp80, Val81, Cys84, Thr85, Ser159, Ser160, Ser163, Phe164 and Tyr403.

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Influence of the electric charge of the mitochondrial outer membrane on the orientation and binding of the pro-apoptotic protein Bax during the activation and oligomerization processes.

Jaure O[a], Amundarain MJ[a], Obiol D[a], Zamarreño F[a], Costabel M[a]
a - Grupo de Biofísica. Departamento de Física, Universida Nacional del Sur (UNS), IFISUR (UNS-CONICET), Bahía Blanca, Agentina.

The crucial event that triggers apoptosis is the activation and translocation to the mitochondrial outer membrane of the pro-apoptotic Bax protein. In healthy cells Bax is found as an inactive monomer in the cytosol or weakly bound to membranes. A transient interaction between Bax and the Bid protein or its truncated version tBid would cause its activation. Once activated, Bax functional regulation includes: conformational change, translocation to the mitochondrial outer membrane and changes in the state of aggregation (formation of dimers and multimers). The oligomerization of Bax leads to permeabilization of the mitochondrial outer membrane, pore formation and the release from the intermembrane space of apoptogenic factors, thus triggering a series of events that culminates in cell death.

In order to deepen our knowledge in the regulation of the mitochondrial pathway of apoptosis, we carried out an analysis of the electrostatic component and the trajectories of molecular dynamics of the system formed by Bax (monomeric and oligomeric states), Bid, tBid and the mitochondrial membrane. The results obtained demonstrate the influence of electrostatic interactions between membrane and the monomeric and oligomeric Bax pro-apoptotic protein; and between Bax and the activator protein Bid/tBid. In addition we determined optimal interaction distances, influence of the ionic strength of the medium, analyzed the distribution of charges in the macromolecules and orientation of the macrodipoles.

The information obtained allowed us to establish an activation model that shows the influence of charged phospholipids on the interaction of Bax with membrane before and after its activation by the Bid/tBid protein. This information, together with the identification of the amino acids involved in the first contact of Bax with the membrane and with its protein counterpart, by means of analysis of specific mutations, made it possible to detect potential modes of interaction that must be further analyzed to determine their importance in the activation of the mitochondrial pathway of apoptosis.

Liquid-liquid phase separation of an RNA polymerase phosphoprotein cofactor drives the formation of viral factories

Salgueiro M[a], Camporeale G[a], Arán M[a], Pelliza L[a], de Oliveira GA[b], Conci J[a], Prat Gay G[a]
a - Fundación Instituto Leloir and IIBBA-CONICET
b - Structural Biology Department, University of Virginia

Mononegavirales order includes viruses of medical relevance, such as ebola, rabies, and respiratory syncytial virus (RSV). Throughout their infective cycle, these viruses form granules where the polymerase complex and viral RNA concentrate. The complex consists of a nucleoprotein (N), a phosphoprotein cofactor (P), the RNA polymerase and a transcription factor. Within the granules viral replication and transcription occurs and, for this reason, they have been called viral factories. Coexpression of N and P is the minimum required system for the formation of viral factories in cells. Like several other biomolecular condensates described in recent years, viral factories form by liquid-liquid phase separation (LLPS). The proteins involved in LLPS have features such as modularity, multivalence, intrinsic disorder, weak interactions and often interact with nucleic acids. RSV P is modular, tetrameric and intrinsically disordered. It has transient secondary structure elements that are more stable at low temperatures and in a tetrameric context. In addition, RSV P establishes local and remote internal contacts. Our hypothesis is that RSV P drives the formation of viral factories. Here we show that RSV P condenses at high concentrations in the presence of a crowding agent, forming spherical, dynamic and reversible droplets. LLPS is favored at low temperatures and occurs at physiological or higher concentrations of NaCl. Stabilization of preformed **α**-helices, induced by TFE, favors LLPS; this suggests that contacts between metastable helices are necessary for condensation. In addition, we observe a strong correlation between a low temperature range conformational transition and the LLPS; likewise, the presence of N dramatically decreases the critical concentration of P for LLPS. These results provide the pysicochemical grounds for understanding how viruses exploit the LLPS phenomenon for compartmentalizing the components and reactions involved in replication and transcription.

NMR ^{1}H-Shielding Constants of hydrogen-bond donor reflect manifestation of the Pauli principle.

Zarycz MNC[a], Fonseca Guerra C[b]
a - Facultad de Química Bioquímica y Farmacia, Universidad Nacional de San Luis, Chacabuco 915, 5700 San Luis, Argentina. IMIBIO-SL (CONICET), Chacabuco 915, 5700 San Luis, Argentina
b - Department of Chemistry and Pharmaceutical Sciences and Amsterdam Center for musticale Modeling (ACMM) Vrije Universiteit

NMR spectroscopy is one of the most useful methods for detection and characterization of hydrogen bond (H-bond) interactions in biological systems. For H-bonds X-H···Y, where X and Y are O or N, it is generally believed that a decrease of 1H-shielding constants relates to a shortening of H-bonds donor-acceptor distance. Here, we investigated computationally the trend of 1H-shielding constants for hydrogen-bonded protons in a series of Guanine C8-substituted GC pair model compounds as a function of the molecular structure. Furthermore, the electron density distribution around the hydrogen atom was analyzed with the Voronoi deformation density (VDD) method. Our findings demonstrate that 1H-shielding values of the hydrogen bond are determined by the depletion of charge around the hydrogen atom which stems from the fact that electrons obey the Pauli exclusion principle.

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Sulfide-mediated reduction of metmyoglobin

Palermo JC[a], Melisa CC[a], Darío E[a], Boubeta FM[a], Bari SE[a]
a - Universidad de Buenos Aires-CONICET, DQIAQF-INQUIMAE, Facultad de Ciencias Exactas y Naturales

Hydrogen sulfide (H_2S) is a gasotransmitter, spotted as a signaling molecule with physiological effects, including cardioprotection, neuroprotection, regulation of blood vessel diameter, among others.[1] Diverse metabolic pathways can produce H_2S endogenously.

The coordination of sulfide to the ferric iron of diverse hemeproteins (HPs) forming complexes $HPFe^{III}(SH^-)$ has been reported.[2] In many cases, coordination of sulfur species to ferric hemeproteins subsequently triggers one-electron reduction to yield the corresponding ferrous form.

The reduction of ferric heme iron by sulfide has been observed for different HPs or heme model compounds, with varying reduction rates and with the concomitant formation of colloidal sulfur.

In organic media, it has been reported that the reduction proceeds by the homolytic cleavage of the coordination complex, yielding the ferrous form and sulfanyl radicals (HS•). So far the molecular mechanism of sulfide-mediated reduction of ferric HPs under biorelevant conditions has not been elucidated. Herein we show our results on the kinetic study of the sulfide mediated reduction of metmyoglobin ($MbFe^{III}$), and the discussion of different mechanistic proposals.

We observed that $MbFe^{III}$ in the presence of Na_2S ($R_{Na2S/Mb}$ = 100 to 400; pH 7.4, anaerobic, 25°C) completely yielded the reduced hemeprotein, $MbFe^{II}$, in the minutes timescale. The intermediacy of of the well characterized $MbFe^{III}(SH^-)$, was observed. The formation of S_8 as a final product of sulfide chemistry suggests the intermediacy of polysulfides H_2S_n (n>1), promoted by the initial formation of radical species (e.g.: HS•).

The formation of $MbFe^{II}$ is characterized by a time-dependent trace that showed an S-shaped profile with a Hill-type time dependency.[3] This kinetic behavior can be assigned to autocatalytic reactions, in which at least one of the products increases the overall reaction rate. A reduction rate was tentatively derived from the initial slow step of the reaction. The experimental results can be interpreted in terms of different mechanism proposals, evaluated using the software COPASI. Given the known reactions of sulfur species in aqueous solution and our current results, we propose the formation of radical species or/and polysulfides as intermediates of a multistep mechanism.

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Suicide probes and Laurdan as tools to assess *in vivo* bacteriocin-membrane interactions.

Rios Colombo NS[a], Chalón MC[a], Dupuy F[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 forming pores over specific target bacteria. Their study is a primary concern, since they are considered alternative antimicrobials. So far, there has been several studies on bacteriocin-membrane interactions, mainly using model membranes. Although *in vitro* approaches are very useful to understand bacteriocins mechanism of action, these models demand to simplify an extremely complex and dynamic system such as the biological membrane. In this work we designed hybrid proteins called "suicide probes" aimed to be expressed in bacterial hosts such as *E. coli*. This is an *in vivo* system that allows to evaluate interactions of bacteriocins with real bacterial membranes, as a tool to complement studies in model membranes. On the other hand, Laurdan is a popular fluorophore, deemed to work extremely well not only in model membranes but also in living cells. It is used to assess changes in membrane fluidity that result from phase transitions. The present work provides a dataset of fluorescence emission spectra and Generalized Polarization (GP) values of Laurdan-labeled *E. coli* bacterial cells, expressing three engineered class II bacteriocins. The results demonstrate that the insertion of different bacteriocins tend to increase membrane order. The arrangement of the lipids towards the gel phase make complete sense if we consider that a pore structure is being formed, where conformational changes are likely to happen in both the peptides and the phospholipids. Additionally, control experiments were performed to evaluate how accurate is Laurdan as an indicator of *E. coli* membrane fluidity changes. It is well known that bacterial cells rapidly adapt to changes in temperature by adjusting the fatty acid composition of the membrane, in order to maintain the homeostatic parameters. Thus, fluorescence spectra of Laurdan-labeled *E. coli* cells are compared, to check the GP behavior in response to different growing temperatures.

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Cell adhesion and migration on linear stiffness gradient hydrogels

Muzzio N[a], von Bilderling C[b,c], Sigaut L[b,a], Pietrasanta L[d,b,a]

a - Instituto de Física de Buenos Aires (IFIBA, CONICET-UBA), Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires

b - Departamento de Física, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires

c - Instituto de Investigaciones Fisicoquímicas Teóricas y Aplicadas (INIFTA, CONICET-UNLP), Departamento de Química, Facultad de Ciencias Exactas, Universidad Nacional de La Plata

d - Centro de Microscopías Avanzadas (CMA), Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires

Mechanical stimuli play a significant role in different physiological and pathological processes such as tissue and cancer development. Cells are constantly exposed to different external mechanical stimuli coming from the extracellular matrix and neighbor cells, which influence several cell functions like adhesion, proliferation, migration, and differentiation. These mechanical stimuli are not always homogeneous and may present spatiotemporal heterogeneities.

In the present study, we examine the results of cell adhesion and migration on polyacrylamide hydrogels with linear stiffness gradients. We adapted the method developed by Hadden *et al.*[1] to fabricate the gels on top of 25 mm diameter cover glasses. The topography and mechanical properties of the gels were assessed by an AFM- based technique: PeakForce Quantitative Nanomechanical Mapping (PFQNM[2]). During image acquisition, force curves were collected and analyzed along with the topography, providing the simultaneous maps of elastic modulus, deformation, and adhesion. A gradient of gel stiffness was observed with values depending on the acrylamide and bis-acrylamide solution concentrations. Cell adhesion on different regions of the gel was examined by Scanning Electron Microscopy and Confocal microscopy. Cell areas and morphologies were dependent on the local mechanical properties of the underlying hydrogel. Live cell imaging was performed to study cell migration patterns, which resulted to be strongly dependent on the slope of the stiffness change along the gel. These results will allow us to explore the stiffness-sensitive expression, localization and dynamics of the mechanosensitive proteins zyxin and vinculin.

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Correlation of cellular traction forces and dissociation kinetics of adhesive protein zyxin by means of multidimensional microscopy

Sigaut L[a], Bianchi M[b], von Bilderling C[c,d], Pietrasanta L[b,a]
a - Departamento de Física, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires and IFIBA, CONICET-UBA, Cuidad Universitaria, Buenos Aires C1428EHA, Argentina
b - Centro de Microscopías Avanzadas (CMA), Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Buenos Aires C1428EHA, Argentina
c - Departamento de Física, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, C1428EHA Buenos Aires, Argentina
d - INIFTA, UNLP, CCT La Plata-CONICET, CC 16, Suc. 4, (1900) La Plata, Argentina

Cells exert traction forces on the extracellular matrix to which they are adhered through the formation of focal adhesion. Spatial-temporal regulation of traction forces is crucial in cell adhesion, migration, cellular division and remodeling of the extracellular matrix. By cultivating cells on polyacrylamide hydrogels of different stiffness we were able to investigate the effects of substrate stiffness on the generation of cellular traction forces by Traction Force Microscopy (TFM), and characterize the molecular dynamic of focal adhesion protein zyxin by Fluorescence Correlation Spectroscopy (FCS) and Fluorescence Recovery After Photobleaching (FRAP). As the rigidity of the substrate increases, we observed an increment of both, cellular traction generation and zyxin residence time at the focal adhesion, while its diffusion would not be altered. Moreover, we found a linear correlation between the traction forces exerted by cells and the residence time of zyxin at the substrate elasticity studied. We found that this correlation persists at the focal adhesion level, even if there is no variation in substrate stiffness, by performing combined TFM and FRAP experiments, revealing that focal adhesions that exert greater traction, present longer residence time for zyxin, i.e. zyxin protein has less probability to dissociate from the focal adhesion.

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Protein nanoparticles as carriers of natural antioxidants

Candido S[a], Alvira F[a,b], Moreno JC[b], Martinez L[a], Alonso S[a]
a - Laboratorio de Bionanotecnologia
b - 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

We have conducted physically and rheological characterization of a protein nanocarrier (bovine serum albumin nanoparticles) to encapsulate antioxidants of natural origin coming from blueberries (Vaccinium Myrtillus). The blueberries are rich in anthocyanin (A). The encapsulation was done with the aim of obtaining a stable formulation of NP BSA + A that can be useful to enrich food or to be injected into the body. Due to BSA NP is formed by an unknown number of individual BSA molecules, computational simulations were performed to estimate this number.

BSA NPs are obtained by a chemical method and stabilized by Gamma irradiation. Antioxidants are obtained by liquid-solid extraction from blueberries. The nanoformulation was studied by, Dynamic Light Scattering (DLS), potential Z, electron transmission microscopy, rheology, UV-VIS and fluorescence spectroscopy at three different temperatures, 8 °C (storage temperature), 25 °C (environment temperature), 37 °C (physiological).

Our results show that the BSA NPs obtained by elution in a molecular exclusion column has a hydrodynamic radius of 28 +/- 5 nm and is composed of approximately 183 BSA molecules. Depending strongly on the temperature.

It was possible to study the interaction of NP BSA + A and discriminate the active site where it occurs (Sudlow I and/or II), by fluorescence. From the rheological tests, it was observed that for all three temperatures the samples tend to a behave as Newtonian type and that there is a correlation between viscosity and temperature values. The morphological changes observed by TEM corroborate the relationship of viscosity values with aggregate formation.

Solving the edge artifact in the SUPPOSe algorithm for super-resolution of fluorescent images

Martínez S[a], Toscani M[b], Martínez OE[b]
a - IMAS, Departamento de Matemática FCEyN UBA - CONICET
b - Laboratorio de Fotónica, Departamento de Física FIUBA - CONICET

Trying to apply SUPPOSe (a super-resolution deconvolution algorithm [1]) to the axonal cytoskeleton (for the actin/spectin) to detect the presence of a periodic structure of periodicity ≈ 180 nm − 190 nm denominated membrane-associated periodic skeleton (MPS) [2,3], we found the appearance of an artifact in the reconstruction that hindered an appropriate recovery of the structure.

It was determined that the artifact arises from the presence of fluorescent molecules outside the registered frame but close enough to the boundary contribute with significant signal inside the frame.

The algorithm presented in [1] for wide-field fluorescent microscopy deconvolution from single acquisition consists on assuming that the measure image is a superposition of point sources of equal intensity. The goal of this method was to find the positions of such sources. This optimization problem was solved using a genetic algorithm with an appropriated fitting function. In this new work a new fitting function is used to remove artifacts arising from the edges.

To test the improved algorithm with an image with known ground truth we constructed a synthetic image similar to MPS consisting of k parallel lines with different periods p. The point spread function (PSF) is Gaussian similar to the one obtained with a wide-field fluorescent microscopy, for this special set-up the PSFwidth=95nm.

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A new scoring function for molecular docking

Villarreal M[a], Quiroga R[a]
a - Instituto de Investigaciones en Físico-Química de Córdoba (INFIQC-CONICET-UNC), Departamento de Matemática y Física, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba

Molecular Docking is a computational method to predict the strength, position and orientation of the ligand binding to a protein, and is a key tool in structure-based drug design. In essence, the method consists of the search for the global maximum of a mathematical function which represents the affinity of ligand at a given position in the protein surface. This mathematical function, termed scoring function, is developed based on different approaches. Among them, there are semi empirical, fully empirical, knowledge and artificial intelligence based methods. In our lab we started to develop an empirical scoring function based on the well-known Vina scoring function. In this work we present the newest version of our scoring function which includes several improvements. In particular we focused in a better atom-typing, hydrogen bond interaction, and solvation terms. The philosophy behind our development was to come up with coarse grained interactions, where the atomic parameters that define the interactions are picked from a predefined and scalonated set of values.

The performance of the scoring function was evaluated in rescoring, redocking and virtual screening tasks. The results show that the new function performs better at the three tasks, when compared with our previous version and also with the original Vina function.

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Binding prediction of agonists and antagonists to serotonin receptor 5-HT3

Issoglio F[a], Zamberlan F[b], **Pallavicini C**[b,c], Defelipe L[d], Tagliazucchi E[b], Marti M[d]
a - Departamento de Química Biológica- FCEN- UBA/ IQUIBICEN-CONICET
b - Departamento de Fisica, FCEN-UBA and IFIBA, CONICET-UBA
c - Laboratorio de Investigaciones Aplicadas a las Neurociencias - FLENI
d - Departamento de Química Biológica, FCEyN, UBA - IQUIBICEN, CONICET

5-HT_3 serotonin receptor is a neurotransmitter-gated ion channel involved in fast intercellular signaling. It also became a common target to the treatment of vomiting and nausea during cancer therapies.

This integral membrane protein is formed by 5 subunits of 580 residues. In addition to the crystal structure of 5HT-3A serotonin receptor reported in 2014, in the past few years more structures were obtained using cryo-electron microscopy (cryoEM), including the apo-structure (closed channel) 2 serotonin bound states (partially closed and open channel) and with the antagonist granisetron.

The main goal of this study is to generate a system able to predict the binding of molecules that could act as agonists or antagonists of serotonin on 5HT-3A receptor.

Different ligands can promote slightly diverse conformations regarding the position of the aminoacids involved in protein-ligand interactions. Conventional molecular docking performed on an open binding site is not capable of reproduce the pose of either serotonin or ranisetron solved by X-Ray or cryoEM.

In order to improve docking results, molecular dynamic simulations with a mixture of water and ethanol was employed to determine solvent sites. Pharmacophorics restraints were defined by combining the co-solvent results with the 5-HT_3 structures solved with serotonin and granisetron, and with pharmacophore models previously proposed by other authors to describe common structure features shared by agonists and antagonists.

Using an open channel conformation of the receptor and the defined pharmacophoric restraints we were able to predict the correct pose of both serotonin and the antagonist granisetron to 5-HT3 receptor, and predict the bound conformation of a set of known agonists and antagonists molecules.

Cell Cycle importance in cell fate definition during diferentiation of mouse embrionic stem cells

Sevlever F[a], Waisman A[b,c], Santiago M[c], Ventura A[a], Guberman A[b]
a - Instituto de Fisiología Biología Molecular y Neurociencias (IFIBYNE). Universidad Nacional de Buenos Aires. CONICET.
b - Departamento de Química Biológica, FCEyN, UBA - IQUIBICEN, CONICET
c - Laboratorio de Investigaciones Aplicadas a las Neurociencias - FLENI

Mouse embryonic stem cells (mESCs) are pluripotent cells that resemble the preimplantation epiblast of the mouse embryo. They can give rise to all the cells of the organism, which makes them interesting in regenerative medicine area. This rise is due of different cell-fate decisions during differentiation. Recently, the cell cycle has gained attention as a key determinant of these cell decisions, based on the observation that cells only respond to differentiation cues during G1 phase. In this work, we aimed to characterize in depth the dynamics of proliferation in mESCs, both while maintaining the pluripotent state and during differentiation. We performed 48 h videos of mESCs stably expressing the FUCCI cell cycle reporters as well as an H2B nuclear probe. We developed a bioinformatic pipeline to extract single-cell dynamics of thousands of cells, with information about their lineage relationships. Analysis of this database confirmed the change in cell cycle dynamics and also showed that G1-L and CC-L are greatly correlated between sister cells, implying that these properties are partially inherited from the parental cell. Along these lines, we applied Grassberger-Procaccia algorithm, what shows that the cycle dynamics is a highly deterministic process where each phase can be well predicted from cell's lineage.

By addressing the complexity of single-cell dynamics of mESCs, we believe our work contributes to a greater understanding of cell cycle connection to fate decisions.

Combining computational tools to study pharmacophore models of the 5-HT3 receptor ligands

Zamberlan F[a], Issoglio F[b], Pallavicini C[a,c], Marti M[b], Tagliazucchi E[a]
a - Departamento de Fisica, FCEN-UBA and IFIBA, CONICET-UBA
b - Departamento de Química Biológica, FCEyN, UBA - IQUIBICEN, CONICET
c - Laboratorio de Investigaciones Aplicadas a las Neurociencias - FLENI

The 5-HT3 receptor it is the only ligand-gated ion channel in the serotonin family and it is present in both central and peripheral nervous system fulfilling different functions and representing a vital pharmacological target for the treatment of diverse diseases.
In the brain, evidence suggests that these receptors could be involved in interactions between serotonin and dopamine systems and therefore addictive behaviour and abuse of alcohol and cocaine in particular.
Nowadays, however, the main interest over this receptor it is due to its interaction in the gastrointestinal tract with chemotherapeutic drugs used for treating cancer leading to nausea and vomiting.
In this work, we combined ligand docking with molecular dynamics simulations to virtually study the interactions of well-known and specifically selected agonists and antagonists with this receptor.
We have fed a graph convolutional network (a powerful machine learning technique specially designed for analyzing graph such as molecules) with the obtained representative poses of those ligands in combination with their respective pharmacophores, abstract descriptions of the molecular structure features responsables for the interaction with biological targets and the following pharmacological response.
The present study shows our conclusions about the geometrical constraints and chemical requirements that a candidate molecule should comply to considered active over this receptor.

Coupling Constant-pH and Accelerated Molecular Dynamics Techniques to Explore the Conformational Landscape of a Mimetic Iron-binder Peptide

Vazquez DS[a], Santos J[b]
a - Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes, Grupo Vinculado de Biología Estructural y Biotecnología – IMBICE-CONICET-CCT La Plata
b - Departamento de Fisiología y Biología Molecular y Celular. Facultad de Ciencias Exactas y Naturales. Universidad de Buenos Aires. C.A.B.A.

One of the most common overlooked points in conventional molecular dynamics simulations is the change in the protonation state of titratable/ionisable residues during calculations. It is well known that protonation state has a very important role in protein structure, stability and function. Usually, the protonation state of each titratable residue is fixed along the simulation and is usually assigned by the standard pKa value of the isolated residue in solution and the impact of fixed-settled protonation states would lead to an inaccurate or inadequate sampling of the native conformational ensemble of the biomolecule. The high degree of conformational freedom in unstructured peptides is a challenging task. Furthermore, the interdependence between accessible conformations and protonation state is of particular interest given that these aspects could together influence the functional role of a specific sequence motif.

In previous work, we grafted the putative iron-binding motif EExxED onto a foreign 16-Mer peptide scaffold. The designed peptide, called GRAP (from GRAfted Peptide), consists of an acidic motif (EExxED) and was able to specifically bind Fe^{3+} with a moderate affinity, an equimolar stoichiometry and the iron-peptide interaction was determined to be entropically driven. A set of mutant peptides of the acidic motif were designed and the metal-free conformational ensemble seems to be more unstructured than GRAP as judged by their circular dichroism (CD) spectra between acidic and neutral pHs evidencing a clear protonation/conformation dependence (Vazquez et al., unpublished results). This scenario makes exciting the study of the interdependence of the protonation state coupled to conformational changes and the ability to bind iron in a helical-restricted context with a simple peptide model. To address this issue, in the present work we coupled the constant-pH molecular simulation scheme with the unbiased conformational sampling aMD technique in explicit solvent for the GRAP and mutant peptides in a broad range of pHs along with the experimental determination of the pH-dependence of the total helical content by CD.

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Cuantitative Prediction of Quantum yields of Fluorescence in Complex System

Diaz Miron G[a], Gonzalez Lebrero MC[a]
a - Departamento de Química Inorgánica, Analítica y Química Física, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires / INQUIMAE-CONICET

The variation of quantum yields and lifetime of fluorescence in proteins is widely exploited in biophysical. Examples of these uses are changes in protein structure, folding/unfolding, substrate or ligand binding, and protein-protein interactions among other.
The quantum yields and excited lifetime of fluorescence are primarily dependent upon the competition between the emission of radiation and various radiationless deexcitation processes. This scenario is further complicated if you want to study this phenomenon in a complex environment, such as solution or protein, where a large number of micro-configurations must be taken into account. That is why there are no methodologies to estimate this important property.

In proteins, the indol group of triptophan is the most used fluorescent cromophore, due to changes in its properties with the enviroment.

In this work, we present a new methodology to calculate quantum yields of indole in different solvent and in various protein.

For this purpouse, we use excited state QM-MM molecular dynamics simulations with Linear Response Time Dependent Density Functional Theory (LR-TDDFT) and Tamm-Dancoff Aproximation (TDA). Additionally, hop probabilities were calculated using Tully's Surface Hopping method (TSH). In our simplified model, we relate the oscillator strength with radiative emission probability through an adjustable parameter, while non-radiative processes rate is related with the hop probability.
The experimental and calculated quantum yields show excelent correlation for all test cases allowing quantitative predictions.

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Development of a reaction diffussion model for the simulation and analysis of the multienzymatic degradation of cellulose

Momoli J[a], Iglesias M[a], Craig PO[a]
a - Departamento de Química Biológica, FCEyN, UBA - IQUIBICEN, CONICET

Biological processes and reactions where the spatial distribution, diffusion, morphological evolution, and accessibility of components play an essential role, are difficult to explore with ordinary differential equations (ODE). These phenomena are better treated in physical terms using numerical methods called "agent-based models", "cellular automata" or “reaction diffusion simulations”. These models are based on diffusible particles within a three-dimensional space, which interact with each other through simple and programmable probabilistic rules according to their proximity and stochastic collisions. The temporal evolution of these systems give rise to complex emergent properties, difficult to anticipate otherwise. The use of these models are particularly useful in the study of multienzymatic systems. In this work, we present the development of a reaction diffusion model for the simulation of the multienzymatic degradation of cellulose. For this purpose, we use the program Readdy to model particles with endoglucanase, exoglucanase, cellulose binding and beta glucosidase activities. Preliminary results for the degradation of a model of crystalline cellulose by a mixture of enzymes show an increase in the degradation for enzymes coupled to multienzymatic particles (artificial cellulosomes) compared to the same enzymes free in solution. The increase observed is similar to the one measured in vitro by our group and others. This model will help us to interpret experimental results for the degradation of cellulose by artificial cellulosomes, and predict the kinetic and structural parameters that are critical for the synergism produced by the colocalization of enzymes. This knowledge will help us to design improvements in multienzymatic complexes for an optimal degradation of cellulose.

Effect of gabaergic phenols on the dynamic and structure of lipid bilayers: a molecular dynamic simulation approach

Miguel V[a], Villarreal M[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, Consejo Nacional de Investigaciones Científicas y Técnicas, Departamento de Química Teórica y Computacional, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba

γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system. $GABA_A$ receptors are activated by GABA and modulated by a wide variety of recognized drugs, including anesthetics and benzodiazepines. GABAergic phenols (GP) like propofol, thymol, chlorothymol, carvacrol and eugenol are positive allosteric modulators of R-$GABA_A$. These GP are lipophilic, therefore their anesthetic activity could be the combined result of their specific interaction with the receptor, as well as nonspecific interactions with the receptor lipidic environment. We used molecular dynamic (MD) simulations to contribute to a description of the molecular events that occur at the membrane as part of the mechanism of general anesthesia. Previous MD simulations indicated that GP interacts with the polar interface of phospholipid bilayer. The presence of GP in a DPPC bilayer has an ordering effect on lipid acyl chains for carbons near the interface. We have now determined GP orientation in the bilayer by defining a set of molecular axes. We have calculated the correlation of the experimental membrane partition coefficients obtained by the IAM–HPLC method (log k_{IAM-W}), with ΔG of partition obtained in biased MD and obtained a value of 0.935. Potential of mean force (PMF) calculations using umbrella sampling were used to characterize the forces that drive propofol partition into the bilayer. This analysis showed that propofol partition is mainly enthalpic driven at the polar region and entropic driven at the hydrocarbon chains. We calculated the GP-water, GP-GP and GP-DPPC non-bonding interactions. We found attractive Lennard-Jones (LJ) interactions between phenol and DPPC, while GP-GP LJ forces were found to be nearly zero. Finally, we determined the first hydration shell for PRF. While in the aquose phase PRF has ~35 water molecules, at the lipid phase there is an average of ~5 water molecules, except at translocations, were water molecules drop to cero. These results confirm that all the GP studied interact with membranes, and exert some alteration of the receptor lipid environment. Thus, it is possible that anesthetic activity of GPs could be the combined result of their interaction with specific receptor proteins (GABA-Rs) but also with the surrounding lipid molecules.

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Effects of lipid composition on defect formation in bilayer membranes

Moyano MS[a], Di Lorenzo HE[a], García S[a], Tapia AP[a], Quiroga AN[a], Nuñez AM[a], Via MA[a], Ferguson M[a]
a - Universidad de Mendoza

Biological membranes are composed of a great variety of lipid species, among the most abundant are the lamellar dioleoylphosphatidylcholine (DOPC) and the non-lamellar dioleoylphosphatidylethanolamine (DOPE) lipids. The lamellar/non-lamellar lipid ratio in a membrane affects its structural characteristics. In this work, we study the effect of this ratio on the formation of hydrophilic transmembrane pores.

We employ a strategy of computer simulation through molecular dynamics to study pore formation in five different systems of DOPC/DOPE lipid bilayers, immersed in water. Each system contains 288 lipid molecules, in varying ratios, with exactly half of the lipids in each leaflet of the membrane. We apply semi-anisotropic stretching in the xy plane in order to generate the desired membrane defects. Subsequently, we follow this by constant area and pressure dynamics to equilibrate the system, thus allowing for the quantitative spatial characterisation of the open pores.

Our preliminary results show that for unitary membranes, i.e. consisting solely of DOPC or DOPE, the formation of the transmembrane pore occurs with similar levels of stretching. In the case of binary membranes, however, a greater proportion of lamellar lipids requires a greater amount of stretching to achieve the same defect than if the non-lamellar lipid is dominant.

In short, we have analysed the poreation profiles of various membrane systems containing differing proportions of lamellar and non-lamellar lipid molecules, and how this ratio changes the mechanical properties of biological membranes.

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Initial computational assessment of the binding mode of Diazepam Binding Inhibitor to $GABA_A$Rs

Amundarain MJ[a], Obiol D[a], Zamarreño F[a], Costabel M[a]
a - Instituto de Física del Sur (IFISUR), Departamento de Física, Universidad Nacional del Sur (UNS), CONICET

$GABA_A$ receptors are the main mediators of the fast inhibitory response in the central nervous system of mammals. These members of the Cys-loop family are activated by g-Aminobutyric acid, and are modulated by a myriad of pharmacologically relevant compounds such as benzodiazepines, neurosteroids and alcohol. Moreover, it has been shown that a 10 kDa protein, named DBI (Diazepam Binding Inhibitor) and small peptides derived from its tryptic digestion interact with $GABA_A$Rs through the benzodiazepines binding site, acting as allosteric modulators. DBI and its peptides are expressed in the brain, both in astrocytes and neurons. However, details about the interaction and the allosteric effects remain unspecified.

The aim of this research is to study the interaction between the small peptides and DBI protein, and the $\alpha_1\beta_2\gamma_2$ $GABA_A$R through *in silico* methods, namely docking and classic molecular dynamics simulations. The structure for the receptor was obtained from homology modeling (based on the β_3 homopentamer PDB ID: 4COF) and DBI was obtained from PDB ID: 2FJ9. Docking simulations were performed with HADDOCK webserver, and GROMACS4.6.5 was employed for the molecular dynamics simulations. The complexes were evaluated through the webservers HyPPI and PRODIGY, in addition to careful inspection of the interactions and standard structure analysis.

First, four peptides were studied: N41-K53, T42-K53, E43-K-53 and R44-K53. R44-K53 (OP peptide) showed the best scores, and a binding mode comprising a stable C-end inside the cavity, which is in agreement with the experimental data available. In addition, the interaction of the whole protein was assessed and a plausible binding mode was found with good scores. The complex was stable during the MD simulation and DBI interacted with loops involved in mechanisms of opening/closing of the channel.

This work presents the first computational assessments of the interaction between $GABA_A$ receptors and DBI protein and peptides.

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Modelling and kinetic analysis of Human Methylenetetrahydrofolate Reductase variants

Cossy Isasi S[a], Gomez H[a]
a - Cátedra de Bioquímica y Biología molecular, Medicina, Facultad de Ciencias Médicas-UNC

Methylentetrahydrofolate reductase (MTHFR, EC 1.5.1.20) converts 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate and participates in the re-methylation of homocysteine to methionine. Since Mudd et al. 1972, enzymatic activity deficiency was found to be prevalent in patients with cardiovascular disease and homocysteinuria. Since then several isoforms were identified that differ in one amino acid and present lower activity in vitro, and the scope of related diseases enlarged comprising cancer and CNS and PNS alterations like autism or multiple sclerosis. We present an advance of the in-silico study to understand the chemical foundations of its pathogenicity. FASTA sequences of the 2 functional wild type isoforms were obtained from the NCBIgene bank (CAB41971 or 1 and AAD17965.1 or 2), and that of isoform 2 mutants, by edition based on the literature: aa A116T, I153M, R157Q, T227M and P251L. Tertiary structures of the catalytic domain were homology modeled with 1v93 of T.thermophilus as template. The structures obtained with the virtual environments that evaluate the similarities with different parameters, Modeller 9.09, SWISS-MODEL, Phyre2 presented 98% of structural identity. Ab initio modelling of the regulatory domain is on the way. The affinity for FAD (flavin adenine dinucleotide) was evaluated by standard flexible docking (implicit solvent) obtaining a scale of interaction energies as affinity estimators. The lowest energies of binding (Kcal / mol) for each mutant in decreasing order were: P251L, -15.47; T227M, -15.20; A116T, -12.8; I153M, -11.8 and R157Q, -10.9. However, the geometries of the different enzyme-substrate complexes that were closer to that of the wild type have another order P251L -14,4; A116T -12.8 and T227M -12.56. The results show that differences in activity should be attributed to the geometric component of the interactions rather than their affinity.

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Molecular dynamics simulation of allosteric effectors binding in vertebrate hemoglobins

Pettinati M[a], Alonso de Armiño D[a], Drusin S[b], Issoglio F[a], Martí M[b], Storz J[c], Estrin D[a]
a - Universidad de Buenos Aires-CONICET, DQIAQF-INQUIMAE, Facultad de Ciencias Exactas y Naturales
b - Departamento de Química Biológica, FCEyN, UBA - IQUIBICEN, CONICET
c - School of Biological Sciences, University of Nebraska, Lincoln, USA.

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). This is true for almost all vertebrates except crocodilians, where the presence of said compounds doesn’t seem to alter Hb-oxygen affinity. It is unknown whether this phenomenon is caused by a reduction in hemoglobin's organic phosphate binding capability, or bonded phosphate's inability to generate the classic allosteric effect due to a difference in protein structure. In order to answer this question, a comparative study between various vertebrate species was performed using molecular dynamics simulation techniques. By analysing how different hemoglobins behave while bonded to an organic phosphate in similar conditions we aim to determine the molecular basis behind this particular evolutionary behaviour, and possibly even pinpoint the specific residues involved in it.

Molecular modeling study of specific inhibitors for the adenylyl cyclase 1 enzyme of the intestinal parasite Giardia *lamblia*.

Vega Hissi E[a], Garro A[a], Zurita A[a], Di Siervi N[b], Enriz R[a]
a - Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis. Instituto Multidisciplinario de Investigaciones Biológicas San Luis (IMIBIOSL-CONICET)
b - Facultad de Farmacia y Bioquímica, UBA. Instituto de Investigaciones Farmacológicas (ININFA-CONICET)

Giardia *lamblia* (G. *lamblia*) is a protozoan intestinal parasite responsible for an infection of great epidemiological and clinical importance known as giardiasis. This disease is the most frequent cause of diarrhea and bad absorption in the world, affecting millions of people every year and that is mainly caused by the ingestion of water contaminated with parasite cysts. There is evidence linking the adaptive differentiation processes of G. *lamblia* with signal transduction mechanisms mediated by cyclic AMP (cAMP). This second messenger would be synthesized by the enzyme adenylyl cyclase (AC: gAC1 and gAC2) which possess very low identity (~ 30%) with respect to their mammalian counterparts. Therefore, they become molecular targets for the development of specific pharmacological agents. As a starting point, we managed to clone, purify and demonstrate the adenylyl cyclase activity in enzymatic assays of the catalytic domain of the enzyme gAC1. In addition, we performed homology modeling, which used the soluble AC of the cyanobacteria S. *platensis* as the best template.

In this work we present enzymatic inhibition assays and theoretical models of interactions between a known S. *platensis* AC inhibitor, 2-catechol estrogen (2CE) and a series of new G. *lamblia* gAC1 inhibitors with novel structural scaffolds. In this way we present a new series of piperidine carboxamide based core derivatives obtained by chemical synthesis. Among these compounds, one stands out (Amj147) that has in its structure a catechol group similar to that of 2CE. This compound shows an inhibitory effect at the μM level, being the most active in the series. Our molecular modeling study reveals that although the catechol group of Amj147 actively participates through interactions with active site ions, the molecular size would result in a mechanism of inhibition different from that of 2CE. This new compound is an excellent starting structure for the design of new specific inhibitors of the gAC1 enzyme.

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Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis. Instituto Multidisciplinario de Investigaciones Biológicas San Luis (IMIBIOSL-CONICET). Facultad de Farmacia y Bioquímica, UBA. Instituto de Investigaciones Farmacológicas (ININFA-CONICET)

Molecular modeling, synthesis and biological evaluation of arylaminopropanone derivatives as potential cholinesterases inhibitors

Garro A[a], Gutiérrez L[a], Parravicini O[a], Rojas S[a], Hudcova A[b], Kroutil A[b], Enriz R[a]
a - Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis; Instituto Multidisciplinario de Investigaciones Biológicas (IMIBIO-SL-CONICET).
b - Department of Chemical Drugs, Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences

A series of novel arylaminopropanone hydrochloride salts with carbamate moiety was synthesized and evaluated as potential AChE and BChE inhibitors. *In vitro* measurements were performed, and the results were compared with those obtained for galanthamine and rivastigmine, drugs currently used in the treatment of neurodegenerative diseases.

The most potent compounds, displayed inhibitory activity against AChE *in vitro*. Their percentage inhibition of AChE was comparable to galanthamine and higher to rivastigmine, with IC_{50} in micromolar range. The structure-activity relationship shows that the incorporation of a piperidine moiety leads to higher inhibitory activity. In comparison, the replacement of piperidine by morpholine significantly decreased the activity against both enzymes. Our results also revealed that compounds with longer alkylamine moiety have higher activity and selectivity to BChE.

The molecular modeling study indicates that these compounds bind to the same active site of AChE as galanthamine, although some other strong molecular interactions were observed. Some of the molecular interactions obtained for the compounds reported here are a little weaker than those found for galanthamine, which would indicate a possible lower affinity for active site of AChE. The differences in activity of piperidine-substituted from alkyl-substituted compounds can be explained by distinct spatial orientation of the basic nitrogen in amine closed in a ring-substructure and amine with alkyl chains. Although the inhibitory activities of these compounds are slightly weaker to those of galanthamine, these compounds show promising potential. They can be taken as starting structures for further molecular modeling and synthetic research.

Polycerasoidol, a natural prenylated benzopyran with a dual PPARα/ PPARγ agonist activity and anti-inflammatory effect.

Garibotto F[a], Suvire F[a], Tosso R[a], Funes M[a], Enriz R[a]
a - Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis; Instituto Multidisciplinario de Investigaciones Biológicas (IMIBIO-SL-CONICET).

Dual peroxisome proliferator-activated receptor-α/γ (PPARα/γ) agonists regulate both lipid and glucose homeostasis under different metabolic conditions and can exert anti-inflammatory activity. We investigated the potential dual PPARα/γ agonism of prenylated benzopyrans polycerasoidol and polycerasoidin and their derivatives for novel drug development.

Nine semisynthetic derivatives were prepared from the natural polycerasoidol and polycerasoidin, which were evaluated for PPAR α, γ, δ and retinoid X receptor-α activity in transactivation assays. Polycerasoidol exhibited potent dual PPARα/γ agonism and low cytotoxicity. Structure-activity relationship studies revealed that a free phenol group at C-6 and a carboxylic acid at C-9’ were key features for dual PPARα/γ agonism activity. Molecular modeling indicated the relevance of these groups for optimal ligand binding to the PPARα and PPARγ domains. In addition, polycerasoidol exhibited a potent anti-inflammatory effect by inhibiting mononuclear leukocyte adhesion to the dysfunctional endothelium in a concentration-dependent manner via RXRα/PPARγ interactions. Therefore, polycerasoidol can be considered a hit-to-lead molecule for the further development of novel dual PPARα/γ agonists capable of preventing cardiovascular events associated with metabolic disorders.

QM and QM-MM investigation of hydropersulfides reactivity towards peroxynitrous acid

Grassano JS[a], Semelak J[a], Zeida A[b], Trujillo M[b], Estrin D[a]
a - Universidad de Buenos Aires-CONICET, DQIAQF-INQUIMAE, Facultad de Ciencias Exactas y Naturales
b - Departamento de Bioquímica and Center for Free Radical and Biomedical Research, Facultad de Medicina, Universidad de la República

Hydropersulfides (RSSH) are thiol (RSH) and hydrogen sulfide (H2S) derivatives, and have been considered to have important biological functions because of their unique chemical properties exhibiting nucleophilic but also electrophilic capability. This nucleophilicity makes hydropersulfides, as well as thiols, prone to react with oxidants as hydroperoxides (ROOH) like hydrogen peroxide (H2O2) or peroxynitrous acid (ONOOH), which are often produced in response to different extracellular stimuli in many cellular types. Moreover, persulfides have been proposed to be stronger nucleophiles than the corresponding thiolates. Despite the growing attention of the biomedical community on this species, many issues regarding the molecular basis of their assumed differential reactivity have not been answered yet. In order to obtain both kinetic and thermodynamic information regarding persulfides and thiolates reactivity towards two-electron oxidants, we have performed *in silico* experiments and compared our results with the available theoretical and experimental data reported so far. Particularly, we studied the reaction of the simplest thiolate and persulfide models (methanethiolate, MeS−, and methanepersulfide, MeSS−) oxidations by ONOOH. First, we performed electronic structure calculations both in vacuo and in an implicit solvent at DFT level of theory. Computations were then repeated using other methods at different theory levels as MP2, and DFT using the more sophisticated ωB97xD and M062X functionals in order to evaluate activation barriers underestimations inherent to pure DFT functionals. Finally, we performed free energy calculations in a multiscale scheme (QM/MM) to obtain free energy profiles by means of umbrella sampling calculations. Our results suggest that persulfides are, at most, slightly stronger nucleophiles than the corresponding thiolates.

Quercetin self-association and hydration. A molecular dynamics study.

Campo MG[a], Corral GM[a]
a - Facultad de Ciencias Exactas y Naturales. UNLPam.

Flavonol quercetin (3, 3', 4', 5, 7-pentahydroxy-flavone), one of the most abundant flavonoids in the human diet, is a potent antioxidant and chelator of metal ions. The hydroxyl groups of QC (quercetin) play a fundamental role in their antioxidant capacity, chelating ability, and interactions with other molecules. Both the hydrophobicity and the ability of the hydroxyl groups to form hydrogen bonds give quercetin a set of significant properties in various phenomena of scientific interest. Thus, the study of their hydration structure is a key factor for understanding the accessibility of other molecules present in the solution to their hydroxyl groups. Phenolic compounds aggregate by H-bonding interactions and pi-stacking. In this work, we used classical molecular dynamics to study hydration, self-association, structural and dynamics properties of quercetin in aqueous solution. The model of quercetin is 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 water model used was SPC/E. The hydration structure studied by analyzing the radial distribution functions, the hydrogen bonds distributions of solvent-solvent and QC-solvent atoms, while the dynamic aspects are analyzed by studying the diffusion coefficient of the molecules in the solution. The catechol hydroxyl groups of the QC show the higher frequency of hydrogen bonding with the solvent although in general, this molecule has a hydrophobic behavior. It is consistent with the fact that these groups are those that are mainly related to the properties of the QC. Also, quercetin molecules are quite prone to π-stacking interactions with themselves (self-association), as predicted by experimental evidence.

Second generation of Benzothiazoles as inhibitors of Girasa B (*E. coli*): Design, synthesis and biological evaluation

Bonvillani A[a], Kikelj D[b], Parravicini O[a], **Enriz R**[a], Gutiérrez L[a]
a - Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis; Instituto Multidisciplinario de Investigaciones Biológicas (IMIBIO-SL-CONICET).
b - University of Ljubljana, Faculty of Pharmacy

The growing appearance of pathogenic bacteria resistant to antibacterial drugs in acquired infections is a serious threat to global health, because the available therapies will no longer be effective in treating these infections (1). Although the rate of bacterial resistance on the medications currently used is on the rise, no new antibacterial drugs appear on the market. Bacterial topoisomerases are enzymes that catalyze changes in DNA topology and are very attractive targets for antibacterial drug discovery (2). DNA gyrase is a tetrameric A2B2 type protein consisting of two GyrA subunits and two GyrB subunits, while topoisomerase IV is composed of two ParC and two ParE subunits (C2E2) that are homologous to GyrA and GyrB, respectively. The GyrA and ParC subunits are involved in DNA transit, while the GyrB and ParE subunits hydrolyze the ATP to obtain the energy necessary for the functioning of the GyrA subunits. At present there are no drugs that inhibit topoisomerase function due to competencies with ATP, this makes the GyrB subunit a new and attractive target for the generation of new and potent antibacterial compounds. This work presents a new series of compounds derived from benzothiazoles designed through the use of molecular modeling tools. We employed techniques of docking, molecular dynamics and calculations QTAIM (Quantum Theory of Atoms In Molecules) which allowed us to explain the different activities of the compounds, as well as the characterization of a new sub-site found in the active site of GyrB. The main interactions observed in this sub-site are those with Ile94, Gly102 and Lys103. The finding of this sub-site allowed us to increase the number of ligand-receptor interactions by improving IC50 values from 58 nM to 20 nM.

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Structural and Vibrational Investigation on Alkaloid N-methylcytisine using FTIR spectroscopy and DFT calculations

Alvarez Escalada FC[a], Ledesma A[b], Romano E[c], Brandan SA[c]
a - Departamento Académico de Química, Facultad de Ciencias Exactas y Tecnologías, FCEyT, Universidad Nacional de Santiago del Estero, UNSE
b - CIBAAL-UNSE- CONICET, Departamento Académico de Química, Facultad de Ciencias Exactas y Tecnologías, Universidad Nacional de Santiago del Estero
c - Cátedra de Química General, Instituto de Química Inorgánica, Facultad de Bioquímica. Química y Farmacia, Universidad Nacional de Tucumán

N-methylcytisine is a quinolizidine alkaloid obtained from plants that is widely used in in the treatment of multiple diseases, such as migraine, insomnia, asthma, or cough. The quasi-aromatic ring in cytisine structure, with similar properties than nicotine, shows an affinity towards the specific subunits of nicotine acetylcholine receptors nAChRs. FTIR spectroscopy combining with Mechanical-Quantum Calculations (DFT level) can be used as tools in the analysis of the structure activity relationship. In this work, we present the analysis of two isomeric forms of N-methylcytisine in gas and aqueous solution phases by using hybrid B3LYP/6-311++G** calculations. The infrared spectrum in solid phase and the UV-Visible spectrum in aqueous solution were registered and the structural analysis was carried out using the Gaussian 09 program. The complete assignments of the IR spectrum and the corresponding force fields in both media were performed by using scaled SQMFF methodology. The reactivities of both forms were predicted in aqueous solution while their charge transference interactions were also characterized in both media by using Natural Bond Orbitals (NBO) calculations.

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Study of oxygen binding in *Mycobacterium tuberculosis* TrHbO when interacting with lipid bilayers

Bringas M[a], Estrin D[a], Capece L[a]
a - Departamento de Química Inorgánica, Analítica y Química Física, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires / INQUIMAE-CONICET

Truncated hemoglobin O (TrHbO) of *Mycobacterium tuberculosis* (*Mt*) is a small heme protein that is known to be vital in the microorganism's capacity to resist microaerobic conditions; GlbO knockout Mt is not able to survive under hypoxic stress. This globin exists mostly as a dimer in solution, and it is able to perform an autokinase activity under low oxygen concentration conditions. The phosphorylation of this protein, which occurs specifically to Thr103, shifts the dimerization equilibrium and favours the monomeric form. The monomeric form of TrHbO is able to interact with lipid bilayers. The association is attributed to electrostatic interactions, provided by multiple arginine residues located in the EF loop and the F helix.

The molecular basis of Mt resistance to hypoxia and the role that TrHbO plays remain unknown. Our aim is to describe both computationally and experimentally the interaction between monomeric phosphorylated TrHbO of Mt with negatively charged membranes. We used Monte Carlo sampling to generate initial structures of the protein-membrane system. From these initial structures, we performed classical molecular dynamics simulations of the wt protein and relevant in silico mutants, to evaluate the conformational changes and mechanical stress that the negative surface can induce on the protein. The results from these simulations suggest that the interaction between the protein and the membrane, which occurs through the F helix -which includes the proximal histidine- might produce a shift in the proximal histidine position and thus affect the O_2 affinity of the protein. We are at the moment performing hybrid QM/MM calculations to confirm this hipothesys.

On the experimental side, we are using monolamellar vesicles as models to study TrHbO in presence of negatively charge lipid membranes. For that purpose, resonant Raman spectroscopy measurements on the carbomonoxy complex will be performed, paying particular attention to the CO frequencies which are known to be particularly sensitive to different distal environment.

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A Molecular Dynamic approach to the differential interaction between unsaturated free fatty acids and the nicotinic acetylcholine receptor

Obiol D[a], Amundarain MJ[a], Jaure O[a], Zamarreño F[a], Antollini S[b], Costabel M[a]
a - Instituto de Física del Sur (IFISUR), Departamento de Física, Universidad Nacional del Sur (UNS), CONICET
b - Instituto de Investigaciones Bioquímicas de Bahía Blanca (INIBIBB). Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur (UNS- CONICET), Bahía Blanca, Argentina

Free fatty acids (FFAs) are important cellular components that increase under certain pathological conditions. One of the effects of FFAs is a protection mechanism through the modulation of ion channels. The activity of the nicotinic acetylcholine receptor (nAchR) is blocked by certain FFAs and its binding site is located at the lipid-protein interface. The objective of this work was to determine, by means of molecular dynamics (MD), the possible points of contact and the effect produced by five different FFAs, located in annular and non-annular sites. The study was carried out on a system composed by a model of AChR from Torpedo marmorata (PDB ID: 2BG9) that was elaborated from PDB ID: 4COF as a template because the e-values were the lowest for each subunit and because it represented the closed desensitized state. The evaluation of the stereochemical parameters for the refined model was improved with respect to 2BG9. The refined model was incorporated into a lipid bilayer composed with a ratio of 1:1:3 of cholesterol, POPA and POPC respectively. We replaced three phospholipids from the lipid bilayer with three of the corresponding free fatty acids: cis-18:1ω-6, cis-18:1ω-9, cis-18:1ω-11, cis-18:1ω-13 and trans-18:1ω-9 in annular or non-annular areas. From the MD runs we obtained the most statistically relevant conformations of each FFAs in each of the systems, we determined the possible contacts with residues of the nAChR and the resulting profile of pore radius. In general, the results show that more contacts are established when FFAs are located in non-annular regions. As expected, contacts are established in a much greater proportion with non-polar residues. cis-18:1ω-11 in non-annular sites leads to conformations that open the pore of the channel, while in annular sites it stabilizes the desensitized state. With cis-18:1ω-13 a similar behavior is observed, although in non-annular sites it produces a pronounced constriction in the extracellular domain.

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Assessing the One-Bond Cα−H Spin-Spin Coupling Constants in Proteins: Pros and Cons of Different Approaches

Arroyuelo A[a], Martin OA[a], Scheraga HA[b], Vila JA[a]
a - IMASL-CONICET, Universidad Nacional de San Luis
b - Baker Laboratory of Chemistry, Cornell University, Ithaca, NY, USA

In the present work we explore three different approaches for the computation of the one-bond spin-spin coupling constants (SSCC) 1JCαH in proteins: DFT calculation, Karplus-like equation and Gaussian Process regression. The main motivation of this work is to select the best method for a fast and accurate computation of the 1JCαH SSCC, for its use on everyday applications in protein structure validation, refinement and/or determination. Initially, we observed a poor agreement between the DFT computed 1JCαH SSCC values and the experimental ones. With a detailed analysis of the causes of this observation we ruled out the choice of functional, basis set, solvent effects and peptide-geometry, as possible sources of error. However, we found that the DFT computed 1JCαH SSCC values depend on both the ψ, φ and χ1 torsional angles and the environment of the peptide model used to compute it. All of which leads to the understanding that the model chosen for the DFT computations is inappropriate. Hence, we discuss different models for solving this issue as well as the non-transferability of said solutions. Additionally, we show that Karplus-like equation and Gaussian Process regression provide faster and more accurate results than DFT-based calculations.

Modeling of Rhodopsin R135L mutant (in)activation as causative of severe “night blindness”

Branda MM[a], **Guérin DMA**[b], Giorgetti A[c], Carloni P[d]
a - Department of Physics, Universidad Nacional de San Luis (UNSL), and Instituto de Física Aplicada (CONICET-UNSL). Av. Ejercito de los Andes 950, 5700 San Luis, Argentina.
b - Instituto Biofisika (CSIC, UPV/EHU) and Universidad del País Vasco, 48940 Leioa, Spain.
c - Dept. Biotechnology, University of Verona, Strada Le Grazie 15, I-37134 Verona, Italy.
d - DIAS-5/INM-9: Computational Biomedicine ± Institute for Advanced Simulation (IAS) /Institute of Neuroscience and Medicine (INM), Forschungszentrum Jülich, Jülich, Germany

Rhodopsin (RHO) is the main prototypical protein of a large family called G-protein coupled receptors (GCPC). RHO is a membrane-bound protein found in disks of retina rod cells, can associate as dimers or bigger homo-oligomers, and its 3-D structure consists of a 7-helix bundle that spans the membrane. A large number of RHO genetic mutations may lead to very severe retinal dysfunction and eventually to impaired dark adaptation (“night blindness”). Elucidating the mutations role in hindering RHO to reaching the active state is key towards understanding the signal transduction mechanism in GPCRs. In this work we use an *all atom*Molecular Dynamic (MD) simulation to model the active state structure of wt bovine RHO and the mutant R135L, both of them in the dimeric form. For this analysis, the protein was embedded into an explicit atom solvated membrane (POPC). A comparative analysis of the 200ns MD run on both models shows that mutant trajectories deviate from those of wt RHO. One parameter that account for these differences is the distance between helix 3 (TM3) and 6 (TM6) at the cytoplasmic side. Another indication of the structural discrepancy is the relative orientation of the monomers. These results are employed to predict the effect these changes could have in binding G-protein, the signaling pathway event following RHO's light-activation. Our conclusion is that this single mutation alters wt RHO structure enough to impede a functional coupling with the G-protein.

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Partition of indocyanine green in model polerized biological membranes: computational simulations approach

Sosa M[a], Del Pópolo M[a], Galassi V[a]
a - Facultad de Ciencias Exactas y Naturales (FCEN), UNCUYO, Mendoza, Argentina

One of the key features of life development is the selective exchange of ions through cell membrane, which gives rise to the so called “membrane potential”. This electrostatic potential is determinant of the interfacial activity regulation, providing the driving force for many fundamental processes such as cell breathing, cardiac activity and nervous synapse. The classical determination methods are inconveniently invasive or of very low resolution. Much effort is currently invested on developing non-electrofisiologic determination methods, based on the use of voltage sensitive dyes (VSD) with electrochromic response.

In this context, we have addressed a detailed computational study of a novel probe: indocyanine green (ICG). This CSV is characterized by its optical activity which lies in the near infrared spectrum. It has also been approved by the FDA for human clinical use. These peculiarities turns ICG into a highly promising molecule for imaging diagnosis, being possible to irradiate and detect through a thin layer of skin, implying lower invasiveness than any other known CSV.

In this work we show a computational study of the interactions of ICG with polarized lipid bilayers of palmitoyloleoylphosphatidylcholine. We parameterized an atomistic force field for the probe, and built model systems with the dye integrated in the interphase. We approached the free energy of partition through umbrella sampling calculations, combined with weighted histogram analysis method (WHAM). The computation of the potential of mean force profile was carried out, for the sake of comparison, in two different systems: one without membrane polarization, and another with ions imbalance such that the transmembrane potential was 1.9V. We analyzed the effect of the polarization on binding energetics and configuration.

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Structural properties of apolipoprotein A1 associated with evolutionary constraints

Masson T[a], Tricerri MA[b], Ramella NA[b], Gisonno RA[b]
a - Instituto de Biotecnología y Biología Molecular (IBBM, UNLP-CONICET)
b - Instituto de Investigaciones Bioquímicas de La Plata (INIBIOLP), CCT- La Plata, CONICET. Facultad de Ciencias Médicas. Universidad Nacional de La Plata.

Apolipoprotein A-I (APOA1) is the main protein of plasma high-density lipoproteins. It is involved in removing excess of cell cholesterol and protect against atherosclerosis. Extensive research supports that the cardio protection attributed to the protein is due to its active role in the reverse cholesterol transport pathway and in the protection against endothelial dysfunction. Several apoA-I mutants have been identified as pathological in familial systemic amyloidosis, with different tissue-specific deposition. Most of the mutations described in patients occur within two “hot spots” in the protein sequence: the N-terminus (residues 26-100) and a short C-terminal sequence (residues 173-178). This tendency highlights the deep coupling between protein sequence and phenotype exhibited by the different mutants.

At the interface of biophysics and molecular evolution there is a set of fundamental processes that shape the molecular function of proteins. Different sites and sectors within a protein sequence evolve at different rates; this evolutionary rate heterogeneity across protein site is useful to study the interplay of functional and structural constraints. Moreover, co-evolution between residues in a protein is a fundamental component for understanding the complex evolutionary design of proteins.

In this work we aimed to deepen our understanding of APOA1 structural design throughout the interrogation of its molecular evolution processes in vertebrate organisms. Our approach involved the phylogenetic reconstruction of APOA1 evolutionary history and the estimation of the amino acid substitution rates across the protein sequence. We also employed multiple sequence alignments (MSA) to calculate residues co-evolution patterns using Direct Coupling Analysis (DCA) and GREMLIN (Generative REgularized ModeLs of proteINs). Our data suggest novel functionally relevant residues and co-evolving pairs inside APOA1 structure, which could help understanding the molecular principles protein aggregation in pathological amyloidosis.

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Theoretical study of the elimination reaction of hydrogen peroxide by Allyl Methyl Sulfide and Diallyl Methyl sulfide, two garlic compounds

Diaz M[a], Vega Hissi E[a], Andrada M[b], Garro Martinez J[a]
a - Facultad de Química Bioquímica y Farmacia, Universidad Nacional de San Luis, Chacabuco 915, 5700 San Luis, Argentina. IMIBIO-SL (CONICET), Chacabuco 915, 5700 San Luis, Argentina
b - Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis, Chacabuco 915, 5700, San Luis, Argentina

The organosulfide compounds are organic species that contain one or more sulfur atoms in its structure. The garlic (Allium *sativum*) have a large number of organosulfide compounds that show different properties. There is evidence of the antiviral, antibacterial, and antiparasitic activity of these compounds. In addition, these compounds decrease the cholesterol levels, inhibit platelet aggregation and tumor growth. Among them, allyl-methyl sulfide (AMS) and diallyl-methyl sulfide (DMS) show a special interest as antioxidant agents. The current investigations on antioxidant species that prevent the oxidative stress, are particularly centered on the reduction of the well known oxygen reactive species (ROS) such as superoxide anion, hydroxyl radical and hydrogen peroxide, that can cause biological damage.

In this work, we carried out theoretical quantum studies of the thermodynamic and kinetic aspects of the elimination reaction of hydrogen peroxide by AMS and DMS.

A conformational analysis and the calculation of Fukui functions allowed the selection of the initial structures of AMS and DMS and the target region where these organosulfide compounds can receive a nucleophilic attack by hydrogen peroxide, respectively. We analyzed three pathways: 1) attack at the sulfide atom through an oxidation reaction; 2) attack over the vinyl group carbon atoms, performing an epoxidation reaction and 3) attack over the vinyl carbon atoms by a hydrogenation reaction. Intrinsic reaction coordinate calculations (IRC) confirmed the transition states proposed and provided the energy profile of the each pathway.

We found that the three reactions analyzed are thermodynamically feasible whereas the sulfur atom oxidation reaction is the pathway kinetically favored.

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Instituto Multidisciplinario de Investigaciones Biológicas (IMIBIO-SL) and Facultad de Química, Bioquímica y Farmacia, UNSL, San Luis.

Triggering Doxorubicin Release from pH-responsive Hydrogels Using the Concentration of Polyamines

Pérez Chávez NA[a], Longo G[a]
a - cInstituto de Investigaciones Fisicoquímicas Teóricas y Aplicadas (INIFTA), CCT- La Plata, CONICET. Universidad Nacional de La Plata.

Near some cancer cells the concentration polyamines is relatively higher than around healthy cells. In this work, we explore the concept a functional biomaterial that can sense polyamine concentration and deliver a therapeutic drug. We develop and apply a molecular theory to describe the adsorption/desorption of doxorubicin to crosslinked poly(methacrylic acid) (PMAA) films in presence of different concentrations of putrescine, spermidine and spermine. Our method allows for a systematic study of the problem, including the effect of pH, salt, amine and drug concentration. Preliminary results show that these hydrogel films have potential as functional biomaterials with the ability to administrate drugs resulting from the stimuli of polyamine concentrations.

8,9-disubstituted pyrrolo[2,1-*a*]isoquinolin-3-ones derivatives, a new series of AChE and BChE inhibitors. Design, synthesis and biological evaluation.

Parravicini O[a], Garro A[a], Gutiérrez L[a], Garibotto F[a], **Andujar S**[a], Cabedo N[b], Enriz R[a]
a - Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis; Instituto Multidisciplinario de Investigaciones Biológicas (IMIBIO-SL-CONICET).
b - Departamento de Farmacología, Facultad de Farmacia, Universidad de Valencia

We have previously reported the synthesis of 8-substituted and 8,9-disubstituted pyrrolo[2,1-*a*]isoquinolinones derivatives[1]. Some of these structures have a slight structural resemblance with rivastigmine, a reversible cholinesterase inhibitor that is used to treat dementia. On the other hand we have recently reported new AChE inhibitors[2] and we have conducted molecular modeling studies that allowed us to understand in some detail the molecular interactions involved in the stabilization of different inhibitor-enzyme complexes for this molecular target. Taking advantage of this information, we asked ourselves if we would be able to design a new structure with inhibitory effects of AChE, taking the pyrroloisoquinoline moiety as starting point.

We conducted a molecular modeling study in four steps. In the first step we carried out a docking study; in the second one we performed simulations using molecular dynamics calculations. With these data, we performed a per-residue analysis and, in the last step, quantum mechanics calculations were made in order to evaluate in details the molecular interactions that stabilize the different ligand-receptor complexes. Based on our molecular modeling study we designed two new structures: 8-chloro-9-phenylcarbamate-1,2,3,5,6,10b-hexahydropyrrolo[2,1-*a*]isoquinolin-3-one and 8,9-diethylcarbamate-1,2,3,5,6,10b-hexahydropyrrolo[2,1-*a*]isoquinolin-3-one. The synthesis of both compounds was carried out and then their inhibitory activities were evaluated. As our simulations predicted both carbamates showed a remarkable inhibitory effect against both AChE and BChE. In fact, these compounds displayed stronger activity than rivastigmine, the compound used as reference.

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Design of new quinolin-2-one-pyrimidine hybrids as sphingosine kinases inhibitors

Vettorazzi M[a], Gutiérrez L[a], Zarycz N[a], Tosso R[a], **Andujar S**[a], Cobo J[b], Enriz R[a]
a - Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis; Instituto Multidisciplinario de Investigaciones Biológicas (IMIBIO-SL-CONICET).
b - Inorganic and Organic Department, University of Jaén

Sphingosine-1-phosphate is now emerging as an important player in cancer, inflammation, autoimmune, neurological and cardiovascular disorders. Abundance evidence in animal and humans cancer models has shown that SphK1 is linked to cancer. Thus, there is a great interest in development new SphK1 inhibitors as a potential new treatment for cancer. In a search for new SphK1 inhibitors we selected the well-known SKI-II inhibitor as the starting structure and we synthesized a new inhibitor structurally related to SKI-II with a significant but moderate inhibitory effect. In a second approach, based on our molecular modeling results, we designed new structures based on the structure of PF-543, the most potent known SphK1 inhibitor. Using this approach we report the design, synthesis and biological evaluation of a new series of compounds with inhibitory activity against both SphK1 and SphK2. These new inhibitors were obtained incorporating new connecting chains between their polar heads and hydrophobic tails.

On the other hand the combined techniques of molecular dynamics simulations and QTAIM calculations provided complete and detailed information about the molecular interactions that stabilize the different complexes of these new inhibitors with the active sites of the SphK1. This information will be useful in the design of new SphK inhibitors.

Hidroxynaphthalenecarboxamides and substituted piperazinylpropandiols, two new series of BRAF inhibitors. A theoretical and experimental study.

Campos L[a], **Garibotto F**[a], Vettorazzi M[a], Angelina E[b], Alvarez S[a], Enriz R[a]
a - Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis; Instituto Multidisciplinario de Investigaciones Biológicas (IMIBIO-SL-CONICET).
b - Laboratorio de Estructura Molecular y Propiedades, Área de Química Física, Departamento de Química, Facultad de Ciencias Exactas y Naturales y Agrimensura, Universidad Nacional del Nordeste

Recently we reported two new structural scaffolds as potential inhibitors of BRAF[1]; both series of compounds were studied in greater depth in the present work. Our results indicate that the new substituted piperazinylpropandiols derivatives evaluated here do not show significantly better activities to that previously reported for the structure chosen as starting structure. In contrast the results obtained for the other series were more positive. We report now new hydroxynaphthalenecarboxamides with significant inhibitory activity on BRAF. In order to better understand these experimental results, we carried out a molecular modeling study using different combined techniques. While the simulations using simple techniques such as docking and DM simulations allowed us to explain which the best structural scaffold, these results is do not allow to explain why compounds with substituents in different spatial positions have similar inhibitory effects. In this sense, the use of MD/QTAIM combined calculations allow to explain in detail the molecular interactions that stabilize the different molecular complexes reported here.

Another interesting contribution of this study is that the different molecular interactions that stabilize the complexes have been analyzed in depth. The QTAIM results indicate that the different spatial dispositions of the substituents (*ortho, meta* and *para*) allow to establish alternative interactions with Asp594 or with Lis483 depending on their spatial arrangement. This result is in agreement to those observed for vemurafenib and dabrafenib. This structural information is important for the design of new inhibitors with this type of structural scaffold.

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Biophysical analysis of the interaction between B30.2 domain of GNIP/TRIM7 and Glycogenin

Muñoz Sosa C[a], Carrizo ME[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 initiation of glycogen biosynthesis involves the self-glucosylation of glycogenin, that synthetizes a maltosaccharide upon which glycogen synthase and branching enzyme continue glucose polymerization. In a yeast two-hybrid screen for proteins that interact with glycogenin, a protein designated GNIP (Glycogenin Interacting Protein) was isolated. GNIP gene generates at least four isoforms, the largest of which (GNIP1) is predicted to have an N-terminal RING domain, a B-box domain, a coiled-coil region (RBCC motif); and a C-terminal B30.2 domain. The GNIP2 isoform, containing the B30.2 domain and a segment of the coiled-coil region, was found to stimulate glycogenin self-glucosylation *in vitro* while the B30.2 domain ($GNIP^{B30.2}$) was reported to be necessary for the interaction with the enzyme.

In the last few years, GNIP has also been named TRIM7 (Tripartite motif containing protein 7) and, as many members of the Trim protein family, has shown to have ubiquitin ligase activity and to be capable of autoubiquitination, with GNIP/$TRIM7^{B30.2}$ having an essential role in this function.

Very recently, we have solved the three-dimensional structure of human GNIP/$TRIM7^{B30.2}$ in two different crystal forms (at 1.6 Å and 1.8 Å resolution). Now, in order to establish the region responsible for the interaction with glycogenin, we have prepared GNIP/$TRIM7^{B30.2}$ mutants in residues selected from the analysis of its structure and the sequence conservation between several B30.2 domains. Here we show preliminary results, which suggest two of these amino acids would be involved in the interaction between GNIP/$TRIM7^{B30.2}$ and human glycogenin.

Coarse grained analysis of the connection between stability, flexibility and foldability of Adenylate Kinase variants from extremophile organisms

Safranchik M[a], Roman EA[b,c], Iglesias Rando MR[a], Craig PO[a]
a - Departamento de Química Biológica, FCEyN, UBA - IQUIBICEN, CONICET
b - IQUIFIB-Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, UBA. Junin 956, 1113 Ciudad de Buenos Aires
c - Laboratorio de Fisiología de Proteínas, IQUIBICEN y Facultad de Ciencias Exactas y Naturales, UBA, Buenos Aires, Argentina

The study of homologous proteins from extremophile organisms represents an excellent system to evaluate the functional dynamics of proteins and their dependence on temperature. In this work we develop a bioinformatic analysis method to evaluate how the stability and conformational flexibility of extremophile variants is related to their foldability and energy frustration. To evaluate the quality of the molecular interactions of the protein variants we used the coarse grained force field of the AWSEM program. The use of this force field also allow us to perform folding simulations of the proteins, and evaluate the thermodynamic profiles of their landscape. The stability, flexibility and foldability analysis of the protein variants was done using two different approaches: 1) An approximate and fast approach using the frustratometer-2.0 program, which is adaptable to the analysis of large databases; 2) An specific and more accurate approach using coarse-grained computer simulations using the program AWSEM-MD. Here we present the results obtained for adenylate kinase homologs from psychrophilic (Bacillus globisporus), mesophilic (Bacillus subtilis) and thermophilic (Bacillus stearothermophilus) organisms. The results illustrate the challenges of this analysis and suggest possible explanations for the expected necessity of improving foldability and flexibility of proteins adapted to low temperatures.

Conformational selective binding of metal fluoride complexes to the Plasma Membrane Ca^{2+}-ATPase and structure stabilization.

Saffioti NA[a], **Riesco AS**[a], de Sautu M[a], Rossi RC[a], Berlin J[b], Ferreira-Gomes MS[a], Rossi JP[a], Mangialavori I[a]

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

b - Department of Pharmacology, Physiology and Neuroscience, New Jersey Medical School, Rutgers University, Newark, NJ, 07103, USA.

The Plasma Membrane Ca^{2+} ATPase (PMCA) is a key participant in cytoplasmic Ca^{2+} regulation. As others P type ATPases, PMCA transports Ca^{2+} by the Albers and Post mechanism. In this, proteins have two main conformations, E_1 and E_2, and phosphorylate from ATP to transport ions against their electrochemical gradient. Recently, we demonstrated that fluoride complexes of aluminium (AlF_x), beryllium (BeF_x) and magnesium (MgF_x) stabilize conformations very similar to the phosphorylated intermediate of PMCA[1].

The aim of this work is to understand what conformational changes occur upon the phosphorylation of PMCA. To this end, we measured the fluorescence of the probe eosin, which binds with high affinity to the nucleotide binding site of PMCA. When the protein binds fluoride complexes, eosin fluorescence decreases, and the same effect is seen when the pump phosphorylates from ATP in presence of Ca^{2+}. However, during transport activity, part of the fluorescence decrease is due to displacement of eosin by ADP from its binding site. Our results show that the ADP and ATP affinity for PMCA in E_2 conformation are 172 ± 28 and 74 ± 17 μM, respectively. The binding of fluoride complexes to PMCA depended strictly on Mg^{2+} concentration. Furthermore, these fluoride complexes only binds to PMCA-Mg^{2+}. This property allowed us to measure the PMCA affinity for Mg^{2+} (2,2 ± 0,4 mM) in E_2 conformation. Finally, we studied thermal stability of PMCA by measuring the enzyme activity as a function of time. Fluoride complexes stabilize PMCA structure and delay activity loss when the protein is incubated at 44°C compared to the free protein. We can conclude that fluoride complexes are ligands of PMCA-Mg^{2+} complex, they set PMCA structure in a conformation analogous to E_2-P, stabilizing it. This property allows to study the PMCA structure in E_2 conformations.

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Cu(I) transport ATPases adapted to different temperatures

Burgardt N[a], Recoulat AA[a], Melian NA[a], Sabeckis L[a], Placenti MA[a], Bredeston L[a], González Flecha L[a]

a - CONICET – Universidad de Buenos Aires. Instituto de Química y Fisicoquímica Biológicas "Prof. Alejandro C. Paladini" (IQUIFIB). Buenos Aires, Argentina.

PIB-1-ATPases, also known as CopA,are integral membrane proteins that actively transport Cu(I) ions through cell membrane coupled to ATP hydrolysis. Their function is essential for intracellular copper homeostasis, so they are widespread distributed in nature. The structure of these proteins consists of four characteristic domains: the heavy metal associated domain (MBD), the catalytic domain (CD), the actuator domain (AD), and the transmembrane (TM) region constituted by eight α-helices. Also, they have several conserved sequence motifs: the phosphorylation site (DKTG), the metal binding site (CXXC), the transmembrane metal binding site (CPC), and some invariable residues of the transmembrane α-helices. In this work we present a comparative characterization of CopAs from the psychrotolerant bacterium B. argentinensis (BaCopA), the mesophilic bacterium L. pneumophila (LpCopA) and the thermophilic archaea A. fulgidus (AfCopA). These proteins have more than 70% similarity and more than 40% identity between pairs, being the soluble metal binding domains the ones with the lowest similarity. The highest identity regions are found in the typical conserved motifs of P-ATPases and in the transmembrane α-helices TM4, TM5 and TM6, where the PIB-1 subgroup conserved residues and the transmembrane Cu(I) site are located. BaCopA (840 aa) has the larger size, followed by AfCopA (804 aa) and finally LpCopA (736 aa). However, the size excluding the MBD presents a different order: BaCopA (650 aa) > LpCopA (644 aa) > AfCopA (621 aa). The differences in size are located in loops between α-helices MA-MB / TM1-TM2 and in loops of the catalytic domains. A comparison of the intraprotein interactions observed in BaCopA (model), LpCopA (3RFU), and AfCopA (3J08) shows that thermophilic CopA have the higher amount of ionic interactions and the lower number of aromatic-aromatic interactions. This suggest that thermal adaptations might modulate the flexibility of these proteins by the length of those loops and the number of intraprotein interactions. Indeed, recombinant purified CopAs show optimal activity at different temperatures (BaCopA<LpCopA<AfCopA), but with similar affinities for ATP. This is the first step for understanding thermal adaptation mechanisms in this membrane protein family.

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Doxycycline neuroprotective effect over taupathies: a novel target for repurposing this antibiotic

Medina L[a,b,c], Vera CC[d], Parrales V[a], Bizat N[a], Raisman-Vozari R[a], Chehín R[b,c]
a - ICM - Institut du Cerveau et de la Moelle Epinière - Hôpital Pitié Salpêtrière
b - IMMCA (Instituto de Investigación en Medicina Molecular y Celular Aplicada)
c - INSIBIO (CONICET-UNT) Instituto Superior de Investigaciones Biológicas, Chacabuco 461 - San Miguel de Tucumán,CP 4000, Tucuman, Argentina
d - INBIONATEC, CONICET, National University of Santiago del Estero. 4206, RN 9 Km 1125, Santiago del Estero, Argentina

Tauopathies are a diverse group of neurodegenerative diseases characterized by a progressive deposition of abnormally phosphorylated tau protein aggregates, in characteristic brain regions. Tau can adopt multiple propagating conformers in vitro, nowadays called “strains". Based on doxycycline neuroprotective effect reported for a Parkinson's disease model, we explored whether doxycycline is able to interact with tau. For this reason, we studied if doxycycline was able to induce changes in tau amyloid aggregation process and phosphorylation patterns. The neuroprotective effect of doxycycline was evaluated on an in vivo models of tauopathy. For the in vitro assays, we used the heparin-induced tau fibrillation model; to perform Transmission Electron Microscopy (TEM), Thioflavin T fluorescence spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), and protease digestion of aggregated tau species; in the presence and in the absence of doxycycline. We performed in vitro phosphorylation of tau using Glycogen synthase kinase-3 beta (GSK3-beta). To evaluate doxycycline neuroprotective effect we used an animal and a cellular tauopathy model: *C. elegans* expressing human tau and SH SY5Y with a P301L mutation, respectively.

Our results show that doxycycline interacts with tau inducing the formation of morphologically differentiated species, observed by TEM. These novel species display different beta-sheet structural arrangement according to FTIR studies and have different protease digestion pattern. Doxycycline does not interfere on the GSK3 beta activity, as tau phosphorylation pattern remains the same. Our data demonstrate that doxycycline could revert the low mobility phenotype induced by the over expression of human tau by the nematode C. elegans. Our results reveal that doxycycline shows a neuroprotective effect over a *C. elegans* tauopathy model, and the mechanism of toxicity reduction would be the interference over tau amyloid fibrillogenesis. The presence of doxycycline might induce a novel and less toxic tau aggregation strain, making this antibiotic a good candidate to be repurposed as neuroprotector for tauopathies.

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Effect of PEG-induced molecular crowding on β-Gal thermal stability.

Rodriguez C[a], Perillo MA[a], **Nolan MV**[a]
a - IIBYT (CONICET - ICTA y Cátedra de Química Biológica, FCEFyN, Universidad Nacional de Córdoba)

The yeast β-galactosidase or lactase [EC 3.2.1.23] (β-Gal) is a soluble enzyme capable of catalyzing lactose hydrolysis into its constitutive monosaccharides: glucose and galactose. This enzyme has a commercial application for lactose hydrolysis in dairy products. Milk processing with b-Gal before milk is commercialized is important to solve nutritional (lactose intolerance) and technological (crystallization of dairy products) problems. In this context, it is important that the activity of β-Gal be evaluated in crowding media systems.

In this work we investigate the effect that molecular crowding induces on thermal stability of β-galactosidase from Kluyveromices lactis. PEG^{6000}, a non-charged highly water-soluble polymer with well-known effects on water dynamics was used to produce the crowded environment.

The effect of PEG on β-Gal thermal stability was studied with two different approaches. In the first one, β-Gal samples both in the absence or in the presence of PEG^{6000} were pre-incubated at different temperatures in a range from 37 to 75 °C. After that, the system was returned to optimal conditions and enzymatic activity was tested. Results obtained showed that β-Gal stability was enhanced in molecular crowded environment. The enzyme maintained its activity when it was pre-incubated at temperatures 5 degrees higher in the presence than in the absence of molecular crowding agent.

In the second approach, the inactivation kinetic was studied: in this type of experiments, the enzyme was pre-incubated at 37 or at 50 °C during different periods of time and after that, the enzymatic activity was measured in optimal conditions. Results obtained show again that molecular crowding conditions protect the enzyme from heat denaturation. In this case, it was observed that the enzyme maintains its activity even when it is subjected for a considerable period of time at high temperature when it is in the presence of the molecular crowding agent.

In both cases, the enzymatic reaction was evaluated by measuring kinetic parameters of β-Gal against an artificial substrate (ONPG).

Changes in protein compactness could be the responsible for the qualitative change behavior observed at the molecular crowding conditions assayed.

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Exploring Human Mitochondrial NFS1 Cysteine Desulfurase Structure-Function Relationships

Sewell E[a], Aran M[b,c], Capece L[d], Santos J[a]
a - Instituto de Química y Fisicoquímica Biológicas Dr. Alejandro Paladini (IQUIFIB-CONICET) Universidad de Buenos Aires
b - Fundación Instituto Leloir, IIBBA-CONICET
c - Plataforma Argentina de Biología Estructural y Metabolómica PLABEM
d - Departamento de Química Inorgánica, Analítica y Química Física, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires / INQUIMAE-CONICET

Iron-sulfur cluster biosynthesis is a complex mitochondrial process that requires a supercomplex formed by five different proteins NFS1, ACP, ISD11, ISCU and FXN. Homodimerization of NFS1 is crucial for its PLP-dependent Cys desulfurase activity. Moreover, the supercomplex is an heterodecamer (two subunits of each protein). ACP-ISD11 heterodimer stabilizes the formation of the NFS1 dimer, whereas FXN produces a strong positive modulation of the enzymatic activity by stimulating -SH group transfer from NFS1 active site to ISCU. In this process, a very conserved loop of NFS1 plays a key-role, presumably establishing communication between the NFS1 and ISCU active sites. We analyzed loop motions by means of molecular dynamics simulations of the supercomplex core NFS1/ACP-ISD11, and we designed loop mutants that modulate the flexibility of this element to explore structure-dynamics-function relationships. Additionally, a double mutant of NFS1 containing a single tryptophan residue (NFS1W97) was prepared. This variant was significantly more sensitive to trypsin, suggesting an increased flexibility. This mutation eliminates a C-terminal Trp that is presumably important for inter-subunit interactions. Accordingly, NFS1W97 exhibited reduced enzymatic activity assessed by measuring [H2S] and [Ala].These results suggest that the function of the supercomplex is governed by intricate relationships between structure and dynamics.

Exploring the surface of the intrinsically disordered protein (IDP) alpha synuclein (AS)

Rial Hawila MR[a], Gómez GE[a], Binolfi A[b], Delfino JM[a]
a - Departamento de Química Biológica e IQUIFIB (UBA-CONICET), Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires
b - Instituto de Biología Molecular y Celular de Rosario, IBR-CONICET-UNR, Rosario, Argentina

The nature and size of the accessible surface area (SASA) of the polypeptide chain plays a pivotal role in protein folding and complex formation. To investigate SASA, we employ diazirine (DZN), a minute precursor of the extremely reactive methylene carbene ($:CH_2$). Methylation signatures left on the polypeptide provide telltale clues on conformation and interactions. The extent of methylation metric (EM) derived directly from mass spectra (ESI-MS or MALDI-TOF) is able to discriminate between native and alternate states. The IDP human alpha synuclein (AS) aggregates into oligomers and amyloid fibrils, constituents of Lewy bodies, a cytosolic hallmark of Parkinson disease. DZN labeling proves particularly fit to analyzing the conformational plasticity inherent to this protein. Unlike well-structured proteins where the methylation signal differs strikingly between native and unfolded states, AS in buffer or equilibrated in 6 M GdmCl displays a similarly enhanced EM value, pointing to the high solvent exposure of AS under normal physiological conditions. Interestingly, compaction of monomeric AS due to calcium binding yields a somewhat decreased EM value. Most remarkably, AS fibrils cause a larger fall in the EM value, a consequence of surface occlusion at an interface. Tryptic fragmentation of AS coupled to MALDI-TOF analysis is revealing methylation patterns at increased resolution. The peptide coverage achieved permits to build a solvent-accessibility sequence profile. Independently, the feasibility to detect methylated products by multidimensional NMR is an approach that does not demand cleavage of the polypeptide, opening a potentially rich source of conformational information. The extent of reaction of ($:CH_2$) at various sites across the surface of AS could be defined by $^{1}H^{15}N$-HSQC spectra. This information illuminates the role played by the constituent parts in the monomeric ensemble as well as the changes observed en route to the fibrillar aggregate.

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

Di Nardo L[a], Val D[b], Castelli ME[b], Rasia R[a]
a - Instituto de Biología Molecular y Celular de Rosario, IBR-CONICET-UNR, Rosario, Argentina
b - Instituto de Procesos Biotecnológicos y Químicos (IPROBYQ-CONICET)

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

Here we present information on metal off-rate constant of the two variants as well as on structural destabilization by denaturants and temperature. We use the methyl and amide/indole fingerprint regions of the ^{1}H NMR spectrum of the protein to follow the processes. A group of signals between -0.25 and -0.32 ppm, among which presumably are the methyl groups of A54 and of I17, shift considerably being useful probes of active site integrity. The near UV CD of the protein shows significant changes between the apo and holo enzyme, as well as upon unfolding. Similar studies on new variants of the protein will help rationalize the effect of the remote mutations present in the industrial enzyme variants on their improvement relative to Bc PLC.

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NTPase activity of Zika virus NS3 helicase: study of the catalytic site of hydrolysis for NTPs and modulation by ssRNA

Mikkelsen E[a], Gebhard L[b], Cababie LA[a], Incicco JJ[a], Rossi RC[a], Gamarnik A[c], Kaufman S[a]
a - Departamento de Química Biológica e IQUIFIB (UBA-CONICET), Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires
b - Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes-CONICET
c - Instituto de Investigaciones Bioquímicas de Buenos Aires (IIBBA-CONICET) y Fundación Instituto Leloir

Zika virus (ZIKV) is a member of the virus family *Flaviviridae* that is responsible for human disease [1]. It's RNA genome encodes structural and non-structural proteins. Among the latter, non-structural protein 3 (NS3) possesses helicase activity [2] and is essential for viral replication [3]. Helicases are molecular motors that couple the mechanical work of unwinding doubled-stranded nucleic acids to the hydrolysis of nucleoside triphosphates (NTPs).

In previous work we established the ability of Zika virus NS3 to catalyze the hydrolysis of four NTPs: ATP, CTP, GTP and UTP and determined the specificity order for these nucleotides. In this work we show that these four nucleotides are hydrolyzed by the enzyme in a single and same site. To address this issue we made use of the fact that if two different substrates yield the same product (as in the present case, where orthophosphate is produced from the four nucleotides) it is possible to distinguish between a kinetic model of competition for the same site from others of multiple catalytic sites by measuring the steady-state velocity of product formation in mixtures of both substrates.

Additionally we studied the effect of single-stranded RNA (ssRNA) on ATPase activity. To this end we performed measurements of ATPase activity in the presence of homopolyribonucleotide polyA. We obtained substrate curves for ATP in the presence of different concentrations of polyA, these curves were well described by equilateral hyperbolas characterized by parameters k_{cat} and $K_{M.}$ The results show an increase in both the turnover rate constant k_{cat} and in K_M for ATP in the presence of polyA.

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Nucleotide-dependent dynamics of the Dengue NS3 helicase

Sarto C[a,b], Kaufman S[c], Estrin D[b], Di Lella S[a], Arrar M[b]
a - Departamento de Química Biológica, FCEyN, UBA - IQUIBICEN, CONICET
b - Departamento de Química Inorgánica, Analítica y Química Física, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires / INQUIMAE-CONICET
c - Instituto de Química y Fisicoquímica Biológicas y Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires-CONICET

Dengue represents a substantial public health burden, particularly in low-resource countries. Non-structural protein 3 is a multifunctional protein critical in the virus life cycle that has been identified as a promising anti-viral drug target. Despite recent crystallographic studies of the DENV NS3 helicase domain (NS3hel), no structural nucleotide-dependent differences have been identified, such that the coupled ATPase and helicase activities of the NS3hel remain mechanistically unclear. Here we use molecular dynamics simulations to explore the nucleotide-dependent conformational landscape of the DENV NS3 helicase and identify substantial changes in the protein flexibility during the ATP hydrolysis cycle. We relate these changes, including a novel open-loop conformation in the absence of ATP, to the specific RNA-protein interactions and proposed translocation models for other monomeric helicases.

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Production of artificial cellulosome to improve lignocellulose degradation: a step towards clean energy

Iglesias Rando MR[a], Gorojovsky N[a], Goldbaum FA[b], Craig PO[a]
a - Departamento de Química Biológica, FCEyN, UBA - IQUIBICEN, CONICET
b - Fundación Instituto Leloir

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 this process. 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. However, the biological production of artificial cellulosomes on an industrial scale has serious limitations. Our goal is to develop artificial cellulosomes using an oligomeric protein scaffold that is highly stable and highly expressed in bacteria for the co-localization 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 cohesin and dockerin modules, complementary fused to the scaffold subunits and the target proteins, respectively. In this work, we focus on the structural and functional characterization of a monospecific cellulosome composed by an endoglucanase. The polymeric display of the endoglucanase on the structural scaffold produce a significant increment in the degradation rate of cellulose compared to the free enzyme. This phenomenon seems to be related to an increase in the binding strength of the multienzymatic complex to the substrate due to an avidity effect. A significant increment in the thermal stability of the enzyme coupled to the scaffold compared to the free enzyme was also observed. It is expected that this technology would be valuable to improve lignocellulose degradation.

Study of correlation between protein sequence similarity and organisms optimal growth temperature.

Hernández Berthet AS[a], Aptekmann AA[b], González-Lebrero RM[c], Noguera ME[b,c], Roman EA[c,a]

a - Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales, Departamento de Química Biológica.

b - Departamento de Fisiología y Biología Molecular y Celular, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires. Instituto de Biociencias, Biotecnología y Biomedicina (iB3)

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

Structural and functional information of proteins are codified in their amino acid sequences. Within a protein family, there are proteins that come from different kinds of organisms: some growth at high temperatures (termophiles), some at middle (mesophiles) and others at low temperatures (psychrophiles). Usually occurs that optimal protein function temperature (OPFT) is close to the optimal growth temperature (OGT) of the organisms that come from. These behaviour is reflected in the sequence differences between orthologs that could affect their function, stability and conformational flexibility. But which of these are more associated to the OGTs? Is there any region more coupled than others? In this work we seek to study the sequences of an Archaea's database to compare the differences between these sequences and the OGT.

To this end, we aligned the sequences of Adenylate kinase, SPT5 elongation factor and Copper-exporting P-type ATPase protein families. Then we compared each possible pair of sequences and quantify their differences, using different substitution matrices. We made comparisons in windows of different size throughout the alignment, or taking positions near in space (using a representative structure of the family as a reference) or taking combinations of random positions of the alignment. Then we made a linear fit of the data and analyzed the correlation's coefficient (R) and Pearson's significance (p). We obtained values of R for each family and analyze those that outliers of more than two or three standard deviations from the average. We obtained positions of significant correlation for each family, and compared the correlation of these positions with bibliographic data.

We found that in the studied families are positions or combinations that have positive correlation with the organisms OGT. Our preliminary results suggest that upon refinement, this method may be used to predict the OGT of a given organism only from sequence data.

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Deleting amino acids 840 to 956 from the Spf1 P5-ATPase does not compromise function

Grenon P[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 biochemical properties of the group P5 on transport ATPases are much less characterized than those of other members of the family. In humans mutations in the P5-ATPases are associated with early onset Parkinson, Neuronal Ceroid Lipofuscinosis and Hereditary Spastic Paraplegia. The primary sequence of P5-ATPases suggests that, similar to other P-ATPases, the molecular architecture of the P5-ATPases consist of an Action (A), Phosphorylation (P), Nucleotide binding (N) and transmembrane (M) domains. P5-ATPases however, have a unique stretch of 60-120 amino acids at the c-terminal end of the P domain (so-called segment ctP) with no homology in other P-ATPases. Previous studies from our group have shown that the Spf1 from *Saccharomyces cerevisiae* is an active ATPase and forms the catalytic phosphoenzyme that characterizes the P-ATPases. With the aim of advancing the knowledge of the importance of the ctP segment we have used site-directed mutagenesis to obtain mutant (ΔctP)Spf1 in which the ctP segment is deleted. (ΔctP)Spf1 exhibited wild type levels of expression, ATP hydrolysis and phosphoenzyme formation. Moreover, (ΔctP)Spf1 was capable to rescue the caffeine sensitivity phenotype of KO Spf1 cells as well as the wild type Spf1. The results indicate that the Spf1 P5-ATPase can perform its basic functions without the need of the ΔctP segment.

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Does stability of the amyloid state depend on its biological activity?

Siri M[a], Herrera M[a], Moyano A[a], Celej MS[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

Proteins can self-assemble into a minimum-energy conformational fibrillar structure known as amyloid. Based on the observation that thermodynamic stability of amyloids formed from disease-related proteins decreases with the length of the polypeptide chain, Knolwes and colleagues (Knowles et al, 2014) proposed that nature optimized 300-500 residues proteins to prevent amyloid formation. The amyloid state however, is not always at the dark side of protein world since many organisms exploit them for functional purposes. Therefore, we challenged the hypothesis stated above by analyzing the thermodynamic stability of pathological, functional and generic amyloids.

We worked with four different proteins: FapC (355 residues), the main component of the functional amyloid identified in the opportunistic pathogen *Pseudomonas aeruginosa*; Hpa 1 (137 residues), that forms the functional amyloid in the plant pathogen *Xanthomonas axonopodis pv. citri*; human a-synuclein (140 residues), a neuronal presynaptic protein responsible for the pathologic amyloid fibrils involved in Parkinson's disease and human serum albumin (585 residues), the most abundant protein in blood that carries a variety of metabolites and does not form amyloids *in vivo*. The amyloids formed *in vitro* were characterized by Thioflavin T fluorescence, FTIR and electron microscopy. Then, their thermodynamic stabilities were evaluated by chemically induced equilibrium denaturation studies. The results will be discussed in the context of the role of the amyloid state in both health and disease.

Reference

Knowles, T. P., Vendruscolo, M., & Dobson, C. M. (2014). *Nature reviews Molecular cell biology*, *15*(6), 384.

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Effect of macromolecular crowding on FapC amyloid formation and stability

Herrera M[a], Siri M[a], Ardanaz E[a], Celej MS[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.

Amyloid is a unique protein quaternary structure formed by the self-assembly of monomeric units into a well-organized cross-b conformation. Although first discovered in the context of diseases, it is now recognized that amyloids can perform physiological functions in several organisms ranging from prokaryotes to humans. Bacteria employ functional amyloids to increase the mechanical robustness and chemical resistance of the biofilm extracellular matrix (EM), which is comprised of hydrated polysaccharides, extracellular DNA and proteins. In the opportunistic pathogen *Pseudonomas aeruginosa*, these amyloid fibrils are composed predominantly of FapC, a protein with high-sequence conservation among the genera. However, little is still known about the mechanism of FapC amyloid formation and its function in *P. aeruginosa* biofilm.

With the aim of understanding the role of FapC fibrils in this complex biological material, we investigated the influence of macromolecular crowding on its fibrillation kinetics and thermodynamic stability. Recombinant FapC from *P. aeruginosa* PAO1 was expressed in *E. coli*. Fibril formation was monitored by Thioflavin-T fluorescence and conformational stability was assessed by chemically induced denaturation equilibrium denaturation studies. The results will be compared with those obtained in diluted conditions. We hope to provide basic knowledge on the biophysical features of FapC amyloid fibrils, laying the foundation for development of new therapeutic interventions to combat biofilm infections.

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In vitro and *in vivo* beta-Gal self-assembling improve the enzymatic activity and stability

Flores SS[a], Clop PD[a], Perillo MA[a], **Nolan MV**[a], Sanchez JM[a]
a - IIBYT (CONICET - ICTA y Cátedra de Química Biológica, FCEFyN, Universidad Nacional de Córdoba)

In our laboratory we are interested in describing the functional plasticity of proteins in their self assembled states driven by oligomerization and/or aggregation.

The objective of this work is to study how the oligomeric/aggregated states of *E. coli* beta galactosidase (β-Gal) modulate the enzymatic activity with respect to the monomeric state.

We achieved self-assembled β-Gal by two strategies: *In vitro*; controlling the presence of $MgCl_2$ in the β-Gal solution, and *in vivo*: isolating aggregate protein in the form of inclusion bodies (IBs) from *E. coli*. The presence of oligomers and/or aggregates were evidenced by means of analytical ultracentrifugation and by quasi-elastic light scattering.

In all the cases the activity was evaluated using lactose as a substrate and quantifying glucose as the hydrolysis product. The reaction conditions were 37° C and pH 6.8 maintained by 0.1 M phosphate buffer. In all self-assembled states, we measured the kinetic parameters by non-linear regression of the experimental data using the minimum square method. Besides we also evaluated the optimum temperature and pH of the enzyme in each format.

We prove that Mg^{+2} is able to stabilize the tetrameric forms of the enzyme but not higher oligomeric states. We could produce and isolate functional IBs of β-Gal of around 1000 nm. We also demonstrate that the catalytic activity increases when the enzyme is self-assembled. On the other hand, studies of β-Gal activity against pH and temperature are similar in the absence or in the presence of $MgCl_2$. However, when we study the pH and temperature profiles of the β-Gal in IBs, we observe their higher stability compared to the soluble enzyme.

Our results contribute to highlighting the effect of protein-protein interaction on the protein functionality beyond the origin and dimension of the protein supramolecular structure.

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Folding of a natural variant of human apolipoprotein A-I associated with atherosclerosis. Micro environment and function.

Gisonno RA[a], Díaz Ludovico I[a], Tricerri MA[a], Ramella NA[a], Garda HA[a], Gonzalez MC[a]
a - Instituto de Investigaciones Bioquímicas de La Plata (INIBIOLP), CCT- La Plata, CONICET. Facultad de Ciencias Médicas. Universidad Nacional de La Plata.

Human apolipoprotein A-I, the major protein fraction of the High density lipoproteins (HDL) has long been considered a protective factor against the development of coronary heart disease. However, either the deletion of a residue (Lys 107) or a chronic pro-inflammatory scenario resulted in the deposition of the protein within atherosclerosis plaques and associated to amyloid deposits. The presence of protein aggregates in this landscape may suggest that a shift from the native conformation should result in a loss of function. To elucidate whether structural changes may be responsible for the protein deposition and misfunction, we compared by biophysical approaches the deletion mutant Lys107-0 structure with respect to the protein with the native sequence (Wt), either freshly resuspended or after controlled oxidation. A red shift in intrinsic Trp fluorescence and a higher binding of Bis ANS indicate a more flexible structure of this mutant, which preserves lipid binding capacity. Structural flexibility seems to be clue to this role, as a lower efficiency in intra-chain cross linking resulted in lower blockage of dimirystoyl phosphatidyl choline (DMPC) clearance.

In order to analyze the effect of oxidation on protein structure and function, Wt and Lys107-0 were oxidized and protein structure reevaluated. Trp and Met oxidation is detected by Mass Spec, and an increased tendency of the proteins to aggregate is observed by microscopy approaches, which is especially evident for the deletion mutant. Nevertheless, lipid clearance and solubilization is not significantly modified, indicating that the integrity of the salt bridge network involving polar residues in the central domain of the protein is not essential to this function. More research will be done to determine the importance of single residues in protein function.

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Kinetic and spectroscopic characterization of two flavodoxin-NADP(H) reductases from *Acinetobacter sp.* Ver3.

Palavecino-Nicotra MA[a], Bortolotti A[b], Sartorio MG[a], Cortez N[a]
a - Instituto de Biología Molecular y Celular de Rosario, IBR-CONICET-UNR, Rosario, Argentina
b - Departamento de microbiología. Facultad de Ciencias Bioquímicas y Farmacéuticas. Universidad Nacional de Rosario. CONICET.

Bacterial ferredoxin/flavodoxin NADP(H)-reductases **(FPR)** are monomeric flavoenzymes carrying non covalently bound **FAD** as cofactor. They are classified in two subclasses that differ in the C-terminus and have been involved in Fe-S clusters reparation after oxidative damage. Although most bacteria contain one of the two subclasses, some species carry both of them. The extremophile UV-resistant *Acinetobacter* sp. Ver3 has both isoenzymes, **FPR1$_{ver3}$** and **FPR2$_{ver3}$**. Our previous experiments proved that these enzymes respond differently to redox stimuli, suggesting distinctive cellular functions. Here we report a biophysical and biochemical characterization of these flavoenzymes. Variances in the FAD environment at the active site were detected using absorption and fluorescence spectra analysis, suggesting particular electronic arrangements for the flavin in each isoenzyme. Kinetic measurements under steady state conditions using a monoelectronic-artificial acceptor, showed a faster electron transfer in FPR1 compared to FPR2 ($kcat1$=79,3 ± 1.3 s^{-1} vs. $kcat2$=12.6 ± 0,1 s^{-1}). A slower transfer was obtained with a di-electronic artificial acceptor ($kcat1$=4.73 ± 0.1 s^{-1} vs. $kcat2$=2.2 ± 0,1 s^{-1}). We cloned, overexpressed and purified a putative redox partner of FPR, **Flavodoxin$_{ver3}$**. When recombinant flavodoxin was used as a protein acceptor, FPR2 had a faster electron transfer than the other enzyme ($kcat2$=1.3 s^{-1} vs. $kcat1$=0.5 s^{-1}). Our findins suggests that the different catalysis observed in these isoenzymes could be related to their specific electronic arrangements in FAD cofactor.

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

Di Nardo L[a], Val D[b], Castelli ME[b], Rasia R[a]
a - Instituto de Biología Molecular y Celular de Rosario, IBR-CONICET-UNR, Rosario, Argentina
b - Instituto de Procesos Biotecnológicos y Químicos (IPROBYQ-CONICET)

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

Here we present information on metal off-rate constant of the two variants as well as on structural destabilization by denaturants and temperature. We use the methyl and amide/indole fingerprint regions of the ^{1}H NMR spectrum of the protein to follow the processes. A group of signals between -0.25 and -0.32 ppm, among which presumably are the methyl groups of A54 and of I17, shift considerably being useful probes of active site integrity. The near UV CD of the protein shows significant changes between the apo and holo enzyme, as well as upon unfolding. Similar studies on new variants of the protein will help rationalize the effect of the remote mutations present in the industrial enzyme variants on their improvement relative to Bc PLC.

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Outline of an Experimental Design Aimed to Detect a Protein A Mirror Image in Solution

Martin OA[a], Vorobjev Y[b], Scheraga HA[c], Vila JA[a]
a - Instituto de Matemática Aplicada San Luis (IMASL), UNSL-CONICET, San Luis, Argentina.
b - Institute of Chemical Biology and Fundamental Medicine of the Siberian Branch of the Russian Academy of Science, Lavrentiev Avenue 8, Novosibirsk 630090, Russia
c - Baker Laboratory of Chemistry and Chemical Biology, Cornell University, Ithaca, NY 14853-1301

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 ~6.2, 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.

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Searching for a hidden intermediate. Thermal Denaturation and Aggregation propensity of Intestinal Fatty Acid Binding Protein (IFABP)

Curto LM[a], Ballatore M[a], Delfino JM[a]
a - Departamento de Química Biológica e IQUIFIB (UBA-CONICET), Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires

IFABP is a 15 kDa protein characterized by a β-barrel fold resembling a clamshell. It consists of two perpendicular five-stranded β-sheets (A-E and F-J) decorated with an intervening helix-turn-helix motif between strands A and B. The addition of TFE (2,2,2-trifluoroethanol) -a structure-promoting co-solvent- at high concentration (~25% v/v TFE) triggers the onset of amyloid-like aggregation. Previous work from our group revealed that, under equilibrium conditions, a low concentration of TFE (up to 15 % v/v) fosters conformational changes akin to those leading to aggregation-prone species. Most significantly, the protein remains functional as attested by its capacity to bind fatty acids. As retaining function is a signature of the native state, it was concluded that this level of co-solvent favors the population of alternative native-like conformations scarcely explored in water.

Interestingly, equilibrium unfolding transitions induced by guanidinium chloride (GdmCl) reveal that, in the presence of TFE, IFABP does not follow a simple two-step folding behavior. Indeed, TFE unveils the existence of intermediate species in IFABP (populated at ~ 1.25 M GdmCl), a classically recognized two-state protein model. As attested by far UV circular dichroism (CD), this hidden intermediate is rich in β structure, although different from that prevalent in native IFABP.

Despite the virtual absence of any structural change evident by far UV CD upon the addition of TFE at low concentration (5 % v/v at 25 °C), the protein becomes susceptible to irreversible thermal unfolding. In view of these facts, we propose that an increase in TFE concentration leads to the accretion in the population of the β-rich intermediate, thus leading to a decreased thermal stability. The main gist of this work points to discovering the connection between the conformational rearrangements exerted by TFE, leading to an imbalance in the population of intermediate species, and their relevance for the thermally induced aggregation propensity of the protein.

Structural characterization of the sensor/transductor protein MecR1 of the *Staphylococcus aureus* β-lactam resistance systems.

Fabbri C[a], Hermoso J[b], Llarrull L[a]
a - Instituto de Biología Molecular y Celular de Rosario, IBR-CONICET-UNR, Rosario, Argentina
b - Instituto de Química Física Rocasolano

Methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged as a globally important pathogen that is resistant to all classes of β-lactam antibiotics and some strains are also resistant to glycopeptides, the last resort antibiotics used to treat MRSA infections. Resistance to β-lactam antibiotics in MRSA is due to the inducible expression of an accessory transpeptidase, PBP2a, with low affinity for most β-lactams and of the serin-β-lactamase PC1. The sensor/transducer proteins MecR1 and BlaR1 regulate the level of expression of PBP2a and PC1, respectively, in response to the presence of the β-lactam antibiotic. However, the intramolecular events that lead to activation of MecR1 and BlaR1 are poorly understood, and the search for inhibitors has been limited by the lack of high-resolution structural information on the full-length proteins.

In order to gain insight into the molecular details of the activation of MecR1 we have evaluated the expression of full-length MecR1 (E205A mutant, which lacks auto-proteolytic activity) as a fusion to the protein Mistic. We have succeeded in overexpressing Mistic-MecR1 in *E. coli* BL21 Star^{TM} (DE3) membranes and we were able to purify it in detergent micelles to homogeneity using affinity chromatography. An assay with the fluorescent penicillin Bocillin-FL showed an active sensor domain in this recombinant protein. In addition, we have obtained polycrystals in a crystallogenesis screening assay. We attempted to optimize the crystallization condition but we have not achieved the formation of individual crystals yet. We are currently exploring our proposed mechanism of activation of MecR1 by β-lactams using site-directed mutagenesis in a *S. aureus* reporter strain and using photoactive amino acids.

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Structural insights into the ligand binding domain of the glucocorticoid receptor: a molecular dynamics study

Alves NRC[a], Pecci A[b,a], Alvarez LD[c,a]
a - Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales, Departamento de Química Biológica.
b - Instituto de Fisiología Biología Molecular y Neurociencias (IFIBYNE). Universidad Nacional de Buenos Aires. CONICET.
c - Unidad de Microanálisis y Métodos Físicos Aplicados a Química Orgánica (UMYMFOR). Universidad Nacional de Buenos Aires. CONICET.

The glucocorticoid receptor (GR) is a ligand-binding dependent transcription factor that ultimately regulates vital biological processes and inflammation response through specific gene expression control, thus representing a notable drug target to explore. Structurally, its ligand binding domain (LBD) harbors the region for the ligand-dependent transcriptional activation function 2 (AF-2), a majorly hydrophobic groove formed by residues from helices H3, H4, and H12, where the H12 position plays a critical role in AF-2 spatial conformation and GR function as a whole. However, the exact mechanisms underlying how regulatory ligands control the H12 structure and dynamics are yet to be elucidated. In this work, we have explored the correlation between ligand identity and GR LBD H12 behavior through different molecular dynamics (MD) simulations. After building diverse GR LBD systems in agonist and non-agonist states, we studied each system's response in the absence or the presence of an agonist ligand (dexamethasone) or an antagonist ligand (RU486) using classical MD simulations. We complemented them with steered MD for assessing the transition between those states, and with the Umbrella Sampling method for free-energy evaluation. On the one hand, successfully obtaining fully-folded non-agonist GR LBD states from the partially-unfolded crystal GR LBD/RU486 underlines the role of the H1 in the GR LBD folding pathway. On the other hand, our results describe the H12 as a dynamic ensemble of conformations whose relative population is in the end determined by the interacting ligand: while dexamethasone privileges only a few poses (determined by a potential energy surface with a deep minimum), RU486 favors a wider H12 conformational amplitude, as indicated by a flatter potential landscape. By characterizing the H12 conformation in different conditions, we provide novel GR LBD models that represent potential targets for rational glucocorticoid drugs design, in aim to accurately modulate GR activity.

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Studies with pyrenyl-labeled apolipoprotein A-I. A multiparametric analysis of Pyrenyl-maleimide.

Tárraga WA[a], González 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 (apo A-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.

Apo A-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, with a mechanism unwell characterized. It also interacts with phospholipids and forms discoidal HDL (dHDL) in different anti-parallel helical arrangement which differ on the proximity of its helices from each other.

The aim of the present study is to obtain information on the apo A-I self-aggregation in solution, especially helices proximities, which may be important for understanding the mechanisms of dHDL 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 apo A-I concentration; and several mathematical models were developed and compared to evaluate the different association types and calculate association constants (Kas) corresponding to the different oligomerization events proposed.

The labeled mutants were stable in solution, as indicated by its 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, highlighting the participation of helices 5 and 10 in the contact regions during certain oligomerization steps. Changes in p-value of monomer emission also reported conformational changes during apo A-I oligomerization. In the case of K133C, we predicted at least two different events of oligomerization, and a model of progressive association seems to be the most suitable to represent this behavior.

Altogether, these results suggest that the self-proximities of helixes 5 and 10, which are necessary to form dHDL, are already present in the soluble conformers of apo A-I.

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The effect of ganglioside oligosaccharide derivatives in the aggregation properties of amyloidogenic proteins

Vasti C[a], Rodriguez P[b], Fidelio GD[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.
b - Ministerio de Ciencia y Tecnología de la Provincia de Córdoba

The aggregation of the β-amyloid protein Aβ(1-42) plays a fundamental role in the pathogenesis of Alzheimer's disease (AD) that is closely linked to neuronal toxicity. It has been described that Aβ(1-42) interacts with gangliosides with a certain affinity. The accumulation of GM1ganglioside complexes with Aβ(1-42) has been reported in brains of patients with AD. Previous studies indicate that both the oligosaccharide portion and the hydrophobic portion of the GM1 participate in the interaction. In order to discern the participation of the oligosaccharide portion, we synthesized new molecules containing only the oligosaccharide portion of GM1 ganglioside (called osGM1) or covalently linked to chitosan, a biocompatible cationic polymer (osGM1-Ch ganglioside like-glycan cluster molecules). This nanoparticle was synthesized by using low molecular weight chitosan (MW~ 100 kD) loaded with 1 µmol osGM1/1.28 mg of chitosan. We have tested the effect of these new molecules against GM1 micelles.

We used β-lactoglobulin (β-lg) as amyloid-like aggregation model. Benzy alcohol induce amyloid fibers Thio T positive (a fluorescent marker of amyloid fibers) in β-lg in aqueous buffer at physiologic pH. We have also used synthetic Aβ(1-42) peptide.

Preliminary results showed that osGM1 has no practically effect on β-lg induced, fibers, GM1 and acidic GM1-H+ micelles have a slight disaggregating effect whereas osGM1-Ch enhances its effect. Over preformed Aβ(1-42) fibers in aqueous solution, osGM1 prevents or delays the time depending self-disaggregating amyloid fibers. Instead, nanoparticle osGM1-Ch has a marked disaggregating effect over preformed Aβ(1-42) fibers but similar effect was also obtained by non-loaded carrier chitosan. Additional experiments are still necessary to attribute some property to the oligosaccharide part of the GM1 ganglioside in its aggregating-disaggregating effect in in-vitro experimental models of amyloids.

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The Marginal Stability of Proteins: How Jiggling and Wiggling of Atoms are Connected to Neutral Evolution

Martin OA[a], Vila JA[a]
a - Instituto de Matemática Aplicada San Luis (IMASL), UNSL-CONICET, San Luis, Argentina.

Here we propose that the upper bound marginal stability of proteins (£ 7.4 kcal/mol) is a universal property that includes biomacromolecular complexes. Consequently, mutations disrupting the marginal stability upper bound will be subject to negative selection and mutations that preserve it will be neutral or nearly neutral, with respect to the marginal stability. Albeit, proteins and biomacromolecular complexes can still evolve under the influence of natural selection.

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Unraveling Calcium/calmodulin-dependent protein kinase 2 delta regulation by Nuclear Magnetic Resonance

Biglione FA[a], Lombardo V[b,a], Sánchez-López C[a], Binolfi A[a,c]
a - Instituto de Biología Molecular y Celular de Rosario, IBR-CONICET-UNR, Rosario, Argentina
b - Centro de Estudios Interdisciplinarios (CEI), Universidad Nacional de Rosario
c - Plataforma Argentina de Biología Estructural y Metabolómica (PLABEM)

Calcium/calmodulin-dependent protein kinase 2 delta isoform (CaMKIIδ) is a highly abundant myocardial enzyme that plays a central role in cellular signaling. CaMKIIδ transmits Ca^{2+} signals to downstream substrates through the activation of its serine/threonine kinase activity in a calcium-bound calmodulin (CaM) dependent manner. Under physiological conditions, CaMKIIδ activity level is finely tuned by the complex interplay between a series of ligand binding, structural and post-translational modification (PTM) events acting on its regulatory domain. In this sense, regulation imbalances leading to CaMKIIδ Ca^{2+}-independent hyperactivation have been related to several cardiac pathologies. Although some of the control mechanisms such as Ca^{2+}/CaM concentrations, CaMKIIδ autophosphorylation and autoinhibitory structural arrangements have been extensively characterized, oxidative regulation by methionine sulfoxide (MetOx) formation has been recently identified as a PTM capable of modulating the switch-like properties of this kinase. In this work, we introduce a new NMR-based approach to delineate CaMKIIδ regulatory phenomena *in vitro* and *in vivo* with atomic resolution. We have designed an isotopically enriched peptide probe (rCaMKIIδ) consisting of the regulatory domain of CaMKIIδ in its reduced or methione-oxidized forms and studied it using high-resolution multidimensional NMR. Our studies suggest that rCaMKIIδ can be used to thoroughly characterize the oxidative regulation in an integrative and site-specific manner. Furthermore, we tested the use of rCaMKIIδ as an *in vivo* NMR reporter in living zebrafish (*Danio rerio)* embryos. We monitored the endogenous reduction of multiple peptide MetOx sites in the native cellular context of a multicellular organism. Altogether, we have developed a novel strategy that will allow us to delineate the regulatory pathways controlling CaMKIIδ activity and their cross-talk under physiological and pathological conditions.

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Unveiling the mechanism of activation of the *vraSRT* system of *Staphylococcus aureus* by β-lactam antibiotics using photoactive compounds

Antinori MB[a], Testero SA[b], Llarrull L[a]
a - Instituto de Biología Molecular y Celular de Rosario, IBR-CONICET-UNR, Rosario, Argentina
b - Instituto de Química Rosario (IQUIR) - CONICET - UNR

Staphylococcus aureus is the leading cause of nosocomial and community-acquired infections. The *vraSRT* system acts as a sentinel that can rapidly sense cell wall peptidoglycan damage and coordinate a response that leads to resistance to β-lactam and glycopeptide antibiotics. VraS is a membrane histidin-kinase and VraR a cytoplasmatic response regulator. However, the rol of VraT, another membrane protein, is yet unknown but essential for the survival of the bacteria. We still do not understand how VraS is activated in response to cell wall-active antibiotics.
The interaction between VraS , VraT and different ampicillin-derived photo-affinity probes was studied. Using a *S. aureus* reporter strain, which has a shuttle vector that allows expression of GFP under the control of the *vraSRT* operator region, we confirmed that the ampicillin photoprobes effectively activate the *vraSRT* system. The photo-affinity probes were used for covalent labeling of VraS and VraT in *E. coli* BL21 Star DE3 spheroplasts. An interaction with VraS was evidenced by a shift in the electrophoretic mobility of the protein. MALDI-TOF/TOF analysis of the purified VraS-photoprobe complexes did not allow the identification of the site of crosslinking. We hypothesized that β-lactams could interact with the extracellular loop of VraS, a peptide not detected by MALDI-TOF/TOF. Hence, we introduced photoactive phenylalanine residues in that loop of VraS and evaluated labeling with the fluorescent penicillin Bocillin-FL. No fluorescent VraS was detected which indicated no direct interaction of the antibiotic with this loop. VraT has an extracellular C-terminal domain, as determined in a Proteinase K susceptibility assay, which does not interact directly with the ampicillin photoprobes.
In conclusion, VraS interacts directly with β-lactams but its extracellular loop is not involved in the recognition. VraT participation in activation of the system is not as a receptor of the antibiotic.

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