VII Jornada de Biofísica

Organitzada per la Secció de Biofísica de la SCB

PROGRAMA I RESUMS DE LES COMUNICACIONS

Amb el suport de:

INSTITUT D´ESTUDIS CATALANS

Sala Nicolau d'Olwer, Institut d'Estudis Catalans
Carrer del Carme, 47. Barcelona

14 de novembre de 2019

PROGRAM

9.30 h – **Registration**

10.00 h – **Welcome Session**

10.15 h –11.15h **Opening Lecture** (sponsored by *Biomolecules (MDPI Editorial Group)*)
SALVADOR VENTURA (UAB, Bellaterra)
Finding new drugs for conformational diseases

11.15-11.45 Coffee Break

11.45 – 13.00 h – **Short Presentations**

11.45 - 12.00 h EKAITZ ERRASTI-MURUGARREN (IRB)
Molecular bases for the asymmetry of substrate interaction in L-amino acid transporters

12.00 - 12.15 h DANIEL SASTRE (UB)
Variable stoichiometries and functional consequences of the Kv1.3-KCNE4 channelosome

12.15 – 12.30 h JOAN RAMON DABAN (UAB)
Multilayer chromatin in mitotic chromosomes and interphase nuclei

12.30 – 12.45 h NÚRIA BENSENY CASES (ALBA-CELLS)
Synchrotron based Infrared Microscopy for the study of Alzheimer Disease.

12.45- 12.50 h MARTA CULLEL DALMAU (UVic)
Exploring the role of integrin glycosylation in wound healing at the single molecule level

12.50 – 12.55 h IRENE ESTADELLA (UB)
Modulation of Kv1.3 endocytosis by Protein Kinase A (PKA)

12.55 – 13.00 h SILVIA CASSINELLI (UB)
Post-translational regulation of the KvB2.1 accessory subunit

13.00 – 13.45 h **Keynote Lecture**
ANTONIO ALCARAZ (Univ. Jaume I, Castelló)
Ion channel Biophysics. From structure to function and vice versa

13.45-15.30h Lunch at the restaurant l'Antic Forn (carrer Pintor Fortuny, 28. Barcelona.)

15.30 – 16.15 h **Meet the Expert**
Dr. Pau Bernadó (CBS, France)
Novel approaches for the structural characterization of complex biological systems

16.20 – 17.00 h **Short Presentations**

16.20 – 16.35 h ELENA ÁLVAREZ-MARIMON (UAB)
Metal cation distribution on fibrillar and non-fibrillar Alzheimer plaques

16.35 – 16.50 h JOSÉ LUIS VÁZQUEZ-IBAR (I2BC – CNRS, France)
ATP2, the essential lipid flippase of malaria parasites. From heterologous production to functional characterization

17.00h – 18.00 h **Closing Lecture**
CARLO MANZO (UVic, Vic)
The spatiotemporal organization of membrane proteins. New insights from artificial intelligence

OPENING LECTURE

Salvador Ventura
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Conformational diseases are pathologies with a great social, economic and personal impact on our society. Some are well-known because they affect a large number of people, like Alzheimer's and Parkinson's diseases, while others are rare disorders, such as familiar amyloidosis. All these diseases remain incurable. They exhibit very different symptoms and each of these disorders is associated with a different protein. However, the problem that these proteins experiment is always the same: at a given time they become insoluble and toxic and they impact the normal physiology of the tissue or organ in which they reside, independently if this is the heart or the brain. In this presentation I will explain the efforts of our research group to find new drugs that can prevent and, hopefully, cure these devastating diseases.

KEYNOTE LECTURE

Ion channel biophysics: from structure to function and vice versa

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Biological membranes not only protect the cell from toxic agents, but also allow the uptake of necessary nutrients and the efflux of waste products. A precise control of membrane permeability is essential for life and therefore is a central issue in Biophysics. Despite intensive investigations on the matter, the molecular mechanisms regulating membrane permeabilization are still poorly understood. Here, we focus on the transport of ions and small metabolites across membranes due to the action of a variety of channel-forming proteins. The hydrophilic pathways created by these molecules maintain the concentration gradients necessary for cell life and enable electrical signaling transmitting specific information throughout any living organism. We explore the correlation between the structure and the function of ion channels in both directions. Firstly, we study proteins with known detailed structural information, such as OmpF from E. Coli and VDAC from mitochondria, which serve as a model system for the testing and development of physical models of different complexity. On the other hand, we also explore proteins of special biomedical interest (channels from clinically relevant animal virus) that need for better characterization.

MEET THE EXPERT

Novel Approaches for the Structural characterization of Complex Biological Systems

Annika Urbanek[1], Matija Popovic[1], Carlos Elena-Real[1], Anna Morató[1], Alejandro Estaña[1,2], Frédéric Allemand[1], Aurélie Fournet[1], Stephane Delbecq[3], Juan Cortés[2], Nathalie Sibille[1], Pau Bernadó[1]

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Homorepeats (HRs), protein fragments composed by the same amino repeated multiple times, are very common in eukaryotes and are involved in key biological processes and multiple pathologies. HRs are enriched in particular biophysical properties that enables them to perform very specialized functions but that can also trigger disease. Despite their interesting properties, the high-resolution structural characterization of HRs has been impaired due to their inherent flexibility and polymeric nature, which give poorly dispersed NMR spectra. Huntingtin (Htt), the causative agent of Huntington's disease (HD), is the prototypical example of a HR hosting protein. Htt has a poly-Glutamine tract of variable length that becomes toxic when the number of consecutive glutamines exceeds 35 (pathological threshold). Moreover, Htt contains two Poly-Proline tracts with 11 and 10 consecutive prolines. The aim of our study is to decipher the structural perturbations exerted by the extension of Poly-Glutamine tract beyond the pathological threshold, and the role that flanking regions, including the Poly-Proline tracts, have in the pathology.
To overcome challenges posed by HRs, we have developed a chemical biology strategy to isotopically label individual glutamines and prolines within HRs by combining nonsense tRNA suppression and cell-free protein synthesis. Our method disentangles the spectroscopic complexity of the HR and has enabled the NMR investigation of two huntingtin exon1 versions with 16 and 46 consecutive glutamines. Implications of these observations to understand the structural bases of HDs, and the future perspectives of the site-specific isotopic labelling will be discussed.

CLOSING LECTURE

The spatiotemporal organization of membrane proteins. New insights from artificial intelligence

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Plasma membrane proteins play important roles in transport and signal transduction. Elucidating the spatiotemporal organization of these proteins provides crucial information to unravel cellular mechanisms of response to external cues.

Recent advances in super-resolution and single-molecule fluorescence microscopy offer unique tools to visualize biological processes at the nanoscale. Such techniques can probe the stoichiometry and interactions of membrane proteins at high spatial and temporal resolution and thus help to decipher the dynamic composition of the plasma membrane or predict the outcome of membrane-based reactions.

Nevertheless, a detailed quantitative description of the molecular mechanisms occurring at the membrane level is still restricted by inherent limitations of these techniques. The use of *ad hoc* calibration platforms combined with artificial intelligence and machine learning tools can overcome these restrictions and accurately measure the stoichiometry and the diffusion of membrane proteins. We will discuss examples of how the combination of these novel approaches can advance our understanding of the complex biological functions of membrane proteins.

SHORT ORAL PRESENTATIONS

Molecular bases for the asymmetry of substrate interaction in L-amino acid transporters

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L-amino acid transporters (LATs) play key roles in human physiology and are implicated in several human pathologies. LATs are asymmetric amino acid exchangers where the low apparent affinity cytoplasmic side controls the exchange of substrates with high apparent affinity on the extracellular side. Here, we report the crystal structures of an LAT, the bacterial alanine-serine-cysteine exchanger (BasC), in a non-occluded inward-facing conformation in both apo and substrate-bound states. We crystallized BasC in complex with a nanobody, which blocks the transporter from the intracellular side, thus unveiling the sidedness of the substrate interaction of BasC. Two conserved residues in human LATs, Tyr 236 and Lys 154, are located in equivalent positions to the Na1 and Na2 sites of sodium-dependent APC superfamily transporters. Functional studies and molecular dynamics (MD) calculations reveal that these residues are key for the asymmetric substrate interaction of BasC and in the homologous human transporter Asc-1.

Variable stoichiometries and functional consequences of the Kv1.3-KCNE4 channelosome

Daniel Sastre[1], Laura Solé [1,2], Sara R. Roig[1,3], Mireia Pérez-Verdaguer[1,4], Magalí Colomer-Molera[1], Michael M. Tamkun[2] and Antonio Felipe[1*].

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The voltage-gated potassium channel Kv1.3 plays a crucial role during the immune response and lymphocyte activation. KCNE4, one of the five members of the KCNE family, binds to Kv1.3 altering both channel activity and membrane expression. KCNE association to Kv channels, especially to Kv7.1, has been subject of studies for the past decades and the stoichiometry of such association still raises an ongoing debate. In this scenario, the negative effect of KCNE4 over Kv1.3 provides a mechanism for fine-tuning the leukocyte physiology. However, the number of KCNE4 subunits modulating Kv1.3 is yet to be determined. The aim of this study was to analyze the functional effects of different fixed Kv1.3-KCNE4 stoichiometries, as well as to determine the most relevant stoichiometry in physiological conditions. We demonstrate that up to four KCNE4 subunits can bind to the same Kv1.3 complex providing variable Kv1.3-KCNE4 stoichiometries with different functional effects. The number of KCNE4 proteins in the channelosome had an additive effect in reducing the abundance of Kv1.3 at the cell surface. However, the presence of a single KCNE4 peptide in the functional complex was sufficient to trigger the cooperative enhancement of the inactivating properties of the channel. This variable architecture, which would depend on the KCNE4 availability, differentially affects the Kv1.3 function. Therefore, the physiological remodeling of KCNE4 and its assembly in different stoichiometries with Kv1.3 would serve as a mechanism to regulate lymphocyte activation.
Supported by MINECO, Spain (BFU2017-87104-R) and Fondo Europeo de Desarrollo Regional (FEDER).

Multilayer chromatin in mitotic chromosomes and interphase nuclei

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Early electron microscopy studies showed the emanation of chromatin fibers from mitotic chromosomes and nuclei swollen with water. Since then, most of the proposed chromosome models have a straightforward relationship with the linear geometry of the chromatin fibers. However, under metaphase ionic condition, our cryo-electron tomography results (1) showed that frozen-hydrated chromatin emanated from mitotic chromosomes is a planar structure that forms multilaminar plates composed of mononucleosome layers, which are interdigitated between them. Synchrotron small-angle X-ray scattering of mitotic chromosomes showed a dominant peak at 6 nm, which can be correlated with the distance between layers and between nucleosomes interacting through their faces. In the 3D reconstructed tomographic volumes, we observed large multilayered plates with widths similar to the chromosome diameter. All these observations reinforce previous studies of our laboratory and support a compact thin-plate model consisting of many chromatin layers stacked along the chromosome axis. Furthermore, we have found that in buffers containing interphase cation concentrations, the chromatin emanated from G1, S, and G2 nuclei also has a planar morphology (2). The plates observed in interphase have a low tendency to form the multilayered structures observed in mitotic chromosomes, suggesting that they are unstacked and more exposed to the medium to facilitate gene expression and replication. Since the size of the topologically associated domains (TADs) detected in Hi-C analyses of interphase cells is similar to the amount of DNA in a chromatin layer (0.5 Mb), we propose that each layer may correspond to a TAD. During mitosis, TADs apparently disappear because the layers become closely stacked and consequently the contacts observed in Hi-C experiments involve distant sequences in the genome.

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(2)Chicano A & Daban JR (2019) FEBS Lett. 593:810-819. https://febs.onlinelibrary.wiley.com/doi/full/10.1002/1873-3468.13370

Synchrotron based Infrared Microscopy for the study of Alzheimer Disease.

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Alzheimer Disease is a neurodegenerative disease and one of the so called conformational diseases. Amyloid plaques are one of the main pathological hallmarks of Alzheimer Disease. Synchrotron-based infrared microscopy makes possible the in situ structural study of such amyloid aggregates in brain. The *in situ* identification of early-stage aggregates could be highly relevant in terms of the characterization of amyloid aggregates that form at non symptomatic stages of the pathology, preceding the consolidation of the final fibrillary structures. The characterization of these aggregates, only found in vitro until now, could be of paramount importance for their importance. In this talk, the characterization of early formation of amyloid aggregates in situ in the brain of APP/PS1 transgenic mice and Octodon degus using Synchrotron-based infrared microscopy is presented. Also, the effect of G4-His-Mal dendrimers (a neurodegenerative protector) in 6-month-old APP/PS1 mice, thus demonstrating putative therapeutic properties of G4-His-Mal dendrimers in AD models. Identification, localization, and characterization using infrared imaging of these non-fibrillary species in the cerebral cortex at early stages of AD progression in transgenic mice point to their relevance as putative pharmacological targets. No less important, early detection of these structures may be useful in the search for markers for non-invasive diagnostic techniques.

Exploring the role of integrin glycosylation in wound healing at the single molecule level

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Cell migration is essential for a variety of physiological processes, including organ homeostasis, immunological response, cancer metastasis and wound healing. During migration, cells interact with the extracellular matrix (ECM) by forming bonds between membrane receptors and ECM ligands.

Integrins are a class of proteins largely involved in cell adhesion and migration. They are glycosylated heterodimeric transmembrane receptors that interact with different components of the extracellular matrix. It is well accepted that integrin's ability to form functional dimers depends upon the presence of *N*-glycans, that can also modulate integrin's conformation. Consequently, cell migratory behavior may depend on integrin *N*-glycosylation, but the mechanism underlying this effect has not been thoroughly investigated.

Since integrin diffusion has been shown to be a valuable proxy for the identification of integrin conformation and its linkage to actin cytoskeleton, we are using single particle tracking experiments to further investigate this mechanism and get insights at the single molecule level. To this aim, we are studying the diffusion of $\alpha_5\beta_1$ integrins in human dermofibroblasts under resting and wound healing conditions and upon different treatments that impact the level of glycosylation.

Preliminary control experiments show an integrin behavior in line with what previously reported [1], thus ensuring on the validity of the procedures. We are currently performing experiment in the presence of different treatments to fully characterize the effect of glycosylation. A better understanding of the molecular mechanisms underlying cell migration in wound healing could produce novel diagnostic and therapeutic approaches.

Modulation of Kv1.3 endocytosis by Protein Kinase A (PKA)

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The voltage-gated potassium channel Kv1.3 plays a special role during the immune response, being crucial for the activation and proliferation of leukocytes. Kv1.3 is considered a potential therapeutic target because an altered expression of the channel has been observed at the onset of many autoimmune diseases.

In this context, understanding the mechanisms involved in the regulation of Kv1.3 deserves considerable attention. The down-stream signaling produced by the channel consists in a balance between the synthesis and forward trafficking mechanisms to the cell membrane and those of internalization and degradation. Therefore, the Kv1.3 turnover influences the inflammatory response and understanding endocytosis is crucial for the knowledge of the molecular physiology of the immune system. Adenosine (Ado), a potent endogenous anti-inflammatory agent, triggers Kv1.3 internalization in leukocytes by stimulating PKC. In addition, Ado also activates PKA-signaling pathways. In this study we have investigated the role of PKA in Kv1.3 turnover. To that end, we examined the channel endocytosis, membrane abundance, ubiquitination and endocytic mechanisms. Our results show that PKA activation increased the number of channels internalized. Moreover, we found that the Kv1.3 endocytosis seems to target the channel to both lysosomal and proteasomal degradation. Therefore, Ado, exerting an effective anti-inflammatory mechanism, mediates two alternative and redundant PKC and PKA-mediated molecular mechanisms modulating the abundance of Kv1.3 at the cell surface.

Keywords: Protein turnover; Endocytosis; Ubiquitination; Protein Kinase A; Voltage-gated potassium channels.

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Post-translational regulation of the Kvß2.1 accessory subunit

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Kvß proteins are mainly known to regulate the voltage dependent K^+ channel (Kv channels). This family of peptides contains three soluble members, Kvß1, 2 and 3. All of them present a four-fold symmetric arrangement, a conserved NADPH binding site and a high aldo-keto reductase homology. Kvß subunits work as chaperone-like proteins, enhancing the channel surface expression and altering electrophysiological properties of some Kv1 and Kv4 channels. In this study, we aimed to elucidate the relationship between Kv1.3 channel and Kvß2.1 within the channel functional oligomeric complex, due to their wide expression in both immune and nervous systems. Kv1.3 and Kvß2.1 may be located in specific membrane microdomains, named lipid rafts. S-Acylation is a post-translational modification, specific for cysteine residues, that targets some soluble proteins to the plasma membrane and lipid rafts. Therefore, we have explored this mechanism in Kvß2.1. By mutating the entire Kvß2.1 pool of cysteines, we identify a basal cysteine-dependent S-Acylation of the peptide. Our results indicate that the largest number of mutated cysteines correlates with a markedly reduced surface expression of the regulatory subunit.

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Metal cation distribution on fibrillar and non-fibrillar Alzheimer plaques

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Alzheimer's Disease (AD) is a neurodegenerative disease characterized by the presence of insoluble deposits of ß-amyloid peptide (senile plaques) and neurofibrillary tangles (NFT) [1].

In vitro studies have described that amyloid fibrils form following a nucleation-dependent polymerization process. Under some physicochemical conditions, such as the presence of metal cations or low pH, however off-pathway amorphous aggregates can be formed, also called Granular Non-fibrillar Aggregates[2,3]. According to the current prevalent idea, it is believed that the more toxic agents are the non-fibrillar aggregated species.

Using Synchrotron-based infrared imaging we could identify in advanced Alzheimer Disease human cortex samples *in situ* two different types of aggregated species: fibrillar amyloid aggregates and unordered non-fibrillar aggregates. Infrared imaging analysis also shows that the lipid associated to the unordered non-fibrillar plaques is oxidized with respect to the controls.

In order to relate the metal cation distribution abnormalities described in AD and the identified Aß amyloid aggregated species we analyzed the same brain spots using nano-X-ray fluorescence (nano-XRF) and X-ray absorption near edge structure (XANES). We found that Fe was highly concentrated in the fibrillar plaques, whereas the concentration of this metal in the non-fibrillar ones was clearly lower, being Fe^{3+} the predominant oxidation state in the fibrillar plaques and F^{2+} on the unordered non-fibrillar plaques.

1. I. Ferrer. *Neurobiol*. 97, 38-51 (2012)
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ATP2, the essential lipid flippase of malaria parasites: from heterologous production to functional characterization

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The spread of malaria parasites resistant to current treatments urges the identification and characterization of essential proteins of *Plasmodium* parasites suitable for drug targeting. Recent studies using gene-deletion tools have demonstrated the irreplaceable role for the parasite's development of ATP2, the *Plasmodium* lipid flippase that belongs to the P4 subfamily of P-type ATPases. In eukaryotes, the phospholipid translocation activity of P4-ATPases is mandatory to maintain the phospholipid distribution and composition of cellular membranes; key in processes like vesicle budding or apoptosis. Moreover, during the intracellular stages of *Plasmodium*, a constant dynamic and transit of phospholipids is required in order to build the intracellular organelles, thus envisaging the relevance of ATP2. To understand the functional role of ATP2 during malaria infection is necessary to unravel its transport mechanism and to identify its substrate(s) and potential inhibitors. We have expressed in *Saccharomyces cerevisiae* the *P. chabaudi* ATP2 ortolog, PcATP2. Lipid flippases form heteromeric complexes with members of the Cdc50 protein family; consequently, we used co-expression experiments followed by co-immune purification using nanobodies against the GFP (nanoGFP) to identify two *P. chabaudi* Cdc50 members that heterodimerize with PcATP2. Moreover, using immobilized nanoGFP in agarose beads we have been able to measure ATPase activity of detergent-solubilized PcATP2/PcCdc50.1 complex bound to the beads. Currently, we are optimizing the purification of PcATP2/PcCdc50.1 using protease digestion to elute the protein from the nanoGFP agarose beads.