Societat Catalana de BIOLOGIA

VII Jornada de Biofísica

Organitzada per la Secció de Biofísica de l'SCB

Coordinador: Pere Garriga

PROGRAMA I RESUMS DE LES COMUNICACIONS

INSTITUT D´ESTUDIS CATALANS

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

Carrer del Carme 47

Barcelona

3 de maig de 2018

Programa

14.30 h Recollida de documentació

14.45 h Introducció: Pere Garriga

15.00 h – 15.45 h *(Compartida amb la Secció de Biologia del Càncer,* ***Sala Gran de Dalt).***

Collective cancer cell invasion by fibroblast forces

Anna Labernadie. IBEC (Barcelona).

15.45 h – 17.15 h Comunicacions

Moderador: Alex Perálvarez-Marín

17.00 h – 17.30 h Pausa cafè

17.30 h – 18.30 h Comunicacions

Moderador: Carlo Manzo

18.30 h – 19.15 h

Biophysical and biochemical approaches to investigate vertebrate phototransduction in health and disease

Alexander Scholten. University of Oldenburg (Germany)

15.45 h – 17.15 h. COMUNICACIONS

Moderador: Alex Perálvarez-Marín

15.45 h – 16.00 h Joan-Ramon Daban **Stacked thin layers of planar chromatin explain the 3D organization of genomic DNA in condensed metaphase chromosomes**

16.00 h – 16.15 h Marc Rico-Pastó, Marco Ribezzi-Crivellar and Felix Ritort **Melting enthalpy and entropy change with single molecule experiments resolution**

16.15 h – 16.30 h Berta Gumí-Audenis, Luca Costa, Fausto Sanz, Marina I. Giannotti **Pulling lipid tubes from model membranes**

16.30 h – 16.45 h A. M. Monge, D. Incarnato, A. Alemany, M. Ribezzi-Crivellari, F. Ritort **Single-molecule characterization of heterogeneous DNA ensembles**

16.45 h – 17.00 h Carlo Manzo **Quantification of protein copy number from super-resolution images**

17.30 h – 18.30 h COMUNICACIONS

Moderador: Carlo Manzo

17.30 h – 17.45 h Alfredo de la Escosura-Muñiz, Kristina Dimitrova, Pere Garriga, Tzanko Tzanov **Electrical evaluation of bacterial pathogen virulence factors using nanopores**

17.45 h – 18.00 h Xavier Viader-Godoy, Maria Manosas, Felix Ritort **Length-dependence of the elastic response of single-stranded DNA**

18.00 h – 18.15 Núria Benseny-Cases, Elena Álvarez-Marimon, Hiram Castillo-Michel, Ester Aso, Margarita Carmona, Marine Cotte, Isidre Ferrer, Josep Cladera **Nano-X-Ray Fluorescence Studies of Alzheimer Disease Amyloid Plaques**

18.15 h – 18.30 h Marta Gironella and Felix Ritort **Relaxational kinetics in red blood cell mechanics: linking physical to biological aging**

Resums

Collective cancer cell invasion by fibroblast forces

Anna Labernadie & Xavier Trepat.

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Cancer-associated fibroblasts (CAFs) promote tumour invasion and metastasis. We show that CAFs exert a physical force on cancer cells that enables their collective invasion. Force transmission is mediated by a heterophilic adhesion involving N-cadherin at the CAF membrane and E-cadherin at the cancer cell membrane. This adhesion is mechanically active; when subjected to force it triggers β-catenin recruitment and adhesion reinforcement dependent on α-catenin/vinculin interaction. Impairment of E-cadherin/N-cadherin adhesion abrogates the ability of CAFs to guide collective cell migration and blocks cancer cell invasion. N-cadherin also mediates repolarization of the CAFs away from the cancer cells. In parallel, nectins and afadin are recruited to the cancer cell/CAF interface and CAF repolarization is afadin dependent. Heterotypic junctions between CAFs and cancer cells are observed in patient-derived material. Together, our findings show that a mechanically active heterophilic adhesion between CAFs and cancer cells enables cooperative tumour invasion.

Stacked thin layers of planar chromatin explain the 3D organization of genomic DNA in condensed metaphase chromosomes

Joan-Ramon Daban

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The 3D organization of genomic DNA in metaphase chromosomes has been one of the most challenging problems in structural biology since the discovery of the double helix. This study shows that chromosome images obtained from typical banded karyotypes and from different multicolor cytogenetic analyses can be used to obtain information about the 3D folding of chromatin within chromosomes. Chromosome bands and the connection surfaces in sister chromatid exchanges and in cancer translocations are planar and orthogonal to the chromosome axis. Chromosome stretching produces band splitting and even the thinnest bands are orthogonal and well defined, indicating that short stretches of DNA can occupy completely the chromosome cross-section. These observations impose strong physical constraints on models that attempt to explain chromatin packaging in chromosomes. The thin-plate model, which was proposed from previous experimental studies of our laboratory (1), consists of many stacked layers of planar chromatin perpendicular to the chromosome axis (2). This is the only model compatible with the observed orientation of bands, with the existence of thin bands (<1Mb), and with band splitting; it is also compatible with the orthogonal orientation and planar geometry of the connection surfaces in chromosome rearrangements. The results obtained provide for the first time a consistent interpretation of the chromosome structural properties that are used in clinical cytogenetics for the diagnosis of hereditary diseases and cancers. A complete description of this work can be found in ref. (3).

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https://www.nature.com/articles/srep14891

Melting enthalpy and entropy change with single molecule experiments resolution

Marc Rico-Pastó[1], Marco Ribezzi-Crivellari[2,3] and Felix Ritort[1,3]

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An accurate knowledge of the thermodynamic properties of nucleic acids as a function of temperature is crucial to predict their structure and stability far away from the physiological temperature. Traditionally, molecular thermodynamic properties, such as free energy, enthalpy and entropy change have been determined by bulk experiments [1]. In the last 20 years, single molecule experiments have become powerful, accurate and bulk complementary methods to characterize thermodynamic parameters such as base pair (bp) energy contributions and folding free energies [2].

We propose a novel method to determine the enthalpy and entropy change from hopping experiments at one unique salt concentration, in contrast with the traditional DSC bulk experiments. We have carried out experiment with three different DNA hairpins, i.e. poly-GC, poly-AT and CD4 (52% GC content), in a temperature range between 5 and 50ºC to measure the enthalpy and entropy change for the folding a GC and AT bp. From our data we have observed a strong temperature dependency, what it means a non-zero heat capacity change, ΔC_p. The measured ΔC_p are, 83±2 , 40±6 and 54±3 cal/K·mol for a GC, AT and CD4 bp. Moreover, we have compared the measured folding free energy at each temperature as $\Delta G = \Delta H - T\Delta S$ with the measured one by subtracting the stretching and orienting contributions using the WLC and FJC models. Finally, an empirical formula to determine the melting temperature for two complementary DNA sequences is presented.

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Pulling lipid tubes from model membranes

Berta Gumí-Audenis[1,2,3], Luca Costa[4], Fausto Sanz[2,1,3], Marina I. Giannotti[3,1,2]

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Several cellular processes, including endocytosis, membrane resealing, signaling and transcription, among others, involve conformational changes such as bending, vesiculation and tabulation. For instance, in endocytosis, the endocytic system needs to generate force enough to form an endocytic vesicle by bending the membrane bilayer. Separation of a membrane segment from the cytoskeleton as well as strong membrane bending are both involved in these mechanisms, which are also associated with the membrane chemical composition and physicochemical properties. This process is energetically comparable to pull out a membrane cylindrical tube by applying a force orthogonal to a small membrane region.[1] Both separation of a membrane segment from the cytoskeleton as well as strong membrane bending are involved in these mechanisms. In this context, these procedures can be mimicked by applying an external force with highly sensitive force transducers such as optical tweezers or atomic force microscopy (AFM).[2] The lipid tube growth is then evidenced on a constant force process in the force-distance curves (Fig. 1). This force is the growing tube force, F_{tube}.

In this work, we compare SLBs with different compositions and prove that the phospholipid state (gel or fluid) as well as the headgroup play a role on the F_{tube}, following the tendency observed on the well-established breakthrough force (F_b) characterization approach.[3,4] In addition, we evaluate the influence of the underlying substrate on F_{tube}, by comparing the tube growth from deposited vesicles and lipid bilayers supported onto silicon or mica substrates. Finally, the influence of the AFM tip chosen to perform the measurements is also studied, considering the tip radius (r_{tip}) and the retracting velocity of the tip away from the sample. We demonstrate that working with SLB models is an intermediate step between a free membrane (blebs) and a cytoskeleton supported membrane.

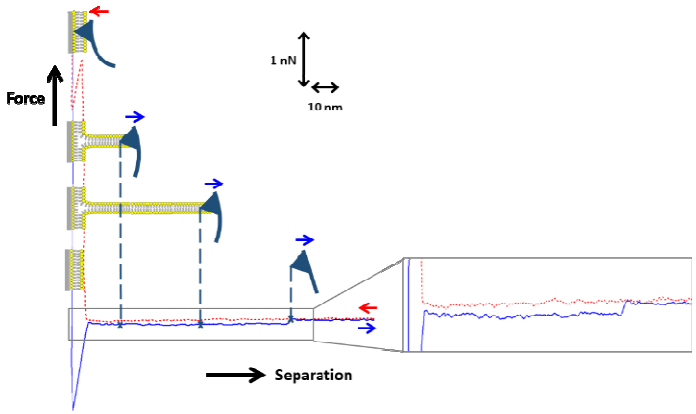

Figure 1: Example of a force-separation curve when performing an AFM-FS measurement on an SLB: approach (red dotted line) and retract (blue line). Schematic representation of the different steps.

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Single-molecule characterization of heterogeneous dna ensembles

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Heterogeneity exists across all spatial scales, from communities down to molecular level. The characterization and quantification of heterogeneous effects, although have been usually overlooked, have turned out to be fundamental in many scientific disciplines, such as cancer research. In this work, we combined single-molecule measurements using optical tweezers with fluctuation theorems to build a novel theoretical framework that allows us to quantify the folding free energy spectrum of the heterogeneous ensemble.

Quantification of protein copy number from super-resolution images

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Single-molecule-based super-resolution microscopy offers researchers a unique tool to visualize biological processes at the nanoscale. Nevertheless, providing a quantitative description of the molecular mechanisms underlying cellular function requires the precise molecular counting of protein copy numbers. Suitable calibration methods – based on the combination of biochemicals and analytical tools – represent a valuable solution to address the challenges of molecular counting using several super-resolution techniques (STORM, STED) in conjunction with immunofluorescence.

Along this line, we have recently proposed a versatile platform for calibrating fluorophore and antibody labeling efficiency based on DNA origami and GFP antibodies to quantify protein copy number in cellular contexts using localization microscopy. The combination of this calibration with image and data analysis methods, besides quantifying the average protein copy number in a cell, allows determining the abundance of various oligomeric states [1]. These quantitative approaches allow accurate studies of the stoichiometry of membrane proteins, nucleoporins and molecular motors.

References:

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Electrical evaluation of bacterial pathogen virulence factors using nanopores

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Bacterial hyaluronidases produced by a number of pathogenic Gram-positive bacteria catalyze the degradation of hyaluronic acid (HA), initiating infections at the skin or the mucosal surfaces. It's known that streptococcus, staphylococcus, streptomyces or clostridium bacteria between others, use this enzyme as a virulence factor to destroy the polysaccharide that holds animal cells together, making easier for the pathogen to spread through the tissues of the host [1]. The interest in the detection of this enzyme is related to two different aspects: i) the evaluation of the secreted levels of enzymes for different bacterial species would allow to discriminate between Gram-positive and Gram-negative bacteria and also to classify them in terms of virulence and ii) the evaluation of the enzyme secretion inhibition would allow to propose novel antimicrobial/antivirulence agents. However, the current available tools for the detection of this enzyme are quite limited. It's a very small protein (60 kDa) which difficult its detection using traditional immunoassays, typically radioimmunoassays (RIA) and enzyme-linked immnusorbent assays (ELISA) that are expensive, time consuming and need hazardous label reagents.

Biosensors in general and the ones based on nanoporous platforms in particular, overcome most of these limitations, since they are rapid, cheap and allow label-free detection [2,3]. In this context, we propose here a novel methodology for hyaluronidase detection on anodized aluminum oxide (AAO) nanoporous membranes. The proposed analytical method based on the electrical monitoring of specific nanochannels blocking/unblocking is shown as a useful tool for the detection of hyaluronidase through immunoassays. This label-free method is rapid and cheap, avoiding sandwich assays and the use of labels. Preliminary results open the way to future applications for virulence evaluation of enzymes as well as for monitoring bacterial infection processes.

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Length-dependence of the elastic response of single-stranded DNA

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Single-stranded DNA (ssDNA) plays a major role in several biological processes, such as replication or transcription. Therefore, it is of fundamental interest to understand the elastic response of this biological polymer. Besides, force spectroscopy techniques have been widely used to study biochemical and enzymatic processes involving DNA. The interpretation of the results obtained by these experiments, such as [1], requires an accurate description of the elastic properties of ssDNA. However, elasticity of ssDNA has been less studied than that of double-stranded DNA, and a large dispersion on the elastic parameters is obtained from different methods and sequences [2].

In this work, we study the elastic properties of ssDNA using molecules with different sequences and lengths comprising 4 orders of magnitude (from 60 bases to 14kbases). Using the inextensible Worm-Like Chain model we proof that the apparent discrepancy found in the previous works arises mainly from the different range of forces used to fit long and short molecules. We have also tested sequences with different pyrimidine/purine content in order to investigate the effect of base stacking, which is known to largely change the elastic properties of homogeneous sequences [3]. Even that the stacking of bases has a minor impact in the elastic response of heterogeneous sequences, we are able to detect base stacking effect at the level of tenths of bases.

References

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Nano-X-Ray Fluorescence Studies of Alzheimer Disease Amyloid Plaques

Núria Benseny-Cases[2], Elena Álvarez-Marimon[1], Hiram Castillo-Michel[3], Ester Aso[4], Margarita Carmona[4], Marine Cotte[3], Isidre Ferrer[4], Josep Cladera[1]

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Alzheimer´s Disease (AD) is characterized by the presence of neurofibrillary tangles (NFT), senile plaques (SP) and changes in the distribution of metal ions[1].

In order to understand the relationship between characteristic secondary structures of Aβ amyloid aggregates, altered distribution of metal ions (Cu, Zn, Fe, Ca) and lipid oxidation, we used the combination of two synchrotron radiation techniques: nano-X-ray fluorescence (nano-XRF) and μFTIR at ESRF beamlines ID16B-NA and ID21, respectively, on human brain tissues of affected AD individuals and healthy controls

On the one hand, synchrotron-based infrared microscopy makes possible the *in-situ* localization (figure 1A) and structural study of amyloid aggregates in relation to other physicochemical parameters, such as tissue oxidation[2]. The infrared data was analysed using Principal Component Analysis (PCA) which allowed us to distinguish between two different types of amyloid aggregates that might correspond with dense core plaques and diffuse plaques. On the same sample areas nano-XRF measurements at 0.2 μm^2 pixel size were done at ID16B-NA (ESRF) in order to measure the metal distribution. The results indicated that Fe, Cu and Zn ion maps co-localize with the plaques with cation content within the plaques well above the level measured outside the plaques. Moreover, when dense and diffuse plaques were compared, the Fe content turned out to be higher in the dense plaques.

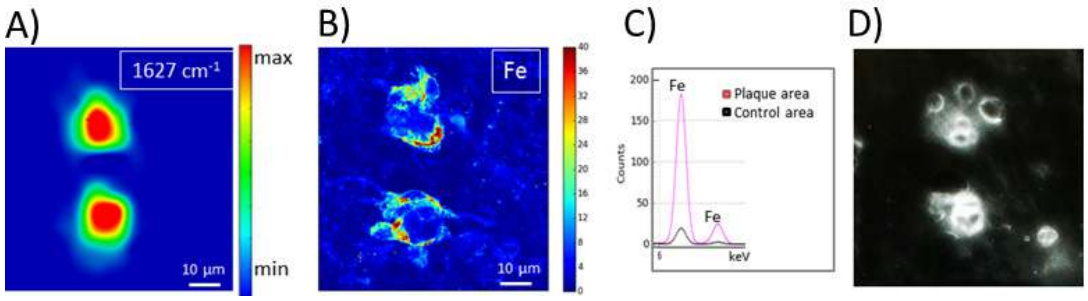

Figure 1. *ADV patient tissue analysis.* A) μFTIR map of the fibrillar β plaques (corresponding to dense plaques). B) Nano-XRF map of the same area representing Fe distribution. C) Fe content on the plaque area is significantly higher to the control area. D) After synchrotron analysis Thioflavin-S dye on the same tissue area confirmed the presence of amyloid plaques.

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Relaxational kinetics in red blood cell mechanics: linking physical to biological aging

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Red blood cells (RBC) are one of the most abundant and simplest cells in human body. Only composed of a lipid bilayer and an spectrin cytoskeleton, their shape, mechanics and aging are fundamental features to understand and treat the majority of blood diseases. In this project we study relaxational processes in the mechanics of RBC using optical tweezers. We use two different approaches in order to understand the viscoelastic response of the RBC: 1) Pulling experiments, where we pull and push the RBC at different maximum forces and different pulling velocities to extract information of the force-distance curves and; 2) Relaxation experiments, where we apply a force jump to the RBC and measure force relaxation. From these two kind of experiments we are able to characterize four different time-scales, three of them related to membrane-cortex interaction, the other one (which is the longest) shows a stiffening of the RBC that we hypothesize it is linked to aging in the RBC. The correlation between the time-scales allows us to globally understand the temporal evolution of RBC and link physical to biological aging.

Biophysical and biochemical approaches to investigate vertebrate phototransduction in health and disease

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Phototransduction means the conversion of light into a biological signal. In the vertebrate retina it takes place in specialized neuronal cells called rod and cone photoreceptor cells. The phototransduction cascade is an archetype of a G-protein coupled cascade. It requires a fine tuned balance between two second messengers: 3',5'-cyclic guanosine monophosphate (cGMP) and Ca^{2+} ions. To maintain this balance and to enable adaptation to different light conditions a system of membrane bound guanylate cyclases (GCs) and GC activating proteins (GCAPs) evolved in photorecptor cells. The main players of this system are GC-E and the GCAP isoforms 1 and 2. In the dark GCAPs sense the high Ca^{2+} concentration and inhibit the enzyme activity of GC-E. After a light stimulus [Ca^{2+}] drops, GCAPs release bound Ca^{2+} ions and switch into an activator state: GC-E produces cGMP. The activation of GC-E by GCAPs occurs in a consecutive manner: GCAP1 release it Ca^{2+} ions first, only after a strong light stimulus and more pronounced drop in [Ca^{2+}] also GCAP2 turns into its activating state. We called this mode of fine tuned adaptation the Ca^{2+}-relay model.

In order to better understand how the GC/GCAP system works on a molecular level we followed two main strategies: the first one included comparative analysis of the two isoform GCAP1 and GCAP2. The second strategy based on the characterization of GCAP1 (and GC-E) mutants that are known to cause inherited retinal degeneration in human. Knowledge about dysfunction in the impaired systems in diseases improved our knowledge on the non impaired wildtype system. Our comparative analysis included biochemical and biophysical approaches and focused on different features of the proteins: activity, structure, Ca^{2+}-affinity and GCAP/GC interaction.

Time-resolved fluorescence spectroscopy on labeled proteins showed us differences in the structural rearrangement of GCAP1 and 2 after Ca^{2+} binding. Circular dichroism was useful to find GCAP1 mutants with changed structure. To determine the Ca^{2+} affinity of the GCAPs we utilized a variety of techniques: $^{45}Ca^{2+}$ binding assay, chelator assay, isothermal titration calorimetry, and surface plasmon resonance. A newly developed technique called back-scattering interferometry gave us new insights into the binding of GCAP1 and 2 to the GC-E.