Societat Catalana de BIOLOGIA

VI Jornada de Biofísica

Organitzada per la Secció de

Biofísica de la SCB

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

14 de desembre de 2016

Programa

14.30 h Recollida de documentació

14.45 h Introducció: Pere Garriga

14.50 h – 16.30 h Comunicacions

Moderador: Joan-Ramon Daban

16.30 h – 17.00 h Pausa cafè

17.00 h – 18.30 h Comunicacions

Moderador: Ramon Barnadas

18.30 h – 19.30 h Conferència convidada

Control of biological activity by light

Pau Gorostiza, Institut de Biotecnologia de Catalunya (IBEC), Barcelona

Presenta: Pere Garriga

14.50 h – 16.30 h. COMUNICACIONS

Moderador: Joan-Ramon Daban

Xavier Viader-Godoy, Joan Camunas-Sole, Felix Ritort. **Length-dependence of the elastic response and secondary structure of single-stranded DNA**

Mercè Tena-Campos, Eva Ramon, Dasiel O. Borroto-Escuela, Kjell Fuxe and Pere Garriga. **Different oligomerization states of brain GPCRs as novel therapeutic targets in depression**

Gisela Cabré, Aida Garrido, Pau Gorostiza, Jordi Hernando, Félix Busqué, Ramon Alibés. **New Azobenzene Derivatives for Light-Induced Control of Neuronal Signalling**

L. Asensio, A.Severino and F.Ritort. **Single molecule study of an RNA thermosensor**

Li-Ying Wang, Vidhya M. Ravi, Gérard Leblanc, Esteve Padrós, Josep Cladera, and Alex Perálvarez-Marín. **Exploring the conformational landscape of the melibiose transporter**

M. Rico-Pasto, M. Ribezzi-Crivellari, and F. Ritort. **Heat capacity change directly measured in single molecule experiments**

María Guadalupe Herrera-Hernández, Eva Ramon and Pere Garriga. **Effects of quercetin on rhodopsin mutants associated with retinitis pigmentosa**

17.00 h – 18.30 h COMUNICACIONS

Moderador: Ramon Barnadas

Garrido-Charles A, Izquierdo-Serra M, Bautista-Barrufet A, Trapero A, Díaz-Tahoces A, Camarero N, Pittolo S, Valbuena S, Pérez-Jiménez A, Gay M, García-Moll A, Rodríguez-Escrich C, Lerma J, de la Villa P, Fernández E, Pericàs MÀ, Llebaria A, Gorostiza P. **Optical control of endogenous receptors and cellular excitability using targeted covalent photoswitches.**

Maribel Marín, Joan Suades, Ramón Barnadas. **Carbonylic metalosurfactants: a new concept in carbon monoxide releasing systems**

B. Gumí-Audenis, F.Carlá, F.Sanz, F.Comin, M.I.Giannotti and L.Costa. **Custom AFM for X-Ray beamlines: *in situ* characterization of lipid bilayers**

Marta Gironella, M. Ribezzi-Crivellari, F. Ritort. **Viscoelastic properties of red blood cells**

Andrea Chicano, Eva Crosas, Benjamin D. Engel, Joaquín Otón, and Joan-Ramon Daban. **3D structure of chromatin plates from metaphase chromosomes by cryo-electron tomography and synchrotron X-ray scattering**

N. Benseny, E. Álvarez-Marimon, H. Castillo-Michel, E. Aso, I. Ferrer, J. Cladera. **Bioimaging Alzheimer's Disease (AD) plaques, lipid oxidation and metal ions distribution by combination of synchrotron radiation spectroscopic techniques: µFTIR and µXRF**

18.30 h – 19.30 h Conferència convidada

Pau Gorostiza. **Control of biological activity with light**

Resums

Length-dependence of the elastic response and secondary structure of single-stranded DNA

Xavier Viader-Godoy[1], Joan Camunas-Soler[1], Felix Ritort[1,2]

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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 how is the elastic response of ssDNA - and to obtain the elastic parameters that define it - and the formation of secondary structures that are modulated by non-specific base pairing and the electrostatic interactions.

Furthermore, force spectroscopy techniques have been widely used to study biochemical and enzimatic processes involving DNA. The interpretation of the results obtained by these experiments, such as [1] requires an accurate description of the elastical properties of ssDNA. However, the elastic properties of ssDNA have been less studied than those of dsDNA, and have been mainly focused on its dependence with the salt concentration [2], despite the discrepancy found in the elastic parameters depending on the length of the molecules studied [2,3].

In this work we study the elastic properties of different molecules of ssDNA (from 60 bases to 14kbases) and the formation of the secondary structure.

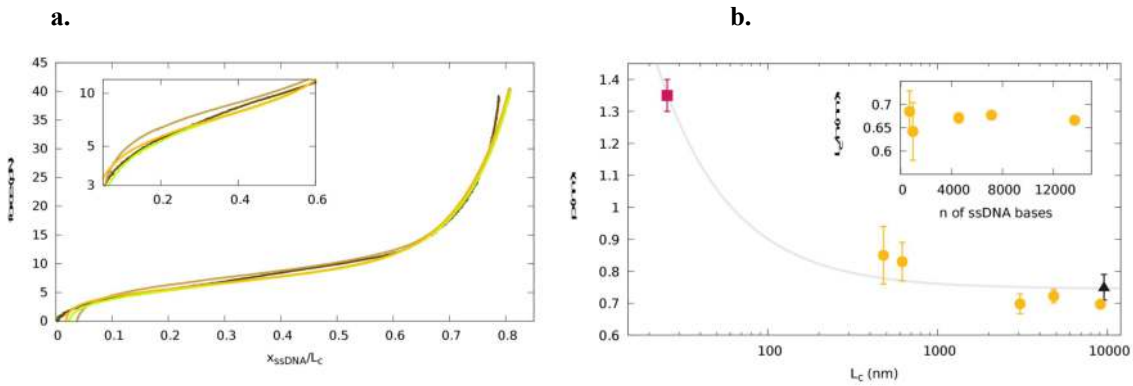

Fig. 1. a. The elastic parameter of the persistence length(p) has a inverse dependence on the total length of the molecule, as shown in this figure where p is represented as a function of the contour length of the molecule. The magenta and black points are from [2] and [1], respectively. **b.** Force-extension curves for different-length hairpins. The behaviour of the secondary structure is length independent, in good agreement with the short-range interactions proposed in [5].

The dependence of the elastic properties found in ssDNA is unexpected, since a inverse dependence is found, which contrasts deeply with the results for dsDNA, which presents a decreasing persistence length for shorter molecules [4]. On the other hand, no dependence on the length has been found for the secondary structure.

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Different oligomerization states of brain GPCRs as novel therapeutic targets in depression

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The serotonin receptor type 1A (5-HT_{1A}), the galanin receptor type 1 ($GalR_1$) and the orphan receptor 39 (GPR39) belong to G-protein-coupled receptors (GPCRs). All three GPCRs share, a relationship with the pathophysiology of unipolar depression associated with the presence of zinc[1,2]. The current paradigm is that these receptors do not function as monomeric units but though complexes involving specific interactions with themselves or other members of the same family. This increases their functional possibilities exponentially, allowing versatility from a fixed number of receptors, and increasing the number of pharmacological approaches[3]. 5-HT_{1A}-$GalR_1$ heterodimerization has been previously described as an antagonistic interaction that could lead to unipolar depression. We have characterized such interaction using Surface Plasmon Resonance spectroscopy in combination with FRET (Förster Resonance Energy Transfer) and we find that this interaction is impaired by zinc. We have also found the following interactions: GPR39-5-HT_{1A}, 5-HT_{1A}-$GalR_1$ and the GPR39-5-HT_{1A}-$GalR_1$ trimer, using the mentioned methodologies together with Gene Reporter Luminescent assays. Monomeric and oligomeric forms have different signaling capacities depending on zinc concentration. Thus, it is proposed that all putative receptor configurations could be present in the human brain, and that the presence of a specific receptor arrangement would be regulated by zinc. Further investigation on the detailed molecular mechanism of the effect of zinc on these complexes should allow the development of new drugs.

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New azobenzene derivatives for light-induced control of neuronal signalling

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The activity of ionotropic glutamate receptors (iGluRs), the main responsible of excitatory currents in the central nervous system, can be remotely controlled by means of light-responsive molecules. This is the case of MAG compounds, which are composed of a **maleimide** moiety for receptor binding, a **photoisomerisable azobenzene group** and a **glutamate agonist**.[1,2]

In our group we are currently developing new MAG switches capable to trigger iGluRs upon two-photon excitation with near infrared light, which enables deeper penetration depths in biological tissues with minimal biological degradation.[3,4] This requires push-pull substitution of the azobenzene core to maximize its two-photon response. In this presentation, new MAG compounds synthesized along these design principles are presented, with which we have demonstrated the optical control of iGluRs.

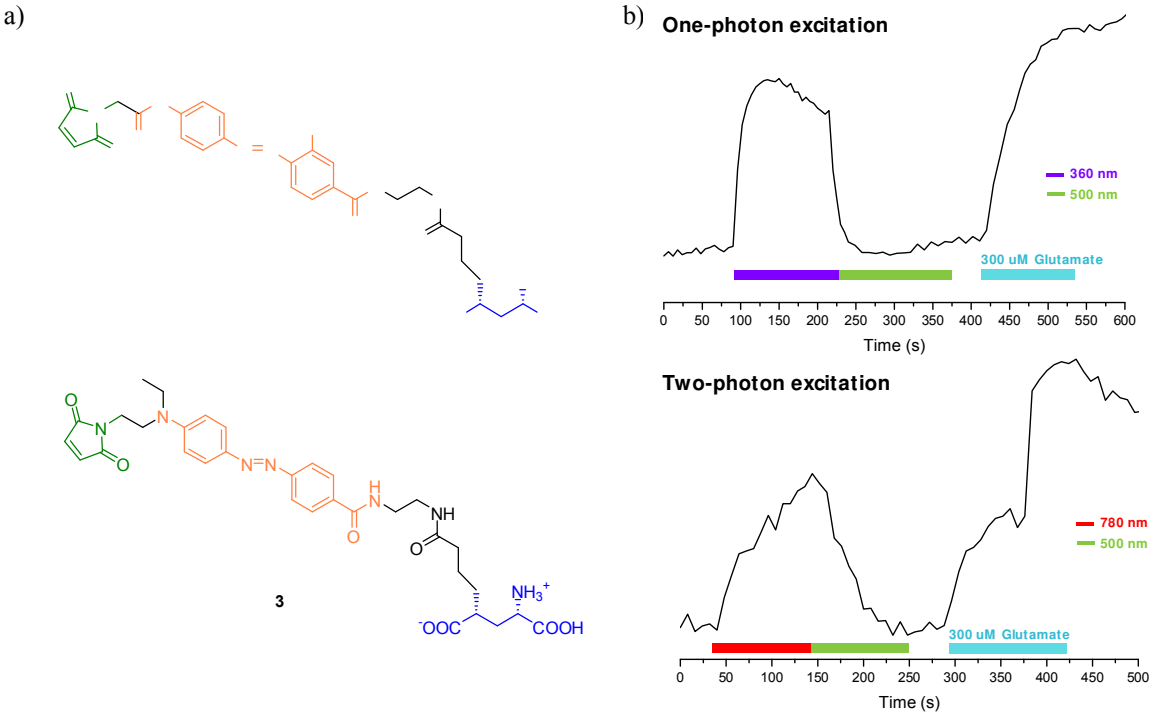

Figure. a) Structure of MAG-derivatives with expected enhanced two-photon absorption; b) Calcium imaging traces of MAG-derivative **2** under one-photon (360 nm) and two-photon (780 nm) excitation to induce ionic channel opening of iGluR.

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Single molecule study of an RNA thermosensor

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Neisseria meningitidis is a human pathogen that has developed subtle mechanisms to evade its host immune system: several genes ensuring its resistance against immune killing are thermoregulated, i.e. increasingly expressed at higher temperature – typically, during host inflammation [1]. The bacterium ability to sense temperature changes is due to special structures occurring in the 5'-UTR region of these genes mRNAs, known as "RNA thermometer" (RNAT) [2]. The *cssA* gene in *Neisseria* is one of such molecules: its zipper-like structure involves base-pairing of the ribosome binding site (RBS) and therefore hinders ribosome binding at low temperature, whereas at higher temperatures the zipper progressively opens ("melts"), the base-pairing of the RBS is destabilized and the ribosome can bind to the mRNA and synthesize the 'immune evasion' proteins.

Here, we studied, by means of laser optical tweezers (LOTs), the wild-type (WT) *cssA* RNAT and a mutant (Δ8), having different thermosensing capabilities. Thanks to the LOTs we can get high accuracy data on the behaviour of single molecules, as we can measure forces in the range of pN and distances in the range of nm, while changing temperature [3,4]. In so-called unzipping experiments, where the molecule is stretched open, we can show that the rupture force of the WT RNAT is higher than that of the Δ8, which is in agreement with the theoretical predictions (MFold) for the sequences. We also observe a high heterogeneity in the unfolding modes of the WT molecule, with a low force branch (3-6 pN) and a high force branch (7-9 pN) featuring a kinetic intermediate (fig. 1, left). A thorough investigation suggests (see notably fig. 1, right) that this intermediate state may play the role of missfolded state on the unfolding pathway.

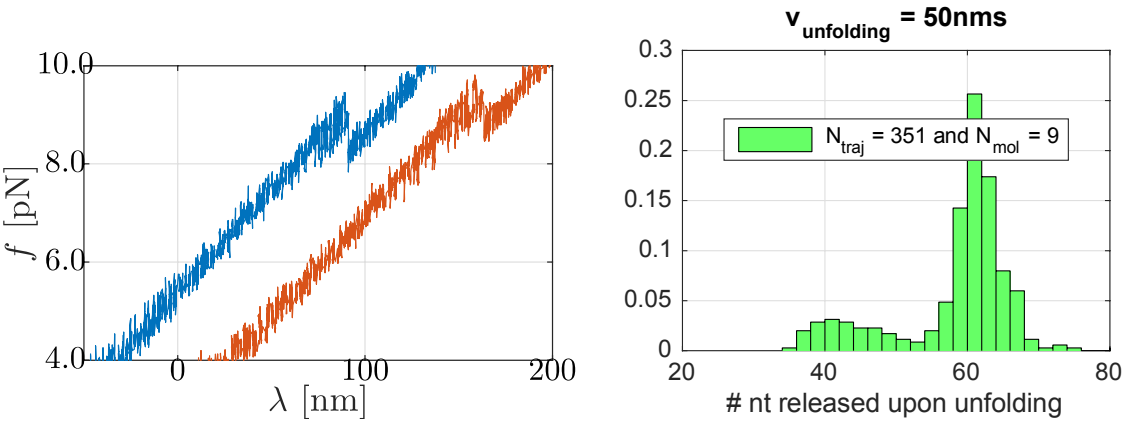

Fig. 1: Left figure features two typical trajectories of the *cssA* RNAT where the intermediate appears as a kinetic state before the unfolding of the putative native structure. In the right there is a distribution plot of the number of nucleotides released in unfolding the *cssA* RNAT: two clear states can be identified. The main mode is compatible with the expected native-unfolded transition, the second could be caused by a missfolded on pathway (stable intermediate).

These preliminary results seem to be consistent with the biologically expected thermosensing properties of the molecules. The analysis of similar unfolding experiments carried out at different temperatures is currently underway.

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Exploring the conformational landscape of the melibiose transporter

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Molecular dynamics simulations have been used to study the alternate access mechanism of the melibiose transporter from *Escherichia coli*. Starting from the outward-facing partially occluded form, 2 out of 12 simulations produced an outward full open form and one partially open, whereas the rest yielded fully or partially occluded forms. The shape of the outward-open form resembles other outward-open conformations of secondary transporters. During the transporter opening, conformational changes in some loops are followed by changes in the periplasm region of transmembrane helix 7. Helical curvature relaxation and unlocking of hydrophobic and ionic locks promote the outward opening of the transporter making accessible the substrate binding site. In particular, FRET studies on mutants of conserved aromatic residues of extracellular loop 4 showed lack of substrate binding, emphasizing the importance of this loop for making crucial interactions that control the opening of the periplasmic side. This study indicates that the alternate access mechanism for the melibiose transporter fits better into a flexible gating mechanism rather than the archetypical helical rigid-body rocker-switch mechanism.

Heat capacity change directly measured in single molecule experiments

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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 have been determined by bulk experiments, e.g. calorimetry [1] and UV absorbance [2]. Melting temperature experiments have been done to determine the heat capacity change [3]. The melting temperature of a molecule, e.g. a DNA hairpin, is defined as the temperature at which half of the DNA strands in the sample are in the double-stranded DNA (dsDNA) and the single-stranded DNA (ssDNA) forms. In the last 20 years, single molecule experiments have become a powerful, accurate and bulk-complementary methods to characterize thermodynamic parameters such as base-pair energy contributions and folding free energies [4].

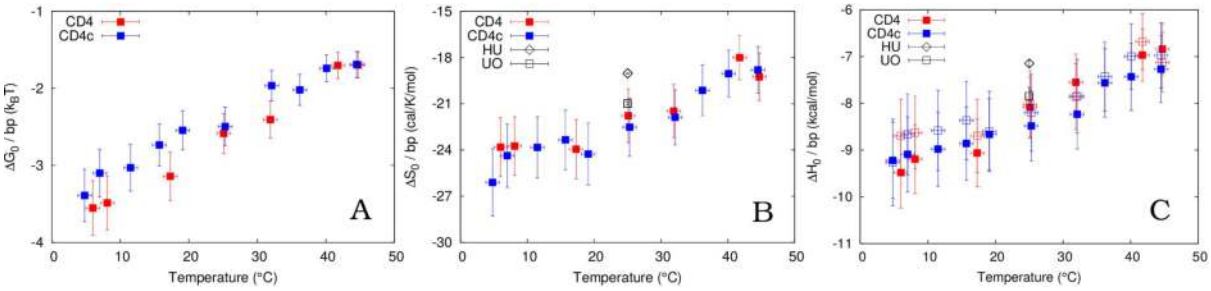

Fig. 1 Folding free energy (A), Entropy (B) and Enthalpy (C) as a function of temperature measured using an Optical Tweezers setup with a temperature controller.

In this work, we propose a novel method to determine the enthalpy, the entropy and the heat capacity change from the force dependence kinetic rates at one unique salt concentration, in contrast with the traditional bulk experiments, where the melting temperature is changed by tunning the salt concentration. From our point of view, this bulk approach left the door opened to an other possible interpretation: It could be that the non zero heat capacity change reported in these previous works was an effect of the salt concentration not to the temperature? With our methodology we overcome this dilemma because keeping constant the salt concentration in all the temperature range we have obtained a non zero heat capacity change.

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Effects of quercetin on rhodopsin mutants associated with retinitis pigmentosa

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Mutations in rhodopsin are associated with retinitis pigmentosa (RP), a group of inherited visual diseases that cause retinal degeneration in which progressive loss of rod and cone photoreceptor cells leads to blindness. A number of studies on rhodopsin RP mutants have been carried out in order to elucidate the molecular mechanisms of the disease as a necessary first step before suitable therapeutic approaches can be developed. Some of the proposed treatments have been based on pharmacological rescue, in which small molecules known as chemical or pharmacological chaperones bind and stabilize misfolded opsins.

Polyphenols are natural compounds with antioxidant activity that have been proposed as useful agents against retinal toxicity (1) but no clear evidence of the effect of this compounds at the visual phototransduction system level has been presented so far.

The aim of the current work was to evaluate the effect of one of such polyphenols, quercetin, on the functional properties of WT rhodopsin and the G90V mutant associated with RP. For this purpose, these receptors were expressed in mammalian COS-1 cells, in the presence of quercetin, regenerated with 9-*cis*-retinal and subsequently immunopurified. After purification, the rhodopsins were characterized by UV-vis and fluorescence spectroscopies and biochemical assays. Functional characterization of the purified receptors was carried out. Differences in photobleaching and acidification were observed for G90V in the presence of quercetin. For both WT rhodopsin and the G90V mutant, the thermal and chemical stability were improved in the presence of quercetin. The Meta II decay for G90V was doubled in the presence of quercetin. Also, the functional assay showed a significant change in the kinetics of transducin activation in the samples treated with quercetin. HPLC-ESI-MS/MS analysis confirmed the presence of quercetin in the purified samples.

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Optical control of endogenous receptors and cellular excitability using targeted covalent photoswitches

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Light-regulated drugs allow remotely photoswitching biological activity and enable plausible therapies based on small molecules. However, only freely diffusible photochromic ligands have been shown to work directly in endogenous receptors and methods for covalent attachment depend on genetic manipulation. Here we introduce a chemical strategy to covalently conjugate and photoswitch the activity of endogenous proteins and demonstrate its application to the kainate receptor channel GluK1. The approach is based on photoswitchable ligands containing a short-lived, highly reactive anchoring group that is targeted at the protein of interest by ligand affinity. These targeted covalent photoswitches (TCPs) constitute a new class of light-regulated drugs and act as prosthetic molecules that photocontrol the activity of GluK1-expressing neurons, and restore photoresponses in degenerated retina. The modularity of TCPs enables the application to different ligands and opens the way to new therapeutic opportunities.

Carbonylic metalosurfactants: a new concept in carbon monoxide releasing systems

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Despite carbon monoxide (CO) is usually considered to be highly toxic for humans, low concentrations of this gas is known to induce beneficial anti-inflammatory, neuroprotective and apoptotic effects.[1] Since the discovery of its therapeutic activities, a great number of studies have been carried out in order to develop carbon monoxide releasing molecules (CORMs) as an alternative method for a save and controlled delivery of this gas. Following this aim we have synthetized two families of molybdenum carbonylic metalosurfactants (MTS) to be used as CORMs in mixed systems formed with phospholipids, specifically phosphatidylcholine (PC). MTS are obtained by means of coordination of tensioactive linear phosphines with different length of their hydrocarbon chains ($Ph_2(CH_2)_nSO_3Na$, with n= 2, 6, 10) to the fragments $\{Mo(CO)_4\}$ and $\{Mo(CO)_5\}$. }. In a recent communication, we reported the amphiphilic properties of these compounds, which exhibit molecular self-assembly in water, forming micellar/bicellar and/or vesicular systems.[2]

Depending on the PC:MTS ratio, different type of aggregates (vesicles, micelles, bicelles, etc) can be obtained when they are prepared by hydration of homogeneous dry films composed by mixtures of PC and MTS. In order to assess their ability as CORMs, the CO release was evaluated spectrophotometrically by measuring the conversion of deoxy myoglobin (DeoxyMb) to carbonmonoxy myoglobin (MbCO).[3] Moreover, the CO release can be accelerated by irradiating the samples with UV or visible light. This study was carried out following the changes in the carbonyl bands of the MTS using FTIR spectrometry.

Toxicity of the mixed systems obtained was determined in human dermal fibroblasts using the XTT assay, showing that our vesicular systems are not toxic in all the evaluated range (0-1mM). On the contrary, pure compounds and micellar systems present more toxicity, being toxic above 100-250 µM, depending on the compound.

In resume, results suggest that mixed PC/MTS systems are good candidates for being used as CORMs.

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Custom AFM for X-Ray beamlines: *in situ* characterization of lipid bilayers

B. Gumí-Audenis[1,2,3,4], F.Carlá[4], F.Sanz[1,2,3], F.Comin[4], M.I.Giannotti[1,2,3] and L.Costa[5]

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The Atomic Force Microscope (AFM) is an instrument to characterize surfaces at the nanoscale at the single molecule level in the direct space and it can also provide information about their mechanical properties. However, the resolution obtained by AFM might be inferior to the one achievable with X-Rays (XR) techniques Indeed, XR techniques such as XR reflectivity (XRR) and Grazing Incidence XR diffraction (GIXD) are powerful tools to characterize surfaces at the nanoscale, providing structural information in the reciprocal space through the interaction between XR and the sample electronic structure. Nevertheless, since those techniques do not require any mechanical interaction with the specimen, mechanical properties cannot be evaluated with XR.
In this work, we present a custom AFM that can be installed as a sample holder for grazing-incidence XR experiments at the solid/gas or solid/liquid interfaces, giving the possibility to study biological samples under physiological conditions [1]. We shall present how the AFM and XR combination allows us to evaluate the radiation damage induced by the beam on DOPC and DPPC supported bilayers (SB) under liquid environment as well as to study the phase transition of DPPC bilayers.

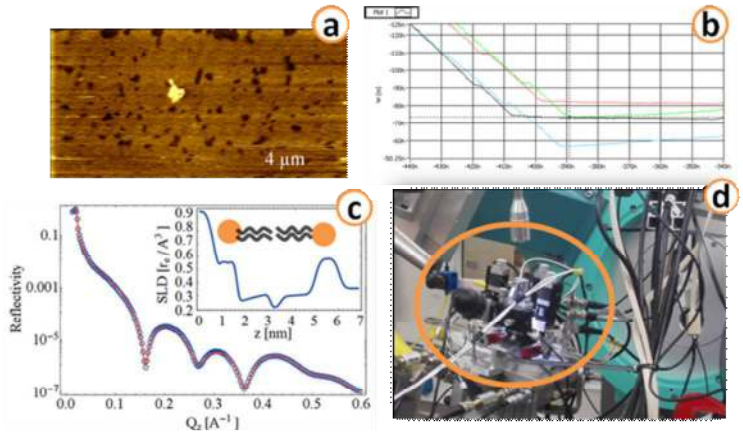

Figure. DPPC SB results using the custom AFM for XR beamlines: a) AFM topography; b) Force-Spectroscopy curves; c) XRR (Inset: Scattering Density Profile); d) Custom AFM for XR beamlines mounted at ID03 (ESRF)

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Viscoelastic properties of red blood cells

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Targeted therapies are one of the most promising advances in the fight against cancer. They require the differentation and characterization of the malignan and healthy cells with hight accuracy. Fluctuation membrane spectroscopy is an extremely reliable technique that allows us to characterize the mechanics of the cells such as the drag coefficient and the membrane stiffness. In order to have a full and precise knowledge of our results we have started studying the mechanics of red blood cells (RBC) whics are one of the simplest cell types. We are able to measure the global and local membrane deformability using two different experimental procedures: (1) Applying a flow to a trapped micron-sized bead attached to the RBC membrane (2) Measuring the force signal fluctuations of the RBC membrane using micron-sized beads coated with fibronectin that bind to the cellular membrane. Once completed this study we will be able to use the gained knowledge to differentiate heterogeneous cellular populations.

3D structure of chromatin plates from metaphase chromosomes by cryo-electron tomography and synchrotron X-ray scattering

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We have used cryo-electron tomography and small-angle X-ray scattering to investigate the chromatin folding in metaphase chromosomes. Cryo-electron tomography has allowed us to study chromosome structure in a vitrified and close-to-native state. Our three-dimensional reconstructions show that frozen-hydrated chromatin emanated from metaphase chromosomes is planar and form multilayered plates, as previously observed in our laboratory using different techniques. Plate thickness measurements show that each single layer is ~10 nm thick, which is equivalent to the nucleosome diameter. These measurements, combined with the observation of many plates contained in the three-dimensional reconstructions, indicate that each plate is formed by a tightly packed single layer of nucleosomes. The same nucleosome organization has been observed in staked layers, but distance measurements in contacting regions between two plates show a thickness of ~16 nm. This distance is smaller than that expected for the sum of two single layers (~20 nm), and indicate that the nucleosomes from both plates are interdigitated and that each layer has an apparent thickness of ~6 nm, as proposed in our thin-plate model for the chromosome internal structure. Plates show dense bars along their cross-sections, which are more or less parallel between them and perpendicular to the surface of the plates. Taking into account the thickness of each layer, these bars could correspond to nucleosomal DNA wrapped around histone octamers. X-ray scattering of whole chromosomes under metaphase ionic conditions shows a main scattering peak at ~6 nm, which can be correlated with the distance between nucleosomes interacting through their faces in interdigitated layers. Furthermore, we have observed very large chromatin structures formed by many stacked plates, which have a width equal to the chromosome diameter. All these observations reinforce previous results of our laboratory and support a compact chromosome model consisting of many interdigitated chromatin layers stacked along the chromosome axis. Our results indicate that nucleosomes are oriented with their flat faces approximately perpendicular to the surface of the plate, allowing a lateral interaction between nucleosomes of adjacent layers which gives stability to the overall structure.

Bioimaging Alzheimer's Disease (AD) plaques, lipid oxidation and metal ions distribution by combination of synchrotron radiation spectroscopic techniques: µFTIR and µXRF

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Alzheimer´s Disease (AD) is a neurodegenerative disease characterized by the presence of neurofibrillary tangles (NFT), senile plaques (SP) and elevated levels of metal ions such as iron, copper and zinc [1]. To deeper understand the relationship between amyloid aggregates, lipid oxidation and metal cation distribution two synchrotron radiation spectroscopic techniques were used on human brain tissues of affected AD individuals at stages V and VI and healthy controls. On one hand, the micro-Fourier Transformed Infrarred (µFTIR) allowed us to localize the aggregated areas of the tissue corresponding to amyloid aggregates and analyze the lipid oxidation of the tissue. In previous studies of the group it was already demonstrated, using µFTIR, the 'in situ' co-localization of AD plaques and lipid oxidation. Interestingly no oxidation is observed in plaques of individuals that do not show signs of dementia [2]. On the other hand, the use of micro-X Ray Fluorescence (µXRF) allowed us to bioimage at high resolution the metal cation distribution. The µXRF results show that Fe, Cu, Zn and Ca ion maps co-localize with the plaques and their surroundings with a cation content in these regions well above the level measured in the controls. Finally, 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.

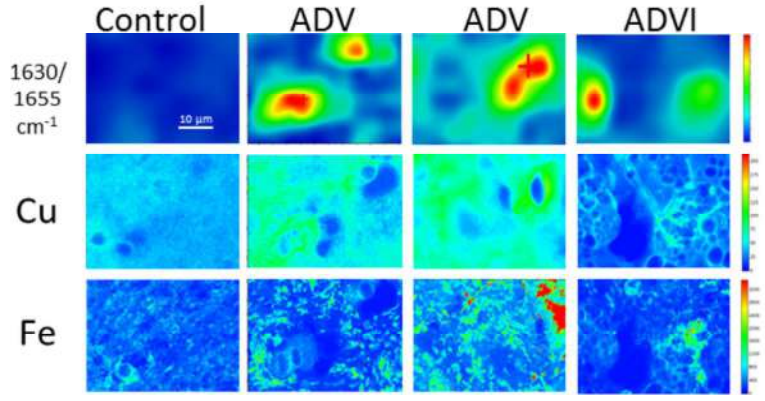

Figure. µFTIR and µXRF maps of a healthy control, ADV-a, ADV-b and AD stage VI tissue sections are shown. In the first row, µFTIR maps of 1630/1655 cm^{-1} ratio show the aggregates in red. Next rows correspond to µXRF of Cu and Fe. For all maps intensities were normalized and expressed as red – maximum (214 Cu cps, 3500 Fe cps) and blue – minimum (0 Cu, Fe cps).

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Control of biological activity by light

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The large number of photoswitchable biomolecules discovered and developed in recent years covers a great variety of cellular functions like catalysis of metabolic processes, cytoskeletal polymerization and motors, nucleic acids dynamics, intracellular signaling and perhaps most dazzlingly membrane excitability, which has been at the focus of optogenetics and photopharmacology. The dream of precisely and remotely photocontrolling every aspect of the cell's inner workings in intact tissue appears within reach and offers the promise of interrogating complex cellular processes to discover their molecular mechanisms. In this talk I will review recent and ongoing projects in the lab focused on light-regulated ligands, including the development of peptide inhibitors of protein-protein interactions, allosteric modulators of G protein-coupled receptors and photoswitchable tethered ligands of ionotropic receptors.