Good Laboratory Practices in Molecular-Scale Biophysics: an ARBRE Symposium

June 15th 2023
Campus Tech La Doua – Villeurbanne (F)

WELCOME TO THE FIRST IN PERSON EVENT OF ARBRE

ARBRE is a non profit charity founded on July 1st 2021, whose statutes and internal bylaws were deposited and approved by the Prefecture de Lyon (France) in October 2021.

ARBRE aims to foster a large-scale pan-European clustering around molecular-scale biophysics, allowing to ally and synergize the power of spectroscopic, hydrodynamic, real-time microfluidic, thermodynamic and single-molecule approaches. In partnership with MOSBRI (https://www.mosbri.eu/), it is the optimal environment for the development of innovative integrative biophysical approaches, at the level of data acquisition, analysis and modelling, as well as for the design of unprecedented and ambitious combinations of methodologies, to decipher more efficiently crucial biological phenomena and to overcome significant biomedical challenges.

ARBRE will provide a platform for scientists to establish early contacts with instrument developers (at the level of concept or prototype), allowing to set-up win-win partnerships that will allow to define and develop together future instrumentation that genuinely meets the needs of the broad biomedical and life sciences communities.

This symposium is the first one of a series of activities, such as Conferences, Workshops and Training Schools, which will favour the exchanges of good practices and continue the success of the COST Action "Between Atom and Cell: Integrating Molecular Biophysics Approaches for Biology and Healthcare (MoBiEU – molecular biophysics in Europe)".

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Amphitheater Marie Curie – Campus Tech La Doua – 43 bd 11 Novembre 1918 – 69100 Villeurbanne – France

9h00 – 10h00: Onsite registration and posters setup

10h-00 – 11h00: Sample quality control, benchmarking & standards – *Chair: Josef Houser*

Welcome, logistics and a couple of words on ARBRE – *Adriana E. Miele* (start streaming)

20' – **Lena Bauernhofer** – University of Graz (AT) - Measuring the cellular copy number and antibody affinity of an oncoprotein in a native lipid-bilayer environment

10' – **Caroline Mas** – IBS Univ. Grenoble Alpes (F) - Analytical Ultracentrifugation Sedimentation Velocity for the Characterization of Recombinant Adeno-Associated Virus Vectors

10' – **Sophie Ayciriex** – ISA UCBL (F) - molecular characterization of lipid metabolism by mass spectrometry and multimodal imaging

10' – **Josef Houser** – Masaryk University Brno (CZ) - Benchmarking in biophysics: Insight from the ARBRE society

10' – Q&A

11h00-11h15 – coffee break

11h15 – 12h25: Macromolecular interactions & tools – *Chair: Paloma Fernandez-Varela*

20' – **Antoine Royant** – ESRF & IBS (F) – Engineering Fluorescent proteins by rational design

20' – **Carlo Travaglni-Allocatelli** – Sapienza University of Rome (I) – Biophysical tools to investigate the structural and dynamic properties of the KH domains of FMRP

10' – **Agnès Hagège** – ISA CNRS (F) - The CE-ICP-MS coupling, a powerful tool for describing protein/metal interactions

10' – **Vincent Chaptal** – MMSB UCBL (F) - Conformational space exploration of cryo-EM structures by variability refinement

10' – Q&A

12h25 – 13h35 – Lunch and poster session

13h35 – 14h25: Technology Alert – *Chair: Maggy Hologne*

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10' – **Dina Nicolaes** – Nicoya – Characterization of Therapeutic Antibodies using Digital SPR

10' – **Amandine Gontier** – Dynamic Biosensors – How to characterize the mode of action and avidity effects of antibodies using switchSENSE® and RT-IC

10' – **Aymeric Audfray** – Malvern – Pushing The Boundaries in Biomolecular Interaction Analysis with the Creoptix WAVEsystem

10' – Q&A

14h25 – 15h25: Lipids, vesicles & membrane proteins – *Chair: Patrick England*

20' – **Valeria Vetri** – ARBRE & University of Palermo (I) – Sensing peptide-membrane interactions using quantitative fluorescence microscopy

10' – **Loïck Moissonier** – MMSB UCBL (F) - Time-resolved structural transitions of the multidrug transporter BmrA using rapid kinetics and time-resolved cryoEM

10' – **Sabrina Romanò** – Univ Cattolica Sacro Cuore (I) - Biophysical techniques in cancer-derived extracellular vesicles: an FT-IR spectroscopy approach

10' – **Isabel Alves** – CMBN CNRS (F) - Impact of membrane lipid polyunsaturation on dopamine D2 receptor ligand binding and signaling

10' – Q&A

15h25 – 15h35 – Tea break & group photo

15h35 – 17h15 – Poster session

15h35 – 17h15 – ARBRE General Assembly for members – *Chair Stefan H. Knauer*

17h15 – 17h30 – Closing remarks (end streaming)

Sample quality control & standards

Molecular characterization of lipid metabolism by mass spectrometry and multimodal imaging

Sophie Ayciriex * [1]

[1] University of Lyon, University Claude Bernard 1 of Lyon, Institute of Analytical Sciences, CNRS, UMR 5280 – University of Lyon, University Claude Bernard 1 of Lyon, Institute of Analytical Sciences, CNRS, UMR 5280 – France

Aquatic ecosystems are constantly exposed to environmental stressors such as chemical micropollutants from the environment or human activities. These contaminants can alter the internal chemical homeostasis of aquatic organisms, visible at the molecular level. At present, the lipid disturbances induced by drug residues, which can be found in river waters, on aquatic species such as *Gammarus fossarum* are not known. A major bottleneck is the lack of molecular information at the genome level in some species of environmental relevance. Multi-Omics approaches are used to characterize molecular responses to toxic exposures in aquatic organisms during vulnerable life cycle stages. In crustaceans, lipids play essential roles in vulnerable stages such as molting, reproduction and development. To access the molecular characterization of lipids in *G. fossarum*, methods employing high-resolution mass spectrometry (Shotgun Lipidomics) and innovative multiplexed approaches in targeted mass spectrometry have been developed and applied in adult organisms (male, female), embryos or in different organs (cephalon, muscle, hepatopancreas). The use of multimodal mass spectrometry imaging (MALDI with ion mobility, ToF-SIMS) enable to localize *in situ* the spatial distribution of lipids in tissue sections of adult gammarids before and after exposure to pravastatin.

Keywords: Lipidomics, mass spectrometry, MS imaging, sample preparation

*Speaker

Measuring the cellular copy number and antibody affinity of an oncoprotein in a native lipid-bilayer environment

Lena Bauernhofer * [1,2,3], Alison Ilsley [4], Sean Devenish [4], Carolyn Vargas [1,2,3], David Glueck [1,2,3], Dagmar Zweytick [1,2,3], Sabrina Riedl [1,2,3], Tuomas Knowles [4,5,6], Sebastian Fiedler [4], Sandro Keller [1,2,3]

[1] Biophysics, Institute of Molecular Biosciences (IMB), NAWI Graz, University of Graz – Austria
[2] BioTechMed-Graz – Austria
[3] Field of Excellence BioHealth, University of Graz – Austria
[4] fluidic analytics – United Kingdom
[5] Centre of Misfolding Diseases, Yusuf Hamied Department of Chemistry, University of Cambridge – United Kingdom
[6] Cavendish Laboratory, Department of Physics, University of Cambridge – United Kingdom

We have devised a novel method for accurately determining the absolute concentrations and affinities of target receptors in native membrane-protein libraries, extracted directly from live cells. To validate our approach, we focused on HER2, a membrane protein overexpressed in several cancers, including gastric, lung, and breast cancer. As a diagnostic marker and a therapeutic target, HER2's role in treatment is of particular interest.

HER2-positive cancer patients are commonly treated with combination immunotherapies, including monoclonal antibodies like trastuzumab and pertuzumab (trade names Herceptin and Perjeta, respectively), which we also used in our experiments. However, not all patients overexpressing HER2 respond to these antibody treatments. This inconsistency may be due to significant variations in the concentration of full-length (binding-competent) HER2 from patient to patient. Thus, quantifying cellular copy numbers of binding-competent HER2 protein could enable more precise personalization of therapeutic strategies for individual patients.

To address this need, we propose a quick and straightforward method for measuring Membrane-Protein Affinity and Copy Number (MAffCoN). We demonstrate the effectiveness of this assay by determining the cellular copy number of the HER2 protein and its binding affinity to trastuzumab and pertuzumab. The key to our method lies in the usage of polymer-encapsulated nanodiscs, which enable the protein to stay embedded in its native lipid-bilayer environment. Utilizing microfluidic diffusional sizing (MDS), we then measured the binding affinity of HER2 to fluorescently labeled trastuzumab, yielding subnanomolar dissociation constants (KD) and absolute numbers of several millions of binding-competent HER2 protein copies in each breast cancer cell (HCC1419 and AU565).

MAffCoN is easily adaptable in standard research laboratories and exhibits the potential for integration into or enhancement of existing drug-discovery workflows. We also foresee potential applications of this assay in diagnostic settings, such as immune profiling, differential diagnostics, or tissue analysis.

*Speaker

Keywords: lipid nanodiscs, membrane proteins, microfluidic diffusional sizing, oncoproteins

Benchmarking in biophysics: Insight from the ARBRE society

Josef Houser * [1]

[1] Masaryk University [Brno] – Czech Republic

Benchmarking (i.e. comparison of quality and performance in a specifically designed way) of instruments and methods is a valuable tool to improve the experiments and services in the field of biophysics. It allows not only to compare the performance of individual laboratories/facilities but also helps to define the strong and weak points in particular measurement set-ups, offers an independent evaluation of individual instruments, and therefore contributes to the retaining of the overall quality of science. Therefore, ARBRE has chosen benchmarking as one of its topics since the very beginning. Within the ARBRE-MOBIEU action, we have organized several runs of benchmarking focused on various methods, e.g. MST (1) or ITC (2). The results were very beneficial and so we would like to continue in these activities further, covering more techniques as well as offering a platform for the design of more specific benchmark studies.

(1) Lopez-Mendez, B. et al. England, P., Jowitt, T.A. Reproducibility and accuracy of microscale thermophoresis in the NanoTemper Monolith: a multi laboratory benchmark study. Eur Biophys J 50, 411-427 (2021). https://doi.org/10.1007/s00249-021-01532-6

(2) Velazquez-Campoy, A. et al. A multi-laboratory benchmark study of isothermal titration calorimetry (ITC) using Ca2+ and Mg2+ binding to EDTA. Eur Biophys J 50, 429-451 (2021). https://doi.org/10.1007/s00249-021-01523-7

Keywords: benchmarking, biophysical methods

*Speaker

Analytical Ultracentrifugation Sedimentation Velocity for the Characterization of Recombinant Adeno-Associated Virus Vectors

Caroline Mas * [1], Sylvie Saleun [2], Aline Le Roy [3], Magalie Penaud-Budloo [2], Oumeya Adjali [2], Blouin Veronique [2], Christine Ebel [3]

[1] Univ. Grenoble Alpes, CNRS, CEA, EMBL, ISBG, F-38000 Grenoble – Centre National de la Recherche Scientifique – France
[2] Nantes Université, CHU Nantes, INSERM, UMR 1089, Translational Research in Gene Therapy, TaRGeT, F-44000 Nantes, France – Institut National de la Santé et de la Recherche Médicale - INSERM – France
[3] Univ. Grenoble Alpes, CNRS, CEA, IBS, F-38000 Grenoble – Centre National de la Recherche Scientifique - CNRS – France

Recombinant adeno-associated virus virus-derived vectors (rAAVs) are among the most used viral delivery system for *in vivo* gene therapies with a good safety profile. However, rAAV production methods often lead to a heterogeneous vector population, in particular with the presence of undesired empty particles. Analytical ultracentrifugation sedimentation velocity (AUC-SV) is considered as the gold analytical technique allowing the measurement of relative amounts of each vector subpopulation and components like particle aggregates, based on their sedimentation coefficients. Principle and practical examples of AUC experiments for rAAVs characterization will be presented. In addition to classical detection at 260 nm, interference optics can provide an independent estimate of weight percentages of the different populations of capsids, and of the genome size incorporated in rAAV particles. Moreover, we used AUC-SV to assess time stability of a rAAV.

Keywords: Adenoassociated virus vector, Analytical Ultracentrifugation, Sedimentation Velocity

*Speaker

Macromolecular Interactions & tools

Conformational space exploration of cryo-EM structures by variability refinement

Vincent Chaptal * [1]

[1] Microbiologie moléculaire et biochimie structurale (MMSB) – Centre National de la Recherche Scientifique - CNRS, Université Claude Bernard Lyon 1, UMR5086, Université Claude Bernard Lyon 1 – France

Cryo-EM observation of biological samples enables visualization of sample heterogeneity, in the form of discrete states that are separatable, or continuous heterogeneity as a result of local protein motion before flash freezing. Variability analysis of this continuous heterogeneity describes the variance between a particle stack and a volume, and results in a map series describing the various steps undertaken by the sample in the particle stack. While this observation is absolutely stunning, it is very hard to pinpoint structural details to elements of the maps. In order to bridge the gap between observation and explanation, we designed a tool that refines an ensemble of structures into all the maps from variability analysis. Using this bundle of structures, it is easy to spot variable parts of the structure, as well as the parts that are not moving. Comparison with molecular dynamics simulations highlight the fact that the movements follow the same directions, albeit with different amplitudes. Ligand can also be investigated using this method. Variability refinement is available in the *Phenix* software suite, accessible under the program name *phenix.varref*.

Keywords: Cryo, EM, Variability analysis, Structure modelling, phenix.varref

*Speaker

The CE-ICP-MS coupling, a powerful tool for describing protein/metal interactions

Agnès Hagège * [1], Coraline Duroux

[1] Institut des Sciences Analytiques (ISA) – CNRS : UMR5280 – 5 rue de la Doua 69100 Villeurbanne cedex, France

For many years, the analytical chemistry community has been interested in protein/metal interactions, in a discipline known as metalloproteomics.
Reliable thermodynamic data describing protein-metal interactions are the subject of numerous publications. Spectroscopic techniques (UV, fluorescence, circular dichroism, NMR, etc.) have proved their capacity in this field. However, these techniques do not always offer the required sensitivities. Moreover, sample consumption (in the range of several tens of μl) limit studies to proteins available in large quantities.
In this context, we have developed a system based on the coupling of capillary electrophoresis (CE) and inductively coupled plasma-mass spectrometry (ICP-MS). In addition to its low sample consumption, the system's versatility in terms of electrolyte allows to approach biological conditions.
With examples drawn from uranium toxicology, copper involvement in Alzheimer disease or bacterial silver resistance, we will illustrate the potential of this tool in protein/metal interactions studies. Particularly, demonstration of its ability to determine complexation constants from a few nanomoles of protein will be done. We will also show how to determine the stoichiometry of complexes and assess the labile/inert character of the interactions ($<$ 2h).

Keywords: protein/metal interactions, capillary electrophoresis, ICP/MS

*Speaker

Engineering fluorescent proteins by rational design

Antoine Royant * [1,2]

[1] Institut de Biologie Structurale (IBS) – Université Grenoble Alpes, Commissariat à l'Énergie Atomique et aux Énergies Alternatives (CEA) - Grenoble, Centre National de la Recherche Scientifique - CNRS – 71, Avenue des Martyrs, F-38000 Grenoble, France
[2] European Synchrotron Radiation Facility (ESRF) – European Synchrotron Radiation Facility (ESRF) – France

The Synchrotron Group of the IBS operates two instruments at the ESRF, the French macromolecular crystallography beamline BM07-FIP2 and the *in crystallo* optical spectroscopy laboratory *ic*OS. The combination of the two techniques is ideal for the study of coloured proteins, in particular for that of fluorescent proteins used in cell imaging. We have been working for 15 years on the structural characterization of bright fluorescent proteins with emission colour ranging from cyan to infrared, in order to identify structural determinants of high fluorescence quantum yield and of other specific properties (photochromism, generation of reactive oxygen species). I will describe our contribution to the development of the bright red fluorescent proteins of the mScarlet family and our efforts to understand the mechanism of photosensitization of the green fluorescent flavoprotein miniSOG.

Keywords: Xray crystallography, optical spectroscopy, fluorescent protein

*Speaker

Biophysical tools to investigate the structural and dynamic properties of the KH domains of FMRP

Carlo Travaglini Allocatelli * [1]

[1] Dip. Scienze Biochimiche, Università degli Studi di Roma "La Sapienza" = Sapienza University [Rome] – Italy

K homology (KH) domains are conserved RNA binding domains often found in proteins playing essential roles in RNA metabolism and regulation by interacting with specific RNA motifs and participating in mRNA splicing, translation, and localization.
FMRP (Fragile X Mental Retardation Protein) is a multidomain protein which plays crucial roles in brain development and physiology and containing three consecutive KH domains (KH0, KH1 and KH2). FMRP deficiency, or the presence of missense mutations in its KH domains, is the cause of the fragile X syndrome (FXS), a genetic disorder characterized by intellectual disability, behavioral problems, and developmental delays in humans. However, although the structure of the isolated KH domains of FMRP is known, structural information of the entire protein is yet unavailable. Moreover, it is still unclear how KH domain mutations impact on the overall structure and/or the physiological roles of FMRP and we do not know if the 3 KH domains act independently or synergistically.
Following a minimalist approach, we started to investigate the stability, folding mechanism, and aggregation propensity of the isolated KH domains KH0 and KH1 and their pathological variants R138Q and G266E, respectively. Our results show interesting differences in the folding mechanism of the wild-type domains and their propensity to form amyloid-like aggregates in vitro. The effects of the pathological mutations on the KH domain architecture have also been investigated by a variety of biophysical techniques, from NMR spectroscopy to SAXS experiments and molecular dynamic simulations, which highlighted the substantial destabilization of the KH1 domain in the G266E variant, ultimately leading to a complete unfolding of the domain.

Keywords: protein folding, protein aggregation, pathological mutations, FMRP

*Speaker

Technology alert

Pushing The Boundaries in Biomolecular Interaction Analysis with the Creoptix WAVEsystem

Aymeric Audfray * [1]

[1] Malvern Panalytical – Malvern Panalytical – France

Biomolecular interactions are crucial in many biological processes and consequently, their characterization is important in many research and development fields like drug discovery. Several label-free technologies are now well established like ITC (Isothermal Titration Calorimetry), for in solution interactions, or SPR-like (Surface plasmon resonance), for interactions on a chip. The GCI (Grating-Coupled Interferometry) technology, developed by Creoptix, brings breakthrough innovations to traditional SPR-like methods. The high sensitivity (very high ligand/analyte weight ratios), the design of the new microfluidic (no risk of clogs, inserted into the chip not in the system) and waveRAPID (only one concentration enough to measure entire kinetics) allow to considerably expand the application domains (small molecules, antibodies, crude extract, membrane proteins, entire viruses,...) of label-free technologies using only one device: The WAVE system (https://www.malvernpanalytical.com/en/products/category/optical-biosensors). This presentation will show the basic principle of GCI, its advantages against traditional interaction analysis techniques and application examples.

Keywords: GCI, Creoptix, Macromolecular interaction

*Speaker

Dynamic Biosensors – How to characterize the mode of action and avidity effects of antibodies using switchSENSE® and RT-IC

Amandine Gontier * [1]

[1] Dynamic Biosensors GmbH – Germany

Dynamic Biosensors – How to characterize the mode of action and avidity effects of antibodies using switchSENSE® and RT-IC

*Speaker

Working smart with FIDA: rapid characterization of proteins and particles directly in crude matrix

Albasini Marion * [1]

[1] Fidabio – Denmark

FIDA technology is a novel technology developed for quantification and biophysical characterization. The readouts from FIDA are a unique combination of structural information as well as functional binding characterization. FIDA is a "first principle" methodology meaning that assay readout can be predicted based on protein structure (PDB files) and Kd's. By being an in-solution technology, FIDA avoids complications related to non-specific surface adsorption.

Based on the absolute and direct in-solution measurements of hydrodynamic radius (Rh in nm), FIDA offers a wealth of information. In every measurement, you have QC parameters including Complex formation, Aggregation, Stickiness and Potential sample loss. A measurement only takes 4 minutes, and a few nL to analyze your sample "as is", i.e., labeled or label-free, no immobilization, no buffer constraints, temperatures from 4-55 deg. C, purified or non-purified, etc.

In this talk, we introduce the fundamentals of FIDA technology and share data on applications for macromolecular interaction and particle characterization with assessment of 8 QC parameters.

Keywords: characterization, particle, protein, membrane protein, IDP, crude matrix, label free, sample QC, macromolecular Interaction, new technology, FIDA, Fida 1, TDA, First principle

*Speaker

Characterization of Therapeutic Antibodies using Digital SPR

Dina Niculaes * [1], Michael Piazza [1], Ryan Denomme [1]

[1] Nicoya Lifesciences Inc. – Canada

Aim/hypothesis: In collaboration with **Sino Biological**, a global leader in research reagents, we demonstrate how Alto is an ideal platform for therapeutics development through crude library screening, binding kinetics characterization, and epitope binning of Sino Biological's influenza products.
Research Design and Methods: Sino Biological reagents:

- Recombinant Influenza A H5N1 Hemagglutinin/HA Protein (Cat# 11048-V08H1)
- Influenza A H5N1 Hemagglutinin/HA Antibodies (Cat# 11048-MM11, 11055-MM08, 11055-RM06, 86001-RM01, 11048-MM04, 11048-MM10, 86001-RM02, 11048-MM03, 11048-RM07, 11048-RM08, 11048-RM09, 11048-MM06, 11048-MM14, 11048-MM01, 11048-MM05)

Nicoya's Alto system uses digital microfluidics (DMF) to deliver automatically diluted sample droplets to SPR sensors for effortless real-time characterization of biomolecular interaction analysis including quantitation, screening, epitope binning, and binding kinetics.
Results: Compared to conventional SPR, Alto reduces operator time and labor, and reduces antigen consumption by about 200x. *Figure 1* shows an example of a screening assay for anti-HA antibodies in serum binding to hemagglutinin (HA) antigen captured on the Alto biosensor.
Alto offers powerful time, labor, and reagent savings for running traditional multi-cycle kinetics (MCK) or single-cycle kinetics (SCK) on up to 48 unique interactions per cartridge. For each analyte, Alto measures five unique concentrations by automatically performing 3-fold serial dilutions.
Alto allows users to design a 16x16 assay to characterize the simultaneous binding of monoclonal antibodies to an antigen, tested in a pairwise manner. In the classical sandwich assay format, the antigen is captured by up to 16 unique surface-coupled antibodies, which is followed by the pairwise binding of solution antibodies. Alto uses only 100 ng of each antibody for the entire experiment.
Conclusions: Alto facilitates crude sample screening, accurate kinetics analysis, and 16x16 epitope binning of Sino Biological's influenza products for accelerated development of antibody therapeutics. By leveraging DMF technology Alto streamlines SPR analysis by automating sample dilutions, eliminating fluidic maintenance, and reducing sample requirements by up to 200x.

*Speaker

Keywords: Digital SPR, kinetic analysis, epitope binning, antibody therapeutics, crude sample screening

Lipids, vesicle & membrane proteins

IMPACT OF MEMBRANE LIPID POLYUNSATURATION ON DOPAMINE D2 RECEPTOR LIGAND BINDING AND SIGNALING

Isabel Alves * [1]

[1] CBMN, UMR 5248 – Université de Bordeaux – France

Increasing evidence supports a relationship between lipid metabolism and mental health. In particular, the biostatus of polyunsaturated fatty acids (PUFAs) correlates with some symptoms of psychiatric disorders, as well as the efficacy of pharmacological treatments. Recent findings highlight a direct association between brain PUFA levels and dopamine transmission, a major neuromodulatory system implicated in the etiology of psychiatric symptoms. However, the mechanisms underlying this relationship are still unknown. Here, in a study going all the way from *in vivo* to molecular scale analysis and implicating several laboratories, among which the CBMN, we demonstrate that membrane enrichment in the w-3 PUFA docosahexaenoic acid (DHA), potentiates ligand binding to the dopamine D2 receptor (D2R), suggesting that DHA acts as an allosteric modulator of this receptor. Molecular dynamics simulations confirm that DHA has a high preference for interaction with the D2R and show that membrane unsaturation selectively enhances the conformational dynamics of the receptor around its second intracellular loop. We find that membrane unsaturation spares G protein activity but potentiates the recruitment of β-arrestin in cells. Furthermore, *in vivo* w-3 PUFA deficiency blunts the behavioral effects of two D2R ligands, quinpirole and aripiprazole. These results highlight the importance of membrane unsaturation for D2R activity and provide a putative mechanism for the ability of PUFAs to enhance antipsychotic efficacy.

Reference: Impact of membrane lipid polyunsaturation on dopamine D2 receptor ligand binding and signaling. Marie-Lise Jobin et al., *Nature Molecular Psychiatry* 2023; https://doi.org/10.1038/s41380-022-01928-6 *in press.*

Contact: Isabel D. Alves, i.alves@cbmn.u-bordeaux.fr

Keywords: receptor, lipids, omega 3, psychiatric disorders

*Speaker

Time-resolved structural transitions of the multidrug transporter BmrA using rapid kinetics and time-resolved cryoEM'

Loïck Moissonnier * [1], Pierre Falson , Vincent Chaptal *

[1] MOISSONNIER – Université Claude Bernard-Lyon I - UCBL (FRANCE) – France

According to the World Health Organization (WHO), antibiotic resistance is a predominant and worrying health problem for humanity due to the emergence of multi-resistant bacteria. The first line of defense of these bacteria is the overexpression of ABC (ATP-Binding Cassette) efflux pumps that expel antibiotics out of the bacteria below their cytotoxicity threshold. The drug is thus inactive and the bacteria can develop, and with this low exposition to the drug can start to acquire resistance by mutating the target. The *Bacillus subtilis* ABC transporter BmrA confers resistance to cervimycin C, an antibiotic secreted by *Streptomyces tendæ*, a natural competitor for the same biotope. BmrA has a broad specificity for a variety of structurally and chemically unrelated molecules conferring to the pump a multidrug efflux capacity.

To better understand the details of the mechanism carried out by BmrA, the team solved its X-ray and cryo-EM structures, the latter in presence of a substrate in pre-release state, never isolated before. These data shed light on how BmrA handles its ligands and expels them. The team recently showed that BmrA structurally deforms in specific regions to accommodate its ligand rhodamine 6G and proposed a new mechanism of structural adaptation to the substrate using intrinsic structural plasticity.

In the present work, we aim at defining and characterizing the biomechanical parameters of the drug efflux mechanism, in a time-resolved manner. I first identified the conditions allowing me to identify the conformational changes biochemically and enzymatically. Then I moved on and characterized structurally the conformational changes using time-resolved cryoEM. This multidisciplinary and integrative approach will give us access to the structural dynamics of the pump in the millisecond to second time-scale, and will further our understanding of drug handling by these transporters.

Keywords: time, resolved cryoEM, fluorescence, membrane proteins, structural dynamics

*Speaker

BIOPHYSICAL TECHNIQUES IN CANCER-DERIVED EXTRACELLULAR VESICLES: AN FT-IR SPECTROSCOPY APPROACH

Sabrina Romanò * [1], Riccardo Di Santo [1], Flavio Di Giacinto [1], Ewa Krasnowska [2], Marco De Spirito [1], Gabriele Ciasca [1]

[1] Università cattolica del Sacro Cuore = Catholic University of the Sacred Heart [Roma] – Italy
[2] Consiglio Nazionale delle Ricerche – Italy

Extracellular vesicles (EVs) are membrane-enclosed vesicles released into the extracellular space by most cell types. Given their key role in cell-cell communication and their specific molecular cargo, EVs are considered an extremely promising source of biomarkers for cancer diseases. The characterization of EVs with biophysical techniques is recently getting much attention due to the possibility of translation in clinical practice. The development of novel and effective methods for the label-free characterization of EVs is still in its infancy. Such practices are highly required to use EVs as tumor biomarkers in liquid biopsies.
Since Fourier Transform Infrared Spectroscopy (FTIR) allows for direct access to the characteristic absorption bands of biomolecules, it has the concrete potential to provide a versatile platform for EVs characterization for diagnostic and classification purposes. Here, we used FTIR in the mid-IR range to investigate the composition of EVs and discriminate their origin from different cellular phenotypes and serum-derived EVs from patients diagnosed with hepatocellular carcinoma (HCC) and healthy subjects. The efficacy of the technique was first tested on an in vitro model using the human colorectal adenocarcinoma intestinal cell line Caco-2 to discriminate EVs from two different cell states through an induced epithelial-mesenchymal reverse transition (EMT). As EVs released into the tumor microenvironment play an important role in the progression of HCC, FTIR spectroscopy was applied to EVs obtained from the serum of patients diagnosed with HCC of metabolic origin and healthy donors.
Statistical and machine learning methods were applied to both models to highlight possible spectral biomarkers of the disease, as well as for testing methods for automated classification. We have demonstrated that mid-IR spectroscopy is a powerful tool to label-free biochemical characterization of EVs with potential diagnostic applications. Our studies may have a positive impact on the development of FTIR spectroscopy as a biophysical tool for EVs-based liquid biopsy for cancer diagnosis.

Keywords: Extracellular vesicles, FT, IR, Cancer disease, Liquid biopsy

*Speaker

Sensing peptide-membrane interactions using quantitative fluorescence microscopy

Sara Anselmo [1], Giuseppe Sancataldo [1], Valeria Vetri * [1]

[1] Università degli studi di Palermo - University of Palermo – Italy

The detailed analysis of peptide–membrane interactions is of fundamental interest in both basic and applied sciences. These interactions are implied in a broad range of functional processes, such as protein trafficking, cellular signaling, and ion channel formation. Moreover, they are believed to play a fundamental role in pathogenic events like the ones related to amyloidosis. Highly dynamic and heterogeneous mechanisms are involved in these phenomena which strongly depend on both the membrane composition, structural details of the peptides (e.g. charge, hydrophobicity, steric hindrance), and on the environmental conditions. Peptides may insert into or lay on the bilayer and/or create intra-membrane pores possibly inducing membrane destabilization, thinning or breakage whit different effects on the biological system.
Here, we report results illustrating the fate of the multifunctional amphiphilic peptide Transportan 10 (TP10) and disentangling different events occurring during the interaction with giant lipid vesicles used as model membrane.
Our study, based on the use of suitable fluorescence reporters, exploits the advantages of Fluorescence lifetime imaging microscopy and phasor plot analysis to distinguish whether the peptide is adsorbed or inserted in the membrane with high spatial resolution. The coupled use of Laurdan and di-4-ANEPPDHQ, fluorescent dyes allowed to highlight events at different depth of phospholipid bilayers: carpetting, insertion and pore formation are observed at different peptide concentrations. Structural and conformational changes of the peptide at the membrane interface are highlighted leading to reorganization at molecular level and progressive dehydration of the membrane.

Keywords: FLIM, Phasor approach, fluorescence, cell penetrating peptide, giant vesicles, transportan 10

*Speaker

Poster Session

A better understanding of the interplay between SilF and SilB proteins in the resistance mechanism of the silver efflux pump

Cyrielle Arrault * [1,2], Yoan Monneau [1,3], Martin Marie [1], Francois-Xavier Cantrelle [4], Emmanuelle Boll [4], Fabien Chirot [2,5], Clothilde Zerbino [5], Olivier Walker [1,2], Maggy Hologne [1,2]

[1] Institut des Sciences Analytiques – CNRS : UMR5280 – France
[2] Université Claude Bernard Lyon 1 – Université de Lyon – France
[3] St Jude Childrenś Research Hospital – United States
[4] Unité de Glycobiologie Structurale et Fonctionnelle - UMR 8576 – Université de Lille, Centre National de la Recherche Scientifique – France
[5] Institut Lumière Matière [Villeurbanne] – Université Claude Bernard Lyon 1, Centre National de la Recherche Scientifique – France

The resitance of Gram-negative bacteria to silver ions is mediated by a silver efflux pump, which mainly relies on a tripartite efflux complex SilCBA, a metallochaperone SilF and an intrinsically disordered protein SilE. However, the precise mechanism by which silver ions are extruded from the cell, and the different roles of SilB, SilF and silE remain poorly understood. To address these questions, we employed Nuclear Magnetic Resonance and Mass spectrometry to investigate the interplay between these proteins. We first solved the solution structures of SilF in its free and Ag +-bound forms and we demonstrated that SilB exhibits two silver binding sites at its N- and C-termini. Conversely to the homologous Cus system, we evidenced that SilF and SilB interact without the presence of silver ions and that the rate of silver dissociation is eight times faster when SilF is bound to SilB, indicating that silver SilE does not bind to either SilF or SilB, regardless of the presence or absence of silver ions, further corrobarating that it merely acts as a buffer that prevents the cell from being overloaded with silver.

Keywords: NMR, silver resistance, silver efflux pump

*Speaker

Highly Accessible OpenSPR™ System for Kinetic Analysis of Molecular Interactions

Nicolas Carvou * [1], Dina Niculaes *

[1], Michael Piazza [1]

[1] Nicoya Lifesciences Inc. – Canada

Surface plasmon resonance (SPR) is a gold standard in biologics research and the development of safe and effective treatments against diseases. The data derived from SPR can help better understand molecular mechanisms and provide key insights into signaling pathways that are critical in advancing drug development.
Nicoya's OpenSPR™ is the world's first benchtop surface plasmon resonance (SPR) instrument and was designed to open access to SPR analysis to research groups looking for flexibility and ease of use.
Thanks to its innovative nanoplasmonic sensor technology, robust hardware, and intuitive software interface, OpenSPR provides real-time, label-free analysis of affinity and kinetics data for a wide range of biomolecular applications. The applications highlighted in this poster include characterization of oligonucleotide interactions, virus-like particles, and antibody-antigen in serum. With its user-friendly interface and compact benchtop footprint, the OpenSPR is the perfect tool for SPR analysis at any level.

Keywords: Surface plasmon resonance, SPR, biophysics, molecular dynamics, protein interactions, Biochemistry, antibody discovery, antibody engineering

*Speaker

switch your SENSE of studying macromolecular interactions

Vincent Morin [1], Jessica Soamalala [1], Paloma Fernandez Varela * [1]

[1] Institute for Integrative Biology of the Cell (I2BC) – CEA, CNRS, Université Paris-Saclay, UMR9198, CEA Saclay, 91191 Gif-sur-Yvette – France

Our team 'Genome Integrity' (IntGen) in collaboration with the platform 'Interaction of macromolecules' (PIM) at the Institute for Integrative Biology of the Cell (I2BC), proposed the swithSENSE technology (Dynamic Biosensors) since 2017. This technique uses nanolevers bound on gold surface to measure macromolecules interactions, in particular DNA/RNA binding proteins. This approach is complementary to other surface methods like BioLayer Interferometry (BLI) and Surface Plasmon Resonance (BLI). In January 2023, the new generation switchSENSE instrument, heliX+, was installed in place of the first generation DRX2. This is the first instrument implemented in France. Among its unique features: 1) It can resolve fastest kinetics with confidence using an advanced microfluidics with two separated circuits (buffer, wash), and a 10 ms data collection (Nemoz, 2018, NSMB, Velours, 2021, EBJ). 2) Molecular interactions can be detected with femto-molar sensitivity. 3) It allows screening in 96 and 384 well plates, and ranking of small molecule inducing conformational changes. 4) It allows to follow enzymatic activities including polymerases, ligases and nucleases. We have recently set up an activity test for the human ligase4 protein, which is involved in the DNA repair pathway of DNA double strand breaks, and analyze the inhibitory effect of small molecules.

Keywords: DNA repair, NHEJ, protein/DNA, protein/protein, protein/small molecules interactions

*Speaker

Micro-Exon Gene (MEG) products: dissecting IDP by NMR

Adriana Erica Miele * [1,2], Stepanka Nedvedova [2,3], Florence Guilliere [2], Jan Dvorak [3], Olivier Walker [2], Maggy Hologne [2]

[1] Department of Biochemical Sciences, Sapienza University of Rome – P.le Aldo Moro 5, 00185 Rome, Italy
[2] Institute of Analytical Sciences, UMR 5280 CNRS UCBL University of Lyon – Université Claude Bernard-Lyon I - UCBL (FRANCE), Centre National de la Recherche Scientifique - CNRS – France
[3] CZU University of Life Sciences - Prague – Czech Republic

The parasitic worm *Schistosoma mansoni* has a complex genome structure, including 7 autosomes and a pair of sexual chromosomes. Among other genes, it expresses a peculiar superfamily of secreted proteins called micro-exon genes (MEG) products. MEGs are encoded by very short exons (6-81 bp), interspersed by long introns (0.5-1.5 kbp) and flanked by transposon-like sequences. We have determined by multidimensional NMR, with normal isotopic abundance, the first 3D structure of 3 isoforms of the MEG 2.1 family, demonstrating their IDP nature and their stickiness. In parallel, we have also analysed all the 87 validated protein sequences and proposed a model for their filiation, duplication and spread over the genome. In the poster we shall also present the strategies we have applied after the failure in heterologous expression in bacteria, yeast, insect cells and cell-free, in order to perform the structural analysis by Circular Dichroism, Dynamic Light Scattering and NMR.

Keywords: IDP, NMR, DLS, CD, parasitic diseases

*Speaker

Characterization of SmVAL13 protein in order to develop a new screening test for Bilharzia

Guillaume Verhaeghe * [1], Florence Guilliere [1], Olivier Walker [1], Adriana Miele [1]

[1] Institut des Sciences Analytiques – Université Claude Bernard Lyon 1, Centre National de la Recherche Scientifique – France

Bilharzia, also known as Schistosomiasis, is a parasitic infection caused by trematode worms of the genus *Schistosoma*. In this research project we study a protein from the specie *Schistosoma mansoni*, which has a complex life cycle. The early symptoms of the disease are common to many other co-endemic parasitic diseases and the available diagnostic tests are unable to detect an early infection. (**1**) This work focuses on the protein called *S.mansoni* Venom Allergen-Like 13 (SmVAL13), because it is expressed more or less at all stage of the life cycle of the parasite (**2**), and released in the host blood stream, thus it would facilitate its detection. Knowing the structure of SmVAL13 and further its interaction with a specific antibody would help to develop a new test based on an antigen-antibody interaction. Such a test must be specific, sensitive, quick, and cheap. It could be used on a large scale to screen at-risk populations for the disease. In the poster we report our preliminary results, on SmVAL13 purification and quality control by SDS-PAGE, dynamic light scattering (DLS), circular dichroism (CD) and NMR.

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Keywords: Bilharzia, SmVAL13, NMR, SDS, PAGE, CD, DLS

*Speaker

List of participants

- Alves Isabel
- Arrault Cyrielle
- Audfray Aymeric
- Ayciriex Sophie
- Bauernhofer Lena
- Carvou Nicolas
- Cesard Elizabeth
- Chaptal Vincent
- Di Primo Carmelo
- Ebel Christine
- Elisabeth Laurent
- England Patrick
- Fernandez Varela Paloma
- Fraccia Tommaso
- Gontier Amandine
- Guilliere Florence
- Hagège Agnès
- Hamacek Josef
- Hologne Maggy
- Houser Josef
- Keller Sandro
- Knauer Stefan
- Leang Anabelle
- López Méndez Blanca
- Maniti Ofelia

- Marion Albasini
- Mas Caroline
- Miele Adriana Erica
- Moissonnier Loïck
- Niculaes Dina
- Offmann Bernard
- Pauleta Sofia
- Roman Szczepanowski
- Romanò Sabrina
- Royant Antoine
- Sarazin Benoît
- Schaffner Irene
- Schuetz Georg
- Sedivy Arthur
- Skowronek Krzysztof
- Verhaeghe Guillaume
- Vetri Valeria
- Walker Olivier
- Walser Reto

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