"Between atom and cell"

MOSBRI
Molecular-Scale Biophysics Research Infrastructure

1st MOSBRI Scientific Conference

20th -22nd June 2022, Institut Pasteur, Paris

Showcasing how our integrated network
can tackle an unusually wide variety of life science research questions

PROGRAMME BOOK

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This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101004806

INSTITUT PASTEUR

WELCOME TO INSTITUT PASTEUR

Molecular-scale biophysics is the study of the architecture, dynamics and interactions of the giant molecules of life (proteins, DNA, RNA, polysaccharides, lipids) via measurements of their physical properties. It is fundamental to both our understanding of how living organisms work, and our ability to block or enhance the function of these molecules, notably for therapeutic purposes. Deciphering the complexity of the behaviour of macromolecular assemblies requires a multi-faceted approach resorting to a large variety of distinct biophysical methods.

MOSBRI (Molecular-Scale Biophysics Research Infrastructure www.mosbri.eu) is a geographically distributed, scientifically and technologically integrated consortium, combining the distinct instrumentation and expertise of 13 academic centres of excellence and 2 industrial partners from 11 different European countries, coordinated by Institut Pasteur (Paris, France) and funded by the Horizon 2020 programme of the European Commission.

The first MOSBRI conference will show how such an integrated network can tackle an unusually wide variety of life science research questions. It will include presentations from MOSBRI partners and Scientific Advisory Board members, as well as from scientists that have already benefitted from the free of charge Trans-National Access (TNA) opportunities that MOSBRI provides.

MOSBRI
Molecular-Scale Biophysics Research Infrastructure

INSTITUT PASTEUR

SPEAKERS

Claudio Canale, Genova, Italy

Tatiana Charnavets and **Jan Dohnalek**, Vestec, Czech Republic

Francesca Cutruzzola, Rome, Italy

Pierre Dorlet, Marseille, France

Patrick England and **Bertrand Raynal**, Paris, France

Maria Garcia Alai and **Osvaldo Burastero**, Hamburg, Germany

Chris Genick, Basel, Switzerland

Rob Gilbert, Oxford, United Kingdom

Søren Vrønning Hoffmann, Aarhus, Denmark

Hajira Ahmed Hotiana, Copenhagen, Denmark

Adela Karhanova, Vestec, Czech Republic

Natalia Markova, Malvern, United Kingdom

Adriana Erica Miele, Lyon, France

Neza Omersa, Ljubljana, Slovenia

Vincent Raussens, Brussels, Belgium

Wouter Roos and **Rifka Vlijm**, Groningen, Netherlands

Juan Sabin, Santiago de Compostela, Spain

Javier Sancho and **Adrian Velazquez Campoy**, Zaragoza, Spain

Anna Sobiepanek, Warsaw, Poland

Rifka Vlijm, Groningen, Netherlands

Eleonore Von Castelmur, Linköping, Sweden

Mark Williams, London, United Kingdom

Barbara Zambelli, Bologna, Italy

SCIENTIFIC ORGANIZING COMMITTEE

Gregor Anderluh

Francesca Cutruzzola

Patrick England

Bertrand Raynal

Javier Sancho

Adrian Velazquez Campoy

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GENERAL INFORMATION

On the Institut Pasteur campus, the "Plan Vigipirate Attentats" is on, so please make sure to have an official ID or passport to enter the campus.

If your registration is fully settled, you will be given a badge that you should wear at all times. The certificate of attendance will be sent after the conference by email.

ACCESS TO THE CONFERENCE CENTER

The only access to the Conference Center is located at
205, rue de Vaugirard, Paris 15th - Pasteur Volontaires metro Line 12

The welcome desk opens at 1:00 pm on Monday, June 20th, 2022.

OPENING HOURS OF THE CONFERENCE CENTER - VAUGIRARD ACCESS

- Monday June 20th, 2022: 1:00 pm - 7:30 pm
- Tuesday June 21st, 2022: 8:00 am - 9:30 pm
- Wednesday June 22nd, 2022: 8:00 am - 1:30 pm

The "Vaugirard" access closes at the indicated times.
Afterwards the exit will be through the main campus exit.

- A cloakroom is available at the auditorium level during all the conference. We ask you not to leave any personal belongings unattended in the auditorium.

- Congress staff assistance is also available during coffee breaks, lunches and cocktails.

GENERAL INFORMATION

PLENARY SESSIONS

Scientific sessions take place in the auditorium of the CIS (Scientific Information Center).

LUNCHES & COFFEE BREAKS

All Coffee breaks and lunches will be served in the hall of CIS. Access to lunches is limited to participants who are registered.

All attendees are invited to the Welcome Reception which will be held on Monday 20th, at 6:35 pm in the hall of the CIS.

FREE ACCESS WIFI

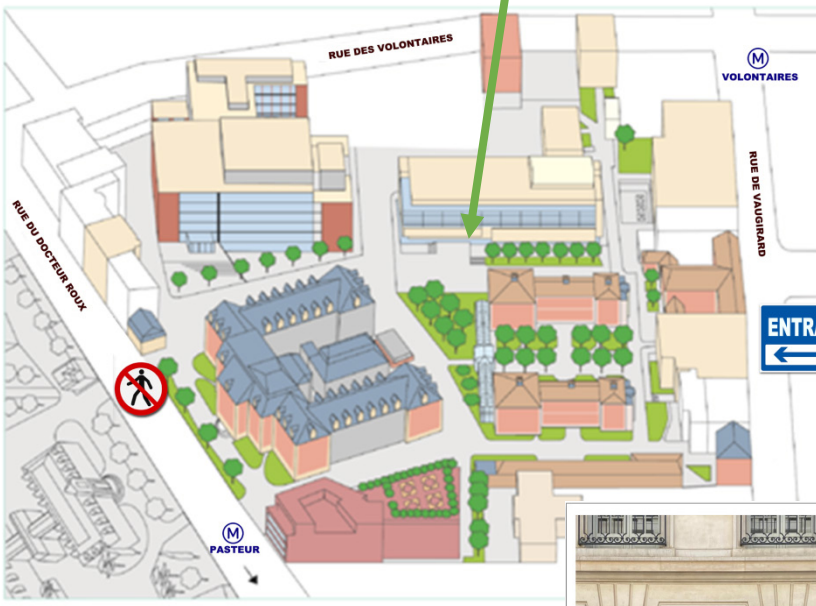
Institut Pasteur Visiteur network

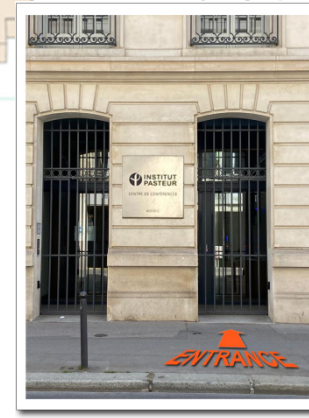
Password : 294106

MAP OF THE CAMPUS

The entrance during the three days of the congress is located at 205, rue de Vaugirard, 75015 Paris

Auditorium CIS
Welcome Desk, Lecture hall, Posters, Cocktail, Lunches and Coffee breaks





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Cyrill Brunner
Bruker Daltonics SPR, Fällanden, Switzerland

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Henrik Jensen
Fidabio, Copenhagen, Denmark

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ORAL COMMUNICATIONS ABSTRACTS

INSTITUT PASTEUR

Pasteur-PFBMI : 20 years old and as dashing as ever!

B. Raynal, S. Brûlé, M. Chevreuil, S. Hoos, P. England*
Molecular Biophysics, Institut Pasteur, Paris, France

For the last 20 years, the Institut Pasteur Molecular Biophysics platform (Pasteur-PFBMI; www.mosbri.eu/partners/pasteur-pfbmi) has always made sure to remain a cutting-edge multi-technological core facility, aiming at potentiating top-level molecular-scale biophysical studies of the properties of biological systems. It federates technical and methodological expertise on 20 instruments, which enable the in-depth characterization of the intrinsic properties of macromolecules and their assemblies and of the interactions in which they are involved. The uniqueness of Pasteur-PFBMI comes from the combination in a single site of a large panel of methodological approaches at expert level, which allows to maximise the type of insights that researchers can obtain about the biological systems they wish to characterize.

In the frame of the MOSBRI TNA scheme, Pasteur-PFBMI offers access to a subset of its instrumentation and expertise, for quality control of purified samples, hydrodynamic characterization of macromolecular assemblies, and kinetic characterization of biomolecular interactions. In a near future, we will also provide scientists worldwide with well-characterized standard samples, notably for training and the evaluation of equipment.

1) Quality control of purified samples. This activity was prompted by the observation that a lot of valuable time is wasted on poor quality samples, confirming the adage "garbage in, garbage out". We have demonstrated that a simple-to-follow workflow, jointly established by the European networks ARBRE-MOBIEU and P4EU, can significantly improve the success rate of research projects.[1,2]

2) Hydrodynamic characterization of the size, shape and architecture of macromolecules and assemblies. We have developed a multi-approach combination to analyze the macroscopic properties of molecular assemblies in solution. Our expertise has allowed to gain insights into key biological processes, such as the pathophysiology of viruses and bacterial toxins [3, 4, 5] or DNA reorganization in mycobacteria[6]…

3) Kinetic characterization of biomolecular interactions. Our longstanding dual expertise in real-time biosensing by surface plasmon resonance (SPR) and biolayer interferometry (BLI) has allowed us to make significant contributions to a wide variety of research projects, on topics such as the development of therapeutic antibodies against targets involved in viral[7] and autoimmune[8] diseases, or the interaction of viral proteins with host membranes[9].

4) Protein standards. We have developed, in collaboration the team of Tom Jowitt (University of Manchester, UK) protein standards[10] that will soon be made available through MOSBRI.

References:

1. **Nat Commun. 2021** 12:2795; **2 Eur Biophys J 2021** 50:453 ; **3** EMBO reports, 2022 e53600; **4** Advanced science 2021 8, 2003630; **5** Frontiers in pharmacology 2021 2538; **6** Structure 2019 27:579; **7** Nature 2016 536:48; **8** Nat Biotechnol 2017 35:977; **9** Science 2017 358:663; **10** Eur Biophys J 2021 50:333

MOSBRI team oral presentations

Synchrotron radiation circular dichroism at MOSBRI partner AU-SRCD

S.V. Hoffmann*, N.C. Jones
ISA; Department of Physics and Astronomy, Aarhus University, Aarhus, Denmark

MOSBRI partner AU-SRCD is based at the Department of Physics & Astronomy at Aarhus University in Denmark. AU-SRCD utilizes synchrotron radiation (SR) produced by the ASTRID2 storage ring, a facility where SR in the UV to the soft X-ray region is produced. Access to the AU-SRCD partner through MOSBRI is offered to advanced Circular Dichroism (CD) beam lines on ASTRID2. Synchrotron Radiation Circular Dichroism (SRCD) spectroscopy offers significant improvements to the well-established method of conventional CD (cCD) spectroscopy. The high photon flux, over a wide range of wavelengths, results in higher signal-to-noise ratios and enables the collection of data at lower wavelengths than possible with cCD spectrometers.

In this talk we present the variety of measurement options at AU-SRCD, the structural informational content of CD and show examples difficult samples where the use of SRCD is highly justified.

TNA beneficiary oral presentations

Characterization of the intrinsically disordered region of human NDRG1, a target for lung cancer therapy

Y. Beniamino[1], V. Cenni[2], E. Mileo[3], S. Ciurli[1], B. Zambelli*[1]
[1]Pharmacy and Biotechnology, University of Bologna, Bologna, Italy [2]Institute of Molecular Genetics, National Research Council, Bologna, Italy [3]CNRS, Bioénergétique et Ingénierie des Protéines, Marseille, France

Nickel compounds, found in cigarette smoke and in fine dusts, are classified as class I lung and nasal carcinogens by IARC and exert their tumorogenic potential by activating the cell hypoxia response. This results in the up-regulation of the human N-myc downstream regulated gene 1 (*h*NDRG1), which is linked to poor prognosis, higher tumor aggressiveness and resistance to chemotherapy in lung cancer.

*h*NDRG1 shares a non-enzymatic a/b hydrolase globular domain with other three members of the *h*NDRG family. In addition, it contains a unique C-terminal sequence of 83 residues, rich in charged residues. This region binds transition metal ions such as Ni(II), is regulated by post-translational phosphorylation and interacts with lipids, suggesting a key role of this region in the physiological function of *h*NDRG1.

In the present work, the structural, biochemical, and biophysical characterization of the C-terminal sequence of *h*NDRG1 (here named *h*NDRG1*C) is reported. Analysis of the sequence assigned it to the family of the intrinsically disordered regions (IDRs). The polypeptide was expressed and purified from *Escherichia coli* and experiments of isothermal titration calorimetry, light scattering, circular dichroism and SDSL-EPR were carried out to establish its metal-binding activity, as well as secondary and quaternary structure. A thorough analysis of the spectroscopic fingerprint of ^{1}H and ^{13}C detected NMR spectra provided detailed information on the effect of pH and Ni(II) binding on the structure. The biophysical data were integrated with the analysis of the Ni(II)-induced expression, subcellular localization and oligomeric states of *h*NDRG1 in a cell line of lung adenocarcinoma. The results are discussed considering the possible role of *h*NDRG1*C in the Ni(II)-driven lung cancer progression.

Company oral presentations

***To affinity and beyond:* New approaches to changing the paradigm in drug discovery**

P. Belcher
Cytiva, Malborough, United States

Drug discovery is becoming slower and more expensive over time, with the efficiency of developing new drugs halving every 9 years, increasing the need for faster, information rich technologies to be applied earlier. While higher throughput label free systems can generate data orders of magnitude greater than previous generations of systems, data analysis has not scaled with instrument throughput. This presentation will outline a machine learning approach that reduces analysis time of SPR data by more than 80% while improving consistency to enable decisions to be made sooner.

Company oral presentations

Light scattering solutions for the characterization of macromolecules

Stéphanie Terme-Ferrari
Wyatt Technology, France

Proteins and related biomacromolecules are complex entities that exhibit fascinating behavior when interacting with other biomolecules. Light scattering provides a simple and effective means for characterizing their essential biophysical properties: molar mass, size, charge, interactions, conjugation and conformation.

Rapid assessments of purity, aggregation and oligomerization may be made using a microwell-plate or microcuvette-based instrument. Studies like formulation screening, temperature stability, colloidal stability or aging process are easily performed.

Multi-Angle Light Scattering coupled with Size Exclusion Chromatography (SEC-MALS) provides accurate molecular weight determination of proteins, oligomers and complexes, regardless of conformation or non-ideal column interactions. That's because SEC-MALS constitutes a rigorous, first-principles analysis of molar mass that does not rely on retention time or calibration with reference molecules. The only function of the SEC column is to separate molecules by size, while MALS determines absolute molar mass of eluting proteins independently.

For samples with challenging separation, the MALS can also be coupled to a Field-Flow Fractionation (FFF), a powerful and unique method to separate by size and characterize all types of analytes ranging in size from 1 nm to 1000 nm.

Invited oral presentations

Biophysical studies of membrane pore formation by bactericidal perforin-2

R. Gilbert
University of Oxford, United Kingdom

Perforin-2 (PFN2, MPEG1) is a key pore-forming protein in mammalian innate immunity, restricting intracellular bacteria proliferation. It forms a membrane-bound pre-pore complex converting to a pore-forming structure upon acidification. A combination of biophysical approaches has been necessary to gain insights into the mechanism of action of PFN2, which has included cryo-electron microscopy, X-ray crystallography, high-speed atomic force microscopy, fluorescence-based activity assays and imaging, and molecular dynamics simulatons. These have allowed determination of structures for pre-pore assemblies and pore-forming complexes, and a tracking of the transition between the two which involves a 180° relative domain rotation while bound to the targeted membrane.

MOSBRI team oral presentations

The EMBL SPC facility

M. Garcia Alai*, O. Burastero
EMBL HH, EMBL, Hamburg, Germany

The EMBL-SPC facility is located next to an integrated infrastructure for structural biology at the PETRA-III ring at DESY Hamburg, open to the international research community. The biophysical platform of EMBL-SPC includes cutting-edge technologies to measure interactions and to precisely determine the stability, shape and size of different biomolecules and biomolecular assemblies. In the context of MOSBRI TNA we offer pipelines for Biophysical characterization of Integral Membrane Proteins and Functionality of membrane transporters. We specialize on Interaction and Time-resolved studies of protein complexes. Evaluation of the data from binding experiments and their fitting is an essential step towards the quantification of binding affinities. Here, we introduce user-friendly online tools to analyze biophysical data from steady-state fluorescence spectroscopy, microscale thermophoresis and differential scanning fluorimetry experiments are presented. The modules of the data-analysis platform (https://spc.embl-hamburg.de/) contain classical thermodynamic models and clear user guidelines for the determination of equilibrium dissociation constants (Kd) and thermal unfolding parameters such as melting temperatures (Tm).

MOSBRI team oral presentations

Combined use of diverse biophysical techniques for the investigation of repetitive extragenic palindromes (REPs) and their associated tyrosine transposases RAYTs

T. Charnavets*, I. Nečasová, B. Schneider
Institute of Biotechnology of the Czech Academy of Sciences, Vestec, Czech Republic

REP elements are noncoding bacterial DNA segments of about 30 nucleotides long that have near palindromic CG-rich sequences. The REP elements are involved in bacterial genomic rearrangements and virulence acquisition, and hence are important elements in bacterial genome evolution. REP elements can be associated with tyrosine transposases (RAYT), which bind REP DNA sequences and their inversion portions (iREP) and catalyze REP and iREP cleavage and transfer to the target site. DNA binding and cleavage by RAYT have been confirmed for *Escherichia coli*, the molecular mechanism of DNA reinsertion has not been fully understood. The genomic sequence analysis identified potential noncoding REP sequences associated with RAYT-encoding genes in other bacterial species. We have characterized biophysical properties of the REP oligonucleotides from six of these bacterial species by means of temperature-dependent circular dichroism and UV spectrometry and differential scanning calorimetry techniques. The RAYT proteins are considered to recognize the REP oligonucleotides in hairpin conformations so that these conformations are biologically relevant. However, our spectroscopic and calorimetric measurements discovered surprising conformational diversity of these palindromic DNA elements in solution. We have succeeded in stabilizing the *H. parasuis* RAYT and been able to measure its thermostability with differential scanning fluorimetry (DSF) and determine the binding affinity to the recognition REP oligonucleotide by microscale thermophoresis (MST) technique. Two REP-related oligonucleotide sequences from *H. parasuis* and *C. hominis* were crystallized and characterized in the liquid and crystal phases.

Our integrated measurements indicate equilibria of multiple conformational species, including architectures compatible with other than hairpin architectures such as antiparallel duplexes and bimolecular tetraplexes. Such structural variability of the REP sequences might be essential for the recognition and interaction with RAYT transposases and represent possibility for regulating RAYT nuclease and transposase activities.

Acknowledgments: This work is supported by grant CZ.1.07/2.3.00/30.0020 from the Ministry of Education of the Czech Republic (MSMT), grant P305/12/1801 from Czech Science Foundation, MEYS CR (LM2018127 and LM2015043); project CIISB for Human Health (CZ.02.1.01/0.0/0.0/16_013/0001776) from the ERDF; UP CIISB (CZ.02.1.01/0.0/0.0/18_046/0015974), *ELIBIO (CZ.02.1.01/0.0/0.0/15_003/0000447),* MOSBRI from EU Horizon 2020 (No. 101004806).

TNA beneficiary oral presentations

The correlation of changes in metabolic and glycosylation pathways by the analysis of biophysical profiles of melanoma cells treated with the computationally predicted drug combinations

A. Sobiepanek*[1], J. Załuski[1], W. Prorok[1], S. Kasarla[1], A. Paone[2], S. Rinaldo[2], F. Cutruzzolà[2], T. Gambin[3], T. Kobiela[1]

[1]Laboratory of Biomolecular Interactions Studies, Chair of Drug and Cosmetics Biotechnology, Faculty of Chemistry, Warsaw University of Technology, Warsaw, Poland [2]Department of Biochemical Sciences "A.Rossi Fanelli", Sapienza University of Rome, Rome, Italy [3]Institute of Computer Science, Faculty of Electronics and Information Technology, Warsaw University of Technology, Warsaw, Poland

The increasing worldwide prevalence of melanoma, one of the most aggressive skin cancers, requires an intensive search for successful therapy. Melanomas are metabolically heterogeneous and they can adapt to utilize a variety of fuels that facilitate tumor progression, and metastasis. Reprogramming of the cellular metabolism has been recognized as a crucial sign of cancer. Novel therapeutic approaches include combining traditional anti-melanoma drugs for targeted signaling pathways together with effectors of the energy metabolism pathway switch. The disturbances in glucose availability can further affect one of the basic mechanisms of post-translational protein modifications, namely glycosylation - a hallmark of more than 50% of human proteome responsible for the development, growth and survival of cells, and organisms. Finding a correlation between the two pathways could bring valuable knowledge to the subject of melanoma metastasis creation.

We have used the SynGeNet prediction to conduct an *in silico* drug screen and identified that vemurafenib (VEM) with dichloroacetate or VEM with pioglitazone combinations applied *in vitro* might be able to prevent melanoma metastasis. To confirm this prediction, we have applied several advanced techniques for the evaluation of the live-cell metabolic assay *via* the Seahorse analyzer, investigation of the enzyme-encoding gene expression connected with several metabolic pathways *via* the RT-qPCR analysis, as well as measurements of the lectin-glycan interaction *via* the quartz crystal microbalance with the dissipation energy monitoring (QCM-D). Our model includes commercial melanoma cell lines derived from different progression stages: WM115 – primary, A375 and WM266-4 – metastatic (all with *BRAF* mutation), and MeWo – metastatic (wild type). As a result, a strong correlation was observed between the cell viability/energy supplied parameters and the glycan viscoelastic index. The selected drug combinations influenced *BRAF*-mutant melanoma cell glycosylation and metabolism, but not the wild-type cell line.

The Seahorse analysis was performed at the Sapienza University of Rome during the TNA visit no. MOSBRI-2021-16 entitled '*Studies on the influence of the energetic metabolism modulation on the glycosylation profile of metastatic melanoma cells with BRAF mutation*'. Further financial support was provided by the Warsaw University of Technology under the program Excellence Initiative: Research University (BIOTECHMED-1 project).

Company oral presentations

Mass photometry - revolutionary biophysical characterization of single molecules

T. De Garay
Refeyn Ltd., Oxford, United Kingdom

Mass photometry is a novel bioanalytical technology that provides single-molecule mass measurements of biomolecules in their native state within minutes without the need for labelling, surface immobilization or big sample quantities. Mass photometry is based on interferometric scattering microscopy, measuring single molecules thanks to an unprecedented level of sensitivity. Its ease of use makes mass photometry the perfect tool for rapid assessments of sample purity and homogeneity, structural integrity or macromolecular interactions across biomolecules ranging from differently sized proteins to DNA and even small viruses, such as AAVs.

In this talk, we will walk over the principle of mass photometry and show its versatility in answering a wide range of biological questions.

Company oral presentations

OpenSPR & Alto: Nicoya's Label-Free Solutions for Biologics Research
Take the complexity out of SPR and empower your team with the data you need

N. Carvou
Nicoya Lifesciences inc., Canada

Surface Plasmon Resonance (SPR) assays are 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 insight into protein interactions and signaling pathways.

OpenSPR is the first commercial benchtop instrument to make SPR accessible and user-friendly while providing (i) high-quality data, (ii) label-free kinetics, and (iii) crude sample compatibility.

Alto is the world's first benchtop high-throughput digitalSPR platform. Its groundbreaking design is user-friendly, versatile, and evolutive. Alto's Digital Microfluidic technology integrates with nanoplasmonic biosensors to allow for:

- Precise handling of 2 µl sample volumes
- Automated serial dilutions
- Crude sample compatibility
- Disposable fluidics
- Minimal maintenance

By attending this talk, you will learn about the advantages of Nicoya's SPR solutions for your research applications.

EPR-MRS site: EPR spectroscopy for the study of biomolecules

MOSBRI team oral presentations

P. Dorlet*, E. Etienne, G. Gerbaud, B. Guigliarelli, V. Belle
BIP UMR 7281, CNRS, AMU, Marseille, France

Electron Paramagnetic Resonance (EPR) spectroscopy is the method of choice for the study of paramagnetic species such as radicals and transition metal ions. In Life sciences, it is of great use for the study of the active sites of metalloproteins, the function of complex redox systems, protein dynamics or protein-protein interactions.

The EPR facility hosted in the laboratory of Bioenergetics and Engineering of Proteins in Marseille (https://bip.cnrs.fr/epr-facility/) is one of the four French EPR centers included in the National Infrastructure Infranalytics and comprises several high-end spectrometers for both continuous-wave and pulse EPR experiments.

The application of EPR to biological systems will be illustrated by examples of ongoing studies from our lab and research projects we are welcoming in the frame of the TNA of the MOSBRI Infrastructure.

MOSBRI team oral presentations

Riboregulation of human serine hydroxymethyltransferase enzymatic activity reveals a novel strategy to shape cell metabolism

F. Cutruzzola*, S. Spizzichino, F. Di Fonzo, F. Romana Liberati, A. Paone, G. Giardina, S. Rinaldo
Department of Biochemical Sciences, Sapienza University of Rome, Italy

RNA binding proteins are known to regulate RNA metabolism and function. A novel mechanism, named riboregulation, suggests that also RNAs can regulate protein's function (1). By studying human Serine hydroxymethyltransferase (SHMT), a key enzyme in the metabolic reprogramming of cancer cells, we demonstrated that an enzyme's catalytic activity can be controlled by RNA (2).

We have also shown in silico and in cell lines that the levels of cellular metabolites (serine/glycine) can be modulated by these RNAs (3).

Using a combination of different approaches, we have unveiled the molecular basis of RNA-mediated allosteric regulation. First, we determined the structure of the SHMT-RNA complex by cryo-EM and studied the RNA-protein interactions by several approaches, including kinetic analysis of the effect of RNA on catalysis. Moving a step forward, we demonstrate that RNA molecules can act as metabolic switches in living cells, controlling the survival of lung cancer cells.

Given that many enzymes in intermediary metabolism are RNA-binding proteins, these results suggest that novel regulatory networks connecting intermediary metabolism and cellular RNAs can be discovered and exploited to control cell fate.

1. Horos R, et al. Cell. 2019; 176:1054¬1067.
2. Guiducci, et al., Nucleic Acids Research, 2019, 47:4240–4254.
3. Monti M, et al. Comput Struct Biotec 2021; 19: 3034¬3

TNA beneficiary oral presentations

Dynamics of microtubule-nucleated multimolecular tau assemblies

A. Karhanova*, V. Siahaan
Biotechnology Institute, BIOCEV, Vestec, Czech Republic

Tau is an intrinsically disordered microtubule-associated protein especially important in neuronal development and function, whose malfunction is associated with a number of neurodegenerative disorders. On microtubules, tau can cooperatively form cohesive superstructures, referred to as condensates or islands, which envelope the microtubule surface, and can shield the surface against binding of other proteins, including severing enzymes. While tau is heavily phospho-regulated, the role of tau phosphorylation in the dynamics of tau envelopes is unclear. Here, using TIRF microscopy and in vitro reconstituted system, we show how tau phosphorylation affects the formation of tau envelopes and their microtubule-protective function.

Company oral presentations

heliX®: The Modular Biosensor for Measuring Interactions from small molecules to cells

A. Gontier
Dynamic Biosensors GmbH, Martinsried/planegg, Germany

switchSENSE® is an automated fluorescence-based biosensor chip technology that employs electrically actuated DNA nanolevers for the real-time measurement of binding kinetics (k_a, k_d) but also affinity vs avidity with a high sensitivity (K_D values down to the fM range).

Fluorescent dyes located on the biosensor surface detect the interaction of ligand and analyte molecules in two different ways. The fluorescence proximity sensing mode detects the binding of molecules in real-time through changes in the dye's local environment. On the other hand, the high frequency dynamic switching mode probes the hydrodynamic friction of the ligand molecule and allows to determine conformational changes and kinetic analysis at the same time. These measurement features are valuable for drug development and hit validation.

The DNA-encoded anchor sequences present on the biochip surface allow the immobilization of a wide range of different molecules or even a combination of different molecules in varying ratios and densities for mimicking the cell surface. Furthermore, the use of two different fluorophores on the same sensor spot allows to monitor two independent signals from two interactions at the same time and determine between affinity and avidity for multi-specific binders.

Thanks to our unique novel **Y-structure DNA** format, competition assays or ternary complex formation characterization can be addressed using Fluorescence Resonance Energy Transfer (FRET) experiments.

In this talk, we demonstrate how to characterize binary and/or ternary complex formation in a single measurement with a Targeted Protein Degradation example such **PROTACs**.

Finally, one new feature coming soon - the **cell**TRAP technology – allowing kinetics directly on cells, will be presented. Association and dissociation kinetics of fluorescently labeled proteins/antibodies are measured directly from the cell surface. For that, new chips with polymer cages were designed to capture single cells.

Company oral presentations

Full control and versatility to allow the direct and real-time visualization of dynamic single-molecule processes essential to life

K. Ben M'barek*, A. Llauró-Portell, F. Payen, M. Hendriks
Lumicks, Amsterdam, The Netherlands

Essential biological processes performed by proteins interacting with DNA/RNA, cell membranes or cytoskeletal protofilaments are key to cell metabolism and life. In fact, detailed insights into these processes provide essential information for understanding the molecular basis of life and the pathological conditions that develop when such processes go awry.

In all of these process force plays a key role, from facilitating the proper folding of a protein to changing the binding affinity of a protein to the DNA. The next scientific breakthrough consists in the actual, direct, real-time observations and measurements of the individual mechanisms and the forces involved in these processes. To this end, structural and functional information, which are often indivisible, need to be studied together in real time and at the molecular level to validate and complete the current biological models. In this complex context, single-molecule technologies offer an exciting opportunity.

Here, we present our efforts for further enabling discoveries in the field of biology and biophysics using both the combination of optical tweezers with correlative fluorescence microscopy (widefield, TIRF, confocal and STED) and label-free Interference Reflection Microscopy (IRM).

We will show how hybrid single-molecule methods can be turned into an easy-to-use, high-throughput and stable instrument that has the ability to open up new venues in many research fields for a broader research community.

Biophysics in Drug Discovery: the Past, Present, and the Future

Invited oral presentations

C. Genick
Novartis Pharma AG, Basel, Switzerland

In Drug Discovery, the end goal is to discover and transform novel chemical material into a selective and ingenious therapeutic. Biophysics has played a unique role in this endeavor for the past 15+ years. Now with the maturation of biophysical approaches, technologies, and their throughput, a new era begins. After a brief review of the history of applied biophysics, let us discuss the challenges of taking biophysics to the next level of demand.

Integrated Structural Biology as a Toolbox to Dissect Viral Entry

MOSBRI team oral presentations

E. Von Castelmur
Department of Physics, Chemistry and Biology, Linköping university, Linköping, Sweden

While the conserved genome organization is one of the defining features of picornaviruses, the 2A proteins are amongst the most divergent. To date there are at least 5 different types identified, with varying roles during infection. Remarkably, there is a group of picornaviruses, including Aichi Virus (AiV) and Parechovirus A (HPeV), whose 2AH/NC proteins share conserved H-box and NC-motifs with the human protein PLA2G16, which we previously identified as a picornavirus host factor. These residues are essential for enzymatic activity for the cellular enzyme as well as for the reported enzymatic role of the viral 2A proteins in RNA replication. We set out to discover whether the 2A proteins are active as lipid modifying enzymes, and have been acquired to allow AiV and HPeV to become independent from the cellular host factor. The crystal structures of the AiV-2A and HPeV1-2A proteins show a similar topology to PLA2G16 in the N-terminus, but diverge in the C-terminal half. AiV-2A preserves the active site configuration, but is inactive as a phospholipase. Remarkably, a topological rearrangement of the C-terminus in HPeV1-2A results in a conformation incompatible with catalysis, despite the conservation of the H-box and NC-motif; consistently the HPeV1-2A protein is inactive as a phospholipase too. Intriguingly, however, HPeV1 is independent of PLAG16 for cellular entry, through an as yet unidentified mechanism. Further work is required and ongoing to trace 2AH/NC proteins' potential role during virus entry and how might they have been repurposed to fulfil new functions in the viral replication cycle.

Company oral presentations

DGE-AUC: Adapting the Power of Density Gradient Separations for the Characterization-in-Solution by Analytical Ultracentrifugation

M. Rouxel
BeckmanCoulter, Villepinte, France

The option to characterize biological structures in density gradients adds a powerful "thermodynamic tool" to the Biophysical-characterization-toolbox. This presentation gives a brief overview and shows application examples.

Company oral presentations

Immunogenic affinity and extracellular vesicle quantification in whole blood with FO-SPR

Filip Delport
FOx Biosystems, Diepenbeek, Belgium

Fiber optic SPR (FO-SPR) combines the benefits of SPR with dip probe sensing. This resulted in some unique benefits in analyzing whole blood samples here highlighted by a study on COVID samples for patient classification. Even more, as SPR allows kinetic affinity measurement this trait could be assessed in the whole blood samples and potentially be applied to clinical severity of disease or immune response to therapies (biological, gene therapy,…). A second unique benefit consists of large particle sensing and isolation here presented with an assay for extracellular vesicle quantification applied on HEK cells and breast cancer serum samples. In conclusion the FOx Biosystems FO-SPR technology can break the limits within bioanalysis and tackle the challenges of diagnostics and therapeutics of the future.

MOSBRI team oral presentations

Biophysical characterization of viral and lipid-based vectors for vaccines and therapeutics with light scattering and calorimetric techniques

N. Markova*, S. Cairns, M. Kaszuba, H. Jankevics Jones

Malvern Panalytical, Malvern, United Kingdom

Particle characterization is ever increasing in its importance, especially for delivery platforms for nucleic acids (mRNA, DNA) such as viral vectors and lipid nanoparticles (LNPs) where their intrinsic structural complexity is integral to their function and successful formulation. During their design, product development and process control, characterization and quality control of physical and chemical sample attributes require the combination of fit-for-purpose complementary analytical tools.

Several analytical techniques can often be applied for measuring the sample attribute, and selection of the most suitable technique to monitor the sample, will depend on the specific properties of the drug delivery vectors, as well as what stage of development the measurement take place, and the measurement's purpose. An in-depth methodological expertise and holistic approach to data analysis are required for robust measurements and to enable an adequate interpretation of experimental findings (Markova et al, 2021).

Here, the combination of complementary label-free biophysical techniques, including dynamic light scattering (DLS), multiangle-DLS (MADLS), electrophoretic light scattering (ELS), nanoparticle tracking analysis (NTA), multiple detection SEC and DSC have been successfully used for the characterization of physical and chemical attributes of viral vectors, mRNA and LNPs encapsulating mRNA. We will discuss methods' performance, applicability, and dynamic range of detection. We will share important methodological considerations which help to ensure reproducibility of data and reliability of data interpretation in the measurements of multiple critical physical−chemical quality attributes, including particle size distribution, polydispersity, particle concentration and nucleic acid payload and structural stability and comparability.

Reference:

1. Markova N., Cairns S., Jankevics Jones H., Kaszuba M., Caputo F., Parot J., (2021) Vaccines 10, 49.

Interactions between membranes and pore-forming toxins

MOSBRI team oral presentations

N. Omersa*, A. Šakanović, G. Anderluh
Department of Molecular Biology and Nanobiotechnology, National Institute of Chemistry, Ljubljana, Slovenia

Protein-membrane interactions are an interesting and challenging topic for scientific investigation. Such interactions are involved in cell-to-cell communication, pathogen attack, host defence, the immune system, and are also fundamental in synthetic biology approaches. We present a recent study on the membrane interactions of the pore-forming toxin perfringolysin O. We have selected and produced several toxin variants by ribosome display technique. We studied their binding to lipid vesicles using surface plasmon resonance and examined how mutations in the cholesterol-binding motif affect binding to phosphatidylcholine membranes supplemented with different amounts of cholesterol. We interpret the distinctive binding properties of the protein variants by a combination of explanations for different membrane properties depending on cholesterol content and different protein specificity with respect to certain amino acids in key positions in the membrane-recognition region of the toxin.

Company oral presentations

Flexibility Meets Throughput: The SPR Pro Platform

C. Brunner
Bruker Daltonics SPR, Fällanden, Switzerland

Surface Plasmon Resonance (SPR) is a label-free, biophysical technique to elucidate the binding kinetics of two interaction partners. The Bruker SPR Pro series is a multiplexing system bringing together the demands for a flexible and efficient system. With up to 32 individually addressable sensor spots the system enables a high degree of flexibility in assay development and application range. A robust microfluidic set-up allows the use of purified and crude samples. Low molecular weight compounds typical for fragment-based drug discovery are no limit for the state-of-the-art detection system. Investigations in binding mode are fully supported with up to eight simultaneously testable condition (e.g. different pH) and an epitope binning module.

The versatile microfluidic set-up allows multiple assay formats optimally suited for both low and high throughput and all drug classes. The platform enables an industry-leading throughput of up to 4400 samples per day and can be integrated into almost all automated environments.

The Sierra SPR Pro platform is a highly flexible system offering broad application possibilities and throughput.

Company oral presentations

Fida 1: The Ultimate Biophysics Tool – Fast and accurate in-solution analysis of proteins and nanoparticles

H. Jensen
Fidabio, Copenhagen, Denmark

Introduction: Fida 1 is a versatile, easy-to-use platform to give you fundamental, quantitative information about the samples you are working with, incl. size, polydispersity, environmental stability, aggregation, viscosity, labelling efficiency, Protein Database Bank correlation etc. It only takes 4 minutes, and you only need four µL of sample material. The samples are analyzed "as is", i.e., labelled or label-free, no immobilization, no buffer constraints, temperatures from 4-55 deg. C, purified or non-purified.

Supported by dedicated support, Fida 1 also offers access to advanced analyses of, amongst others, membrane proteins, targeted protein degradation, liquid-liquid phase separation, VHH clone selection, bi-specifics, immunogenicity, exosomes, AAVs, etc.

Though a new technology, the Fida 1 is already adopted by several large pharmaceutical companies and leading international research institutions.

Key Learnings:

- Flow-Induced-Dispersion-Analysis is a unique way of getting fundamental information about parameters essential for understanding the entities you are working with.
- Fida 1 enables advanced analysis of complex constructs and complicated interactions.
- The use of the Fida 1 platform is supported by easy-to-use software. To generate data requires only a few hours of training is needed.

AFFINImeter: A software to analyze molecular recognition processes from experimental data

J. Sabin*[1], A. Piñeiro[1-2]
[1]*AFFINImeter, Santiago de Compostela, Spain* [2]*Santiago de Compostela University, Santiago De Compostela, Spain*

Spectroscopy techniques such as Nuclear Magnetic Resonance, MicroScale Thermophoresis, and, especially, Isothermal Titration Calorimetry (ITC) provide sensibility enough to detect complex mechanisms of interaction where multisite binding, conformation changes, dimerization, or cooperativity may be involved. The lack of analytical tools to translate this data into relevant information make the decision-making in research programs more difficult and expensive.

We present AFFINImeter; a software platform able to accurately analyze such complicated experiments. AFFINImeter allows to design personalized binding models directly in chemical language, through an original user-friendly interface and to perform a robust global fitting of several isotherms obtained with the same system under different experimental conditions.

AFFINImeter provides a complete data processing and analysis, starting from raw ITC files (calculation of baselines, correction of the signal noise, and integration the heat peaks). Additionally, it offers KinITC, a unique method to obtain kinetic information from ITC data.

These features open the possibility of exploring new applications of the ITC technology and the possibility of obtaining a fully thermodynamic and kinetic characterization of an interaction in a single ITC experiment.

MOSBRI team oral presentations

Automated STED nanoscopy allows to study cell division without synchronization and reveals how the assembly and constriction of ESCRT-III polymers aid archaeal cell divisiona

F. Mol[1], T. Burgers[1], F. Hurtig[2], B. Baum[2-3], R. Vlijm*[1]
[1]Zernike institute, Rijksuniversiteit Groningen, The Netherlands [2]MRC-Laboratory for Molecular Cell Biology, University College London, London and MRC-Laboratory of Molecular Biology, Cambridge, United Kingdom [3]Institute for the Physics of Living Systems, University College London, United Kingdom

Stimulated Emission Depletion (STED) microscopy is a super-resolution light-microscopy technique with a spatial resolution of ~30nm in cells, well beyond the diffraction limit of 250nm, typical in confocal microscopy. Compared to techniques with similar resolution, STED has a high temporal resolution, and does not require time-consuming data processing as it is a direct-imaging technique. Although these advantages in principle allow high-throughput imaging, the full potential to obtain large datasets is currently not utilized due to elaborate manual microscope handling for optimal image settings. We therefore developed an automated STED-imaging workflow, significantly improving the throughput: from a few tens of measurement in a work-day, to hundreds of images unsupervised overnight. This increased efficiency makes this state-of-the-art method more affordable and accessible. Perhaps even more important, the automation allows for a more objective data collection as the variety within a sample now is recorded and quantified, and a potential microscopist bias in the selection of cells/structures is prevented by objective selection criteria. The combination of fast, low-resolution mapping of the sample followed by STED imaging of selected structures also allows to obtain sufficient data on rare or transient structures which are otherwise difficult to measure. One concrete application is the study of cell division machinery, which typically requires synchronization methods to increase the otherwise small fraction of cells in mitosis. Using our automated imaging we are now able to obtain sufficient data of unaltered wild-type cells, avoiding potential synchronization artefacts. We used this method to study the change in composition and organisation ESCRT-III co-polymers in dividing archaeal cells. This is important because, although ESCRT-III polymers play a conserved role in driving membrane constriction and scission in cooperation with VPS4 across the tree of life, their mode of action is not well understood. By revealing the detailed organisation of ESCRT-III homologues (CdvB, CdvB1 and CdvB2) in the context of co-polymeric division rings at an unprecedented resolution, we identified spatial patterning of the polymers that had been previously missed by imaging at lower resolution, which has important implications for our understanding of the mechanism by which ESCRT-III and Vps4 deform and cut membranes.

TNA beneficiary oral presentations

Human Glucose-Regulated Protein 94 (GRP94): Protein Characterization and Functional Analysis

H. Hotiana
Department of Biomedical Sciences, Membrane Protein Biology Group, University of Copenhagen, Denmark

Proteins are workhorses of the living cell and play critical roles in all aspects of human function such as regulation, movement, transport and function. Due to this, protein malfunction is also often responsible for a variety of defects and diseases giving rise to the need of studying proteins in detail both to understand their structure and function. However, an initial requirement for such studies is the ability to produce significant quantity and quality of protein biomolecules for biochemical characterization, use in industrial processes and development of commercial goods. Given the mere complexity of the biological pathways involving proteins, protein analysis remains one of the most complex and challenging fields of study. Therefore, setting up efficient and reproducible expression and purification pipelines for different types of proteins is a prerequisite for all such studies.

Human Glucose-Regulated Protein 94 (hGRP94) is the HSP90-like protein in the lumen of the endoplasmic reticulum where it chaperones secreted and membrane proteins. As a chaperone, GRP94 plays essential roles in development and physiology of multiple multicellular organisms. Recently, hGRP94 was also shown to be vital for correct proinsulin folding and maturation, significantly increasing insulin production levels. In-vitro studies to understand the molecular basis of how hGRP94 interacts with and stabilizes proinsulin are dependent on the ability to overproduce functional hGRP94.

We carried out an extensive array of assessments on the physiochemical properties including detailed analysis of molecular weight, purity/impurity, homo- and heterogeneity and post-translational modifications of hGRP94 and its mutants. These studies allowed us to establish the most efficient production pipeline for recombinantly produced hGRP94. Additionally, to evaluate the functional state of the overproduced hGRP94 we utilized nDSF based analysis to quantify binding affinities against different nucleotides based on thermal shifts.

Company oral presentations

Pushing the Boundaries in Biomolecular Interaction Analysis with GCI and waveRAPID

Edward Fitzgerald
Creoptix, Wädenswil, Switzerland

GCI is a surface-based, label-free biosensing technique. When target molecules (e.g. proteins) are attached to the sensor surface, binding of analytes leads to an increase in mass and hence to a change in the refractive index within the evanescent field near the surface. In GCI, refractive index changes on a sensor surface are measured as time-dependent phase-shift signals. The long light to-sample interaction length of the waveguide provides intrinsically high signal-to-noise levels for improved sensitivity. With high sensitivity, the ability to resolve extremely rapid dissociating kinetics and innate compatibility with high molecularweight ratios, the Creoptix® WAVEsystem's GCI technology improves fragment-based screening and kinetic analysis of smallmolecules to accelerate drug development. Paired with no-clog

WAVEchips®, a wide range of molecules can be immobilized using various chemistries.

Company oral presentations

Measure molecular interactions with Spectral Shift to minimize assay development time

T. Bartoschik
Nanotemper Technologies GmbH, Munich, Germany

Knowing the strength of an interaction between key players is crucial to understanding the details behind how biological systems work. The new Monolith X instrument provides the flexibility to work with all different types of molecules and samples, e.g., proteins, small molecules, nucleic acids, and more. Especially it has been shown to obtain results on difficult interactions, including membrane proteins, IDPs, or PROTACs. The method is immobilization free, requires only a few microliters of sample per data point, and experiments can be done in any buffer, including crude lysate or serum. For the interaction analysis, Monolith X features the latest innovation for measuring binding affinities: Spectral Shift technology. This method is based on a well-known phenomenon where organic fluorophores report changes in their chemical microenvironment by slight modifications of their emission spectrum, e.g., changes in their overall fluorescence intensity or blue- or red-wavelength shifts. Monolith X exploits this phenomenon by performing ratiometric measurements at two distinct emission wavelengths of a labeled target molecule in the presence of various concentrations of an unlabeled ligand to derive the affinity constant (K_d) for the interaction. The instrument allows for a way to resolve even sub-nanometer spectral shifts, making this approach highly sensitive towards ligand binding events — independent of conformational changes or changes in mass and size upon binding. Moreover, Spectral Shift is highly robust against sample impurities and aggregates and provides high quality data with minimal assay development time.

For more than ten years, NanoTemper Technologies has built products for straightforward interaction analysis known for their ease of use and broad applicability. With the development of Spectral Shift technology, scientists have access to a completely new way of measuring molecular binding events that will allow them to characterize even the most challenging interactions successfully.

Company oral presentations

With Octet® systems, you don't have to choose between SPR and BLI

C. Quetard
Sartorius, Dourdan, France

Besides its well-known Octet® BLI systems, Sartorius has just launched the Octet® SF3 instrument. You can now have what made the success of the Octet, ease of use, throughput, reliability, data quality and low-cost per data point and go for SPR detection.

Here we will review some of the unique technical features of both, our Octet® BLI and Octet® SPR systems.

Multiple Perspectives on Multiple States

MOSBRI team oral presentations

M. Williams
Institute of Structural and Molecular Biology, Department of Biological Sciences, Birkbeck, University of London, United Kingdom

Most analyses of biomolecular association focus on the simplest possible case of a binary interaction in which two molecules can be in one of two states: bound or unbound. Accurate measurement and robust interpretation of the data concerning these two states can themselves be challenging problems, however, many biological processes are more complex; involving multiple interacting molecular species each of which can be in more than one state. The presence of a multiplicity of states makes disentangling the physical parameters corresponding to changes between states substantially more difficult. Consequently, our ability to fully analyse more complex biological processes has been historically limited. However, new methods for enumerating individual species (such as native mass spectrometry or photometry), for analysing such data, together with approaches for combining data from biophysical experiments that offer distinct views of a process are beginning to open up clearer views of the affinities, cooperativity, and other thermodynamic properties of complex biological systems.

MOSBRI team oral presentations

Characterization of misfolded protein aggregates through AFM-STED correlative nanoscopy

C. Canale*[1], S. Jadavi[2-1], P. Bianchini[2], A. Diaspro[1-2]
[1]*Department of Physics, University of Genoa, Genova, Italy* [2]*Nanophysics Department, Istituto Italiano di Tecnologia, Genova, Italy*

In the last decades, new technical approaches brought optical fluorescence microscopy beyond the diffraction limit. Super-resolution (SR) microscopy revealed details of biological processes at the previously inaccessible molecular scale.

We coupled an AFM with a stimulated emission depletion (STED) microscope, applying them to the study of amyloid aggregates formation.

In our first work, we investigated the aggregation of insulin, and of the Alzheimer's disease-related Aβ1-42, and Aβ1-40 peptides. We adopted standard procedures to induce fibrillation, and label the peptides. We considered dye-to-protein ratios frequently used and reported in the literature. The results define the same scenario: STED microscopy detects only a part of the fibrillar aggregates, showing that labeled peptides contribute only to forming a subset of fibrils and supporting the hypothesis that labeled molecules follow only selected aggregation pathways. More recently, we investigated the aggregation process of α-synuclein (α-syn), a peptide involved in the neurodegenerative cascade in Parkinson's disease. We used a site-specific labeling method, binding the fluorophore at the C-terminal domain, scarcely involved in the fibrillization process. With this approach, we obtained a homogeneous distribution of fluorescent fibrils. Our study indicates that the adoption of a site-specific labeling method is important to avoid influencing the molecular mechanisms. The integrated AFM-STED system candidates as a gold standard method for monitoring the eventual influence of fluorophores on molecular processes.

Invited oral presentations

ARBRE: a Europe-wide connexion hub for molecular-scale biophysics research

A. Miele
UMR 5280 Institut des Sciences Analytiques, Université Claude Bernard Lyon 1, Villeurbanne, France

The Association of Resources for Biophysical Research in Europe (ARBRE) is a recently established charity, registered in Lyon in October 2021.

The aim of ARBRE is to promote the research field of molecular-scale biophysics and to bring together laboratories, core facilities and platforms, primarily in Europe, which study biological macromolecules and assemblies as a whole, at an intermediate level between atomic-resolution structural descriptions and cellular-level observations.

ARBRE aims at building an optimal environment for the development of innovative integrative biophysical approaches, at the level of data acquisition, analysis and modelling.

To pursue these objectives, the members of ARBRE will engage in several working groups in order to:

- disseminate and exchange knowledge and expertise, notably through the organization and support of training activities and meetings;
- organize benchmarking studies and establish Good Laboratory Practices (GLP) as well as Standard Operation Procedures (SOP);
- establish contacts with instrument developers and, possibly, contribute to the development of novel methodologies and technologies, by improving and pushing existing biophysical instrumentation beyond state-of-the-art.

During the talk I shall present the business plan and how ARBRE can find its place among research infrastructures and scientific societies.

https://www.arbre-biophysics.eu

Infrared spectroscopy, a versatile tool

V. Raussens*, J. Waeytens, E. Goormaghtigh
Center for Structural Biology and Bioinformatics, Laboratory for Structure and Function of Biological Membranes, Faculty of Sciences, Université Libre de Bruxelles, Brussels, Belgium

After a short introduction on the laboratory and the available equipment, we will describe the interest of infrared spectroscopy in study of biomolecules and especially proteins. Fourier transform infrared (FTIR) spectroscopy is fast (a few minutes), required minute amounts of samples (10-100 ng) and study proteins without any external labeling or chemical modifications. Our laboratory is using mainly the attenuated total reflection (ATR) sampling method which allow an easy study of membrane proteins in their native lipidic environment but also poorly or insoluble proteins like amyloids. Information about post-translational modifications like glycosylation or phosphorylation can be acquired also at the same time. We will describe a new high throughput method for FTIR, using an infrared microscope, developed in the framework of the Robotein® platform in collaboration with the University of Liège (Belgium). Finally, we will show some new advances in infrared spectroscopy allowing to record high quality spectrum in aqueous solution even in the presence of highly concentrated buffer. These new methodologies are now available and included in the TNA offer provided by the laboratory.

MOSBRI team oral presentations

Protein stability and stabilization by ligands and by protein engineering: calorimetric and computational studies and tools

A. Velazquez-Campoy, J. Sancho*
Institute for Biocomputation and Physics of Complex Systems, University of Zaragoza, Spain

Proteins are key agents for cellular function and molecules of great value in biotechnology and biomedicine. Some proteins perform their functions through their unfolded conformations, but most do so after adopting a compact conformation that remains in equilibrium with the unfolded one.

The conformational stability of a protein (the difference in free energy between the folded and unfolded conformation) is the key thermodynamic property that determines the fraction of active protein molecules. It is therefore very useful to learn how to determine and how to modify it. *Ex vivo* solution conditions pose a challenge for protein activity, as they tend to reduce the intrinsic stability, which is often low. Fortunately, current biophysical knowledge of proteins allows us to assess and increase their stability by judicious addition of ligands, by modifying solvent conditions, or by engineering changes in the amino acid sequence.

We will show the experimental infrastructure available at BIFI-LACRIMA for evaluating protein stability, mainly, calorimetry (differential scanning calorimetry) and spectroscopy (circular dichroism, fluorescence, differential scanning fluorimetry, absorbance), as well as the different assays that can be performed and the information that is experimentally accessible.

We will illustrate with examples from our experimental work at BIFI-LACRIMA how calorimetric and spectroscopic techniques can be combined to obtain an accurate description of the conformational landscape of simple monomeric two-state proteins or of more complex proteins that populate equilibrium intermediates or are oligomeric. We will also illustrate how ligand binding can be used to increase protein stability and to discover new bioactive compounds.

On the other hand, we will present our computational approaches to study the effect of point mutations on protein stability with examples of variants in disease-related proteins. Finally, we will present a new computational tool (Protposer) designed to facilitate rational protein stabilization. Protposer performs a fully automated analysis of PDB files and proposes point mutations with a high probability of being stabilizing.

POSTER ABSTRACTS

1P MOSBRI team posters

TNA: Apply for access to laboratories of excellence in MOSBRI

S.V. Hoffmann*[1], N.C. Jones[1], E. von Castelmur[2], D. Derbyshire[2], M. Sunnerhagen[2]
[1]ISA; Department of Physics and Astronomy, Aarhus University, Aarhus, Denmark [2]Department of Physics, Chemistry and Biology, Linköping university, Sweden

MOSBRI provides trans-national (TNA) access to molecular-scale biophysics instrumentation and expertise, aiming at studying biological systems at an intermediate level between atomic-resolution structural descriptions and cellular-scale observations.

The MOSBRI programme offers TNA to 13 European biophysical laboratories, widely distributed over Europe and selected to ensure a comprehensive portfolio of technologies and expertise. Access to the MOSBRI TNA sites is based on a proposal submitted via the MOSBRI web-site.

MOSBRI has a range of access modalities:

MOSBRI pipelines: This means an integrated access to a synergistic set of biophysical instruments and technologies. This will allow the TNA user to fully exploit the expertise of the TNA site to tackle advanced questions.

Access to instruments and methodologies: This proposal submission method may be used if you have a focused research question and already know which instrument/methodology your project needs access to.

Project maturation: If you are unsure which pipeline or instrument suite will best answer your project's scientific question, the MOSBRI's moderator panel experts can offer to guide you via project maturation.

In this poster we will present what MOSBRI TNA can offer and explain our different access modalities in detail.

The Institut Pasteur Molecular Biophysics platform

B. Raynal, S. Brûlé, M. Chevreuil, S. Hoos, P. England*
Molecular Biophysics, Institut Pasteur, Paris, France

Created in 2002, the Institut Pasteur Molecular Biophysics platform (Pasteur-PFBMI; www.mosbri.eu/partners/pasteur-pfbmi) is a cutting-edge technological core facility, which aims at potentiating top-level molecular-scale biophysical studies of the properties of biological systems, however complex they are. The study of pathogens, their interactions with their targets, the host response and the prevention and treatment of infectious diseases is one of its major priorities, but it applies its expertise to any biological question posed by scientists working in any academic or industrial institution in Europe and worldwide, notably through the MOSBRI TNA scheme.

The technologies and expertise of Pasteur-PFBMI focus on the four following areas:

1) Hydrodynamic characterization of the size, shape and architecture of macromolecules and assemblies. We have developed a multi-approach combination to analyse the macroscopic properties of molecular assemblies in solution, using an integrated set of methodologies: analytical ultracentrifugation (AUC), dynamic light scattering (DLS), mass photometry, static light scattering (SEC-LS), small-angle X-ray scattering (SAXS) and viscometry/Taylor Dispersion.

2) Kinetic and thermodynamic characterization of biomolecular interactions. Our expertise focuses notably on the structure-function study of protein-protein interactions, using approaches such as micro-scale thermophoresis (MST), surface plasmon resonance (SPR), biolayer interferometry (BLI) and isothermal titration calorimetry (ITC).

3) Spectroscopic characterization of the folding, dynamics and stability of macromolecules: circular dichroism (CD), differential scanning fluorimetry (DSF) and calorimetry (DSC), and fluorescence spectroscopy.

4) Quality control of purified samples. This activity is organized as a turnkey service and was the first of its kind in Europe. It was prompted by the observation that a lot of valuable time is unfortunately wasted on poor quality samples, confirming the adage "garbage in, garbage out". In 2014, with the aim of improving the quality of scientific results by improving the quality of input samples, we set-up, through a partnership between the European networks ARBRE-MOBIEU (molecular biophysics) and P4EU (protein production and purification), a simple-to-follow workflow based on an ensemble of widely-available physico-chemical methodologies, that allows to assess the essential properties that any protein sample should fulfil, whatever its downstream application, i.e. purity, integrity, homogeneity and activity.

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Synchrotron radiation circular dichroism at MOSBRI partner AU-SRCD

N.C. Jones*, S. Vrønning Hoffmann
ISA, Department of Physics and Astronomy, Aarhus University, Aarhus c, Denmark

MOSBRI partner AU-SRCD is based at the Department of Physics & Astronomy at Aarhus University in Denmark. AU-SRCD utilizes synchrotron radiation (SR) produced by the ASTRID2 storage ring, a facility where SR in the UV to the soft X-ray region is produced. Access to the AU-SRCD partner through MOSBRI is offered to advanced Circular Dichroism (CD) beam lines on ASTRID2. Synchrotron Radiation Circular Dichroism (SRCD) spectroscopy offers significant improvements to the well-established method of conventional CD (cCD) spectroscopy. The high photon flux, over a wide range of wavelengths, results in higher signal-to-noise ratios and enables the collection of data at lower wavelengths than possible with cCD spectrometers.

In this poster we present the variety of measurement options at AU-SRCD, the structural informational content of CD and show examples difficult samples where the use of SRCD is highly justified.

Infrared spectroscopy, a TNA offer

V. Raussens*, J. Waeytens, E. Goormaghtigh
Center for Structural Biology and Bioinformatics, Laboratory for Structure and Function of Biological Membranes, Faculty of Sciences, Université Libre de Bruxelles, Brussels, Belgium

Our laboratory and its available equipment, more than 4 FTIR spectrometers with various accessories and highly sensitive MCT detectors, one FTIR microscope with a 128x128 FPA detector, offers a complete infrared analysis solution to our TNA visitors. We developed infrared expertise in the analysis of biomolecules over decades now, both in the recording and the interpretation of spectra. We have developed our own analysis program. The interest of infrared spectroscopy in the study of biomolecules and especially proteins is numerous. Fourier transform infrared (FTIR) spectroscopy is fast (a few minutes), required minute amounts of samples (10-100 ng) and study proteins without any external labeling or chemical modifications. Our laboratory is using mainly the attenuated total reflection (ATR) sampling method which allow an easy study of membrane proteins in their native lipidic environment but also poorly or insoluble proteins like amyloids. Information about post-translational modifications like glycosylation or phosphorylation can be acquired also at the same time. In the case of membrane proteins, linear dichroism allows the determination of orientation of the proteins and the lipids. For amyloids, we can distinguish oligomers from fibrils and to follow aggregation kinetics. We also developed a new high throughput method for FTIR, combining an infrared microscope and an array jet protein printer. This was developed in the framework of the Robotein® platform in collaboration with Prof. A. Matagne at the University of Liège (Belgium). Finally, we recently receive an AQS^{3}PRO spectrometer from RedShiftBio. We are now the European reference laboratory for this new technology. This spectrometer can record high quality spectrum in aqueous solution even in the presence of highly concentrated buffer. These new methodologies are now available and included in the TNA offer provided by the laboratory.

Transnational Access at LAMBS facility

C. Canale*[1], P. Bianchini[2], A. Diaspro[1-2]
[1]Department of Physics, University of Genova, Italy [2]Department of Nanophysics, Istituto Italiano di Tecnologia, Genova, Italy

The Laboratory for Advanced Microscopy Bioimaging Spectroscopy (LAMBS) is located in Genoa (Italy) and is based on the collaboration between two institutions well consolidated in the Genoa Area: the University of Genoa and Istituto Italiano di Tecnologia (IIT).

The infrastructure has two different installations: The first, DIFILAB, is a new laboratory at the Physics Department of the University of Genoa, and the second, is the Nanoscopy Lab at IIT.

DIFILAB is an integrated research laboratory designed and built as part of the ministerial project "Departments of Excellence, 2018-22" to achieve the goal of enhancing research on biomedical science and nanotechnology. Nanoscopy Lab deals with the development of novel technologies and instruments for advanced diagnostics at the nanometer scale. The two laboratories are collaborating on several projects in the field of microscopy and spectroscopy.

We offer the opportunity to exploit our technologies and expertise in the frame of MOSBRI Transnational Access (TNA). In particular, advanced optical fluorescence techniques such as confocal and multiphoton resonant scanner microscopy, N-STORM, N-SIM, and STED super-resolution microscopy, fast-FLIM, and custom-made IML-SPIM. Furthermore, the LAMBS facility is equipped with advanced integrated systems for the acquisition of correlative AFM-STED images, and AFM-FLIM images.

Molecular Biophysics research in Groningen

W. Roos*, R. Vlijm, P.C.A. van Der Wel
Zernike institute, Rijksuniversiteit Groningen, Groningen, The Netherlands

The RUG-BP infrastructure is located at the Zernike institute of the Rijksuniversiteit Groningen in the Netherlands and comprises the experimental biophysics efforts of the institute. RUG-BP has state-of-the-art equipment for all offered techniques and research output illustrates that we perform top-level research using these techniques. In particular we will discuss the following techniques that are on offer in Groningen. (i) (High Speed) AFM: We provide access to both traditional as well as High speed AFM. Measurements are predominantly performed in liquid. (ii) Optical Tweezers: With this set-up we are able to trap one or two beads and to measure/exert pN forces and nm displacements. (iii) ssNMR: The solid state NMR set-up probes changes in secondary structure and molecular dynamics upon aggregation or self-assembly of macromolecules (e.g. proteins). (iv) STED microscopy: Our super resolution fluorescence microscopes are both used for fixed cell and live cell experiments. On this poster we will present these different techniques and provide examples of the type of research we perform with it.

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The Sample Preparation & Characterization facility at EMBL Hamburg

M. Garcia Alai, D. Ruiz Carrillo, S. Niebling, A. Struve Garcia*
EMBL Hamburg, Hamburg, Germany

The EMBL Sample Preparation & Characterization (SPC) facility is located within the DESY Campus in Hamburg, Germany. Our main task is supporting academic and industry researchers carrying out structural biology studies. In addition to that, our facility has a strong record in developing and implementing new technologies and approaches in the biophysics field.

Our high-throughput crystallization (HTX) laboratory offers a large array of commercial crystallization screens as well as customized screens for optimization of initial hits. Located next to the EMBL beamlines, users have access to automated crystal harvesting and data processing for streamlined crystallization experiments and synchrotron data collection. In addition, we offer assistance to perform SAXS batch measurements with near-real-time outputs of macromolecular structural parameters and low resolution solution-state structures.

The biophysical platform of the SPC includes cutting-edge technologies to measure biomolecular interactions and to precisely determine the stability, shape and size of different biomolecules and biomolecular assemblies. We offer a wide range of services, from initial protein quality control to more sophisticated biophysical approaches. Our online data analysis platform, eSPC, is available at spc.embl-hamburg.de and allows users to analyze and visualize biophysical data from anywhere in the world.

The SPC core facility is a partner of the MOlecular-Scale Biophysics Research Infrastructure (MOSBRI) and a member of other complementary research networks like iNEXT-Discovery, INSTRUCT-Eric, DFG and HALOS. These networks offer researchers from different fields to get fully-funded access to a wide range of methods to support their scientific projects.

ProLInC – 'Protein folding and Ligand Interaction Core' facility

A transnational access site for MOSBRI – 'Molecular Scale Biophysics Research Infrastructure'

A. Ahlner, E. Von Castelmur, P. Hammarstrom, S. Nystrom, M. Sunnerhagen, V. Morad, D. Derbyshire*
ProLInC - IFM, Linköping University, Linköping, Sweden

Understanding the biological function of proteins, and their interactions, requires the ability to fully characterise individual proteins and said interactions in isolation. Such 'targeted' experimentation is plagued with assay artefacts often resulting in confusion and misinterpretation, particularly in instances of highly focused questions or structural analyses where test samples are devoid of context.

Each technique will produce a biased view of the question addressed. What if the technique is inappropriate or the sample flawed? Ultimately all approaches have shortcomings. Artefacts and biases arising from one technique, or poorly behaving samples, can only be circumvented by adopting orthogonal methodology.

The **PRO**tein folding and **L**igand **IN**teraction **C**ore facility (ProLInC) at Linköping University has been established as a resource integrating a diverse (and complementary) array of techniques for the complete characterization of proteins and their complexes: shape, structure, stability, affinity etc. Together with on-site expertise, the facility aims not only to provide access to instrumentation but also to facilitate experimental design and assist in the interpretation of results.

ProLinC forms part of **SMILE**, an initiative to improve Small and Medium Enterprises (SME's) access to experimental and computational infrastructures and deepen collaboration between academia and business. The facility also provides an entry point to the EU-program for molecular biophysics: **MOLecular-Scale Biophysics Research Infrastructure (**MOSBRI), offering international access to instrumentation and expertise for biophysical approaches.

Students, staff, and external researchers alike gain user access to a wide range of equipment with support and advice where required. Users can verify (and complement) previously obtained results as well as completely characterise their protein samples (or complex thereof). ProLInC staff are available for user-focused discussions to advise on appropriate technique(s) to address the question at hand and assist in experimental design and optimization.

The poster will endeavour to provide a flavour of the instruments and techniques ProLInC offers highlighting some activities carried out by visiting scientists.

https://liu.se/en/research/prolinc https://liu.se/en/research/smile www.mosbri.eu

Molecular interactions in the Department of Molecular Biology and Nanobiotechnology

N. Omersa*, K. Pirc, M. Kisovec, G. Anderluh
Department of Molecular Biology and Nanobiotechnology, National Institute of Chemistry, Ljubljana, Slovenia

Knowledge of interactions between molecules can help us understand various processes in biology. How can we quantitatively assess such interactions and what conclusions can be drawn from biophysical approaches to measuring molecular interactions? In the Department of Molecular Biology and Nanobiotechnology at the National Institute of Chemistry, Slovenia, we specialize in the preparation of lipid membrane systems and recombinant proteins and their characterization (e.g., circular dichroism, cryo-electron microscopy) and use various approaches to study molecular interactions such as surface plasmon resonance, isothermal titration calorimetry, microscale thermophoresis, and quartz crystal microbalance. These approaches are useful for screening and identifying small or large molecule binders, monitoring regulatory pathways, optimizing the development of assays to study interactions between molecules, developing new products, etc. In addition, a well-characterized molecular interaction can be informative to infer a biological function of interactants or to evaluate the biological relevance of a particular target. We provide access to these techniques and our expertise, with a focus on protein-membrane interactions, through MOSBRI Trans-National Access.

The ISMB Protein Crystallography and Biophysics Centre (BiophysX)

N. Pinotsis*, M.A. Williams
Institute of Structural and Molecular Biology, Department of Biological Sciences, Birkbeck, University of London, London, United Kingdom

BiophysX is a core facility of the UCL/Birkbeck Institute of Structural and Molecular Biology (ISMB). The facility, housed at Birkbeck, provides access to a wide range of instrumentation and expertise covering the characterization of protein stability, crystallization and biomolecular interactions. Amongst the Centre's instrument are state-of-the-art liquid handling equipment, automated imaging systems for monitoring aggregation or crystal growth, temperature-controlled CD and fluorescence spectrometers, isothermal and differential scanning calorimeters, biolayer interferometry, dynamic light scattering and multi-angle light scattering attached to size exclusion chromatography for accurate sizing of molecular species.

The Centre is experienced in working with a wide range of users. In addition to long experience of helping structural biologists, we have users engaged in protein engineering, ligand discovery, antibody therapeutic development, chemical kinetics in confined environments and the assembly of protein and nucleic acid membrane spanning complexes. As a partner in MOSBRI, BiophysX is providing free Trans-National Access to European academic and industrial researchers.

Metal cofactor structure in a tungsten dependent formate dehydrogenase: Determination through EPR spectroscopy, isotopic enrichment and DFT calculations

A. Oliveira[1], F. Biaso[2], I. Pereira[1], B. Guigliarelli*[2]
[1]ITQB, University of Lisbon, Lisbon, Portugal [2]BIP-UMR7281, Aix-Marseille University, Marseille, France

Molybdenum (Mo) and tungsten (W) enzymes are found in virtually all living organisms where they catalyse a wide diversity of redox reactions involved in major biogeochemical cycles. Among them, bacterial formate dehydrogenases (Fdh) attract much attention due to their activity of CO_2 reduction to formate. These enzymes harbour a large Mo/W-*bis* pyranopterin guanosine dinucleotide cofactor in which the metal ion is coordinated by the four sulfur atoms of the two pterin rings, by an amino-acid of the polypeptide chain (Ser, Asp, Cys/SeCys) and by a sixth ligand that can be oxygen or sulfur. During catalysis, the metal ion cycles between the +IV and +VI redox states, but in spite of numerous crystallographic and spectroscopic studies, the structure of active site intermediates and catalytic mechanisms are still largely debated [Grimaldi *et al.*, RSC 2016]. Recently, a tungsten dependent Fdh exhibiting remarkable properties of CO_2 reduction activity and O_2 tolerance has been characterized in the bacterium *Desulfovibrio vulgaris* Hildenborough [Oliveira *et al.*, ACS Catalysis 2020]. Although the structure of the enzyme has been determined by X-ray crystallography in the fully oxidized (W^{VI}) and fully reduced (W^{IV}) states, the structure of the W^{V} intermediates remains unknown. As the W(V) state is paramagnetic (S=1/2), we have developed an approach based on EPR spectroscopy to address this question. Surprisingly, depending on the substrate or reductant used, different W^{V} species have been identified by EPR. By using a specific ^{77}Se enrichment of the enzyme, we demonstrate that the conserved SeCys is a direct ligand of the W atom in all the W^{V} catalytic intermediates. In addition, by combining g-tensor analysis and DFT calculations of model compounds, we show that in these species the W(V) ion has the same 6-ligands coordination sphere and that the magnetic parameter differences result from small variations of bond angles between the four sulfur ligands of the two pterin rings. These results confirm the stability of the six-ligand coordination of the W ion along the catalytic cycle and the implications on the Fdh's catalytic mechanism will be discussed.

Survey on Needs for Data Standardization and Databases in Molecular Biophysics

J. Dohnálek*[1], J. Stránský[1], M. Williams[2], J. Sabín[3], J. Černý[1], M. Malý[1], P. England[4]
[1]Institute of Biotechnology of the Czech Academy of Sciences, Vestec, Czech Republic [2]Birkbeck College, University of London, London, United Kingdom [3]Software 4 Science Developments, Santiago De Compostela, Spain [4]Institut Pasteur, Paris, France

Recent years have seen a growth of the need for proper scientific data management (not only) in natural sciences. Methods of molecular biophysics lack data format standards, computational tools enabling analysis, conversion, and interoperability, and open access repositories or databases covering raw and processed data. In line with the European support of activities aiming at enabling or improvement of scientific data management the project MOSBRI (https://www.mosbri.eu/) has taken on the task of trying to improve biophysical data management. For a selection of biophysical techniques this should result in a pilot open access database, adhering to the FAIR principles of making scientific data accessible (FAIR = Findable + Accessible + Interoperable + Reusable).

To map the landscape of molecular biophysics techniques regarding perceived needs for data standards and repositories we have performed a survey, focused mainly on the techniques available under the MOSBRI transnational access programme.

The electronic Survey on the needs for biophysical data standardization and databases containing 23 questions yielded 104 complete questionnaires. The respondents were mainly academics from universities and public research institutions across the world. The results provide insights into several areas: identification of the more heavily used techniques, the extent of biophysicists experience with scientific databases and with data management, the perceived needs for data standardization, improvement of data handling tools, and the perceived necessity of databases for the individual techniques.

While some techniques are heavily used but their need for data format standardization is not felt to be high (e.g. UV/VIS spectroscopy), several techniques have many users who have identified a significant need for data standards and accessibility (e.g. circular dichroism, isothermal titration calorimetry, surface plasmon resonance).

The main outcomes of the Survey will be presented as well as the analysis of internal correlations providing insights into the respondents' experience with open access to data and perception of the data standardization needs in the field of molecular biophysics.

This work is supported by the project MOSBRI – Molecular Scale Biophysics Research Infrastructure of the Horizon 2020 research and innovation programme of the European Union, no. 101004806.

Live cell analysis of energy metabolism using the Hyp-ACB (Hypoxic Analysis of Cell Behavior) platform, a MOSBRI instrumentation for TranNational Access

S. Rinaldo*, C. Scribani Rossi, F. Liberati, C. Di Lucente, A. Paone, F. Cutruzzolà
Biochemical Sciences, Laboratory affiliated to Istituto Pasteur Italia - Fondazione Cenci Bolognetti, Sapienza University of Rome, Rome, Italy

The Hyp-ACB platform is a complex infrastructure able to measure, under normoxic and hypoxic conditions, the metabolic activity of living cells, with particular attention to the respiratory activity. The respiratory and glycolytic activity can be measured by means of Seahorse instrument within a pipeline allowing cell handling, growth, treatment, and respiratory measurements under controlled oxygen tensions.

The variable and controlled oxygen tension reproduces more closely the cell or tissue microenvironment(s) in both physiological and pathological conditions. Evolution of multicellular organisms from their single-cell ancestors required the development of an ability to sense changes in oxygen tension. The normal levels of oxygen supply can enormously vary in different environments. In present-day tissues and multicellular community such as biofilms, normoxic levels are often well below the atmospheric oxygen tension of about 21%, ranging on average between 5-15% (often referred to as physioxic levels).

It is becoming increasingly clear that the cellular effects of exposure to low-oxygen tensions also represent a pernicious facet of many diseases, such as cancer [1, 2], cardiovascular disease, dementia, and diabetes [3]. Hypoxic conditions are also widespread in many environmental settings (natural and artificial), most often as a consequence of pollution and eutrophication, and characterize also industrial settings, as for example food industry.

Given the high impact of such technique, we have accepted and completed several TNA visits in the first year of MOSBRI activity; we have explored many experimental set-ups including hypoxia, nutrient supplementation, bacteria profiling and drug pre-treatment.

An overview of the most relevant applications with this platform with both Eukaryotic and Prokaryotic cells, under different oxygen tensions, will be presented.

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BIFI-LACRIMA: Biophysical Instrumentation for Protein Stability and Interactions

A. Velazquez-Campoy[1-2-3-4], O. Abian[1-2-3-4], J.A. Carrodeguas[1-2], N. Cremades[1-2], B. Antolí[1], Y. Vergara[1], S. Vega[1], J. Sancho*[1-2-3]
[1]Institute of Biocomputation and Physics of Complex Systems (BIFI), Joint Unit GBsC-CSIC-BIFI, Universidad de Zaragoza, Spain [2]Department of Biochemistry and Molecular and Cell Biology, Universidad de Zaragoza, Spain [3]Aragon Institute for Health Research (IIS Aragon), Spain [4]Biomedical Research Networking Centre for Liver and Digestive Diseases (CIBERehd), Spain

The Institute of Biocomputation and Physics of Complex Systems (BIFI, University of Zaragoza, https://www.bifi.es) is a research centre founded in 2002 to create an interdisciplinary environment in which scientists with diverse backgrounds and expertise could approach challenging problems at the interface of Physics, Biology and other scientific disciplines. Thus, combining theory, numerical simulations and experimentation we attempt to solve specific issues with important societal impacts such as the design of new drugs or a better understanding of social collective phenomena.

Many of the systems under study are related to Cellular and Molecular Biology. In particular, we are interested in understanding how proteins behave (structure, function and regulation) and in using that knowledge for tackling biotechnological and biomedical challenging problems: 1) stabilization, formulation and quality control of proteins and biologics, 2) identification and optimization of bioactive compounds; and 3) development of diagnostic biomarkers. For those tasks we develop and improve experimental approaches, as well as models and data analysis methodologies.

LACRIMA (Advanced Laboratory for Screening and Molecular Interactions in Aragon) is the experimental facility for Biochemistry, Molecular and Cell Biology, and Biophysics located at BIFI. Within MOSBRI, LACRIMA offers instrumentation for elucidating, assessing and tailoring protein stability (conformational landscape, equilibrium and kinetic stability of proteins and biologics) and studying and interpreting protein interactions (functional landscape, thermodynamic interaction parameters, cooperative phenomena and allostery), as well as performing in vitro assays with isolated proteins and cell-based assays:

- Calorimetry: VP-ITC, Auto-iTC200, VP-DSC, and Auto-PEAQ-DSC (MicroCal, Malvern-Panalytical)
- Differential scanning fluorimetry: Mx3005p (Agilent)
- Microscale thermophoresis: Monolith NT.115Pico (NanoTemper)
- Spectroscopy: Chirascan spectropolarimeter (Applied Photophysics), Cary Eclipse fluorimeter (Agilent), NanoStar Dynapro, and DynaPro Plate Reader III (Wyatt Technology)
- Multimode plate readers: FluoDia T70 (PTI), Synergy HT (BioTek), CLARIOStar, and FLUOStar (BMG Labtech)
- Fluorescence microscopy: DMI 6000B (Leica)
- Time-resolved single molecule spectroscopy: MicroTime 200 (PicoQuant)

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Fragment Screening of an MBP-Fusion Protein from Gene to Structure

P. Schambel, M. Vitorino, F. Ciesielski, P. Ciapetti*
NovAliX, Strasbourg, France

The design principles of a novel 1,000 ligand library (NovA-Frag) are described showing the wide coverage of chemical space especially into 3D. The compounds in the library were pooled in cocktails suitable for NMR screening thus reducing time and protein needed.

Screening by ligand observed NMR at 800 MHz using a QCI-cryoprobe in cocktails of 5 were performed on a 24 kDa soluble enzyme-MBP fusion protein. The hit distribution of 3 NMR experiments is shown. Controls were used to remove MBP binders and establish the binders to the protein of interest.

Hit confirmation by X-ray crystallography allowed the identification of 2 new binding sites, so the project was structurally enabled and pursued by SAR by catalogue.

Smart HumAfFt coated SPIONs for theranostic applications

L. Affatigato*, A. Martorana, A. Bonamore, A. Boffi, V. Militello, M. Licciardi
[1]*Department of Physics and Chemistry - Emilio Segrè, University, Palermo, Italy* [2]*Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), University, Palermo, Italy* [3]*Department of Biochemistry, Sapienza University, Roma, Italy*

Among various nanostructures, Superparamagnetic Iron Oxide Nanoparticles (SPIONs), particularly magnetite (Fe_3O_4) and meghamite (Fe_2O_3) nanoparticles, are used primarily in cancer theranostic applications such as magnetic resonance imaging (MRI) and magnetic hyperthermia due to their significant magnetic properties and biocompatibility. It is important that the SPIONs are internalized by cancer cells, as this can increase the contrast-enhancing effect of the particles (through the accumulation of more SPIONs in the cancerous tissue), allow for longer effective imaging times and facilitate interesting applications such as cell tagging and tracking (even through multiple generations) (Rosen et al., 2012). Both organic polymers and inorganic materials are extensively used for encapsulating the SPIONs and improving their biocompatibility, increasing the cellular uptake, enhancing the circulation of SPIONs and preventing protein corona adsorption (Licciardi et al., 2019). Internalization is also desirable for the implementation of various therapeutic techniques such as drug delivery and hyperthermia (Kandasamy & Maity, 2015). For the first time, ferritin coated SPIONs were investigated as nanotools for site targeted theranostic applications. We synthetized a new nanocarrier, the Humanized Archaeoglobus fulgidus ferritin (HumAfFt), an engineered ferritin characterized by the peculiar salt-triggered assembly-disassembly of the hyperthermophile Archaeoglobus fulgidus ferritin and which is successfully endowed with the human H homopolymer recognition sequence by the transferrin receptor (TfR1 o CD71), overexpressed in many cancer cells in response to the increased demand of iron (Palombarini et al., 2020). We first disassembled the HumAfFt and then we reassembled the nanoparticle in the presence of 10 nm SPIONs (HumAfFt-SPION ratio= 1), by monitoring the concentration of $MgCl_2$. The DLS measurements with SEM-EDX and AFM analyzes demonstrated that we successfully coated the Fe_3O_4 nanoparticles with the HumAfFt. The ability of this synthetized nanosystem to discriminate cancer cells from the healthy ones was demonstrated by in vitro studies.

DNA condensation and protection by the *Myxococcus xanthus* Encapsulin

A.V. Almeida*[1-2], T. Calmeiro[3], N.C. Jones[4], S. V. Hoffmann[4], E. Fortunato[3], A. S. Pereira[1-2], P. Tavares[1-2]

[1]UCIBIO – Applied Molecular Biosciences Unit, Lisbon, Portugal [2]UCIBIO, Caparica, Portugal [3]Associate Laboratory i4HB - Institute for Health and Bioeconomy, Lisbon, Portugal [4]CENIMAT/i3N, Department of Material Science, Lis, Portugal [5]ISA, Department of Physics and Astronomy, Aarhus, Denmark

Spatial organization is an essential intracellular mechanism used to control complex biochemical reactions. Due to the absence of intracellular lipid-based membranes in prokaryotes, protein nanocages emerged as a solution to generate and maintain distinct controlled environments.

Encapsulins are icosahedral protein nanocages found in Bacteria and Archaea, composed of multiple identical subunits that self-assemble to form a hollow symmetric sphere-like structure capable of harboring different types of cargo. Depending on the sequence and number of subunits these proteins cages can display a large range of internal cavity sizes.

The function of Encapsulin systems is related with the cargo proteins encapsulated within them. *Myxococcus (M.) xanthus* encapsulin (EncA) is a 32 nm wide cage protein with 180 identical subunits and three distinct cargo proteins encoded in its operon: EncB, EncC and EncD. EncC is predicted to contain Ferritin-like domains, suggesting a potential for this complex (EncAC) to act as an enhanced large compartment for iron storage (up to 30,000 atoms) in the form of a mineral core, similar to the proteins from the Ferritin family.

Our results demonstrate the binding and protection of supercoiled plasmid DNA (pUC19) by EncA both with and without its cargo protein. Using Electrophoretic Mobility Shift Assays (EMSA) and Atomic Force Microscopy (AFM) we monitored the conversion of pUC19 from its free supercoiled conformation to a more relaxed form with a beads-on-a-string morphology when forming a complex with EncA. At higher concentrations EncA self-aggregates, further condensing the DNA. This binding and condensation event protected the DNA from enzymatic digestion by DNase I. Additionally, changes in the overall secondary structure of the protein upon binding to pUC19 were evaluated using Synchrotron Radiation Circular Dichroism (SRCD). Although the secondary structure of EncA is unaltered by the interaction with pUC19, the melting temperature of the protein (Tm) slightly increases from 76 ± 1 °C to 79mmon feature of encapsulin systems or if it is limited to *M. xanthus* encapsulin. The confirmation of the relevance of these findings in vivo would also be an important follow-up experiment.

Fluorescence Lifetime Imaging Microsocpy (FLIM) to study peptide-membrane interaction

S. Anselmo*[1], G. Sancataldo[1], V. Foderà[2], V. Vetri[1]
[1]1Dipartimento di Fisica e Chimica – Emilio Segré, University of Palermo, Palermo, Italy [2]Department of Pharmacy, University of Copenhagen, Copenhagen, Denmark

The detailed analysis of peptide-membrane interaction, which is regulated by the balance of general forces (hydrophobic, electrostatic, Van der Waals intractions), is of great interest as it underlies functional and pathogenic phenomena. Such interactions involve multiple complex mechanisms, which are strongly dependent on both the membrane lipid composition and structural details (e.g. charge, hydrophobicity, steric hindrance) of the bioactive peptide chains, and may furthermore change depending on environmental conditions (temperature, pH, presence of co-solvents). Due to the complexity of these highly dynamic and spatially heterogeneous processes, a mechanistic description of these phenomena is still far from being achieved.

Here, combining spectroscopic and fluorescence microscopy techniques, we are able to analyse the fate of the multifunctional amphiphilic peptide Transportan 10 (TP10) and its effects on model membranes and to gain information on the occurring events with molecular details. Our study, based on the use of suitable fluorescence reporters, exploits the advantages of phasor plot analysis of Fluorescence Lifetime Imaging (FLIM) measurements to distinguish peptide internalization from absorption in the membrane with high spatial resolution and to highlight the consequential membrane modifications in terms of fluidity and hydration. By means of the use of Laurdan and di-4-ANEPPDHQ, fluorescent dyes that sense physico-chemical aspects of the membranes at different length scales, we could also analyzed what happens at different depth of phospholipid bilayers.

Results indicate how the complementary use of multiple molecular reporters and FLIM analysis, by means of phasor approach, may highlight diverging aspects of such complex phenomenon as peptide-membrane interaction allowing the possibility of following dynamic events in real time without sample manipulation. Subresolution details are achieved regarding lipid organisation changes in the membrane which also reveal pores formation in conditions where mofications in membrane morphology at the micronscale are not observed.

Biochemical Characterization of metalloproteins involved in *Neisseria gonorrhoeae* proliferation in the host

D. Barreiro*[1-2], R. Oliveira[1-2], S. Pauleta[1-2]
[1]*Microbial Stress Research Laboratory, Applied Molecular Biosciences Unit (UCIBIO@FCT-NOVA), Lisbonne, Portugal* [2]*Associate Laboratory i4HB - Institute for Health and Bioeconomy (i4HB@FCT-NOVA), Lisbonne, Portugal*

Pathogens are typically exposed to environmental stresses in a host organism, such as oxidative stress induced by the host immune system, through reactive oxygen and nitrogen species, and/or low levels of oxygen. *Neisseria gonorrhoeae* is the obligate human pathogen responsible for the sexually transmitted disease gonorrhoea. Due to its high level of resistance against antibiotic and lack of a vaccine it is considered an urgent health problem,[1] and thus other targets need to be identified for the design of antibiotics. In this case we focus on the anaerobic respiration of *N. gonorrhoeae* that has a truncated denitrification pathway comprised by an outer-membrane copper-nitrite reductase (AniA) and an inner-membrane nitric oxide reductase (qNOR) that catalyse the reduction of NO_2^- to NO and NO to N_2O, respectively.[2] AniA was shown to be essential for colonization as it uses NO_2^- as an alternative electron acceptor to O_2,[3] while preventing cell exfoliation with the generated NO enhancing bacteria-host cell attachment.[4]

We present the biochemical and spectroscopic characterization of AniA and cytochrome c_2, reported to be involved in NO_2^- reduction pathway as a putative electron donor to AniA.[5] UV-visible and ^{1}H-NMR spectroscopies showed that Cytc_2 is His-Met coordinated in a HS/LS-equilibrium at biological pH and room temperature. Circular dichroism indicates that Cytc_2 has a mixed secondary nature, conversely to other class I *c*-type cytochromes. UV-visible and EPR spectra of AniA classifies it as a blueish-green CuNiR. Reoxidation in the presence of NO_2^- at pH 6.0 indicates that AniA is catalytically active, decreasing its activity at neutral and basic pH. Thermostability by CD indicates that AniA is hyperthermophilic, as copper centres coordination sphere are still assembled at 90ºC.

Essential transcription regulator Rta of Epstein-Barr virus - functional and structural implications for new antiviral strategy

T. Brom*, T. Janovic, M. Stojaspal, E. Kurilova, S. Chludilova, C. Hofr
LifeB, FGP, National Centre for Biomolecular Research, Faculty of Science, Masaryk University, Brno, Czech Republic

The Epstein-Barr virus (EBV) is one of the most common human viruses that infects more than 90% of the world population during their lifetime. EBV causes 200,000 cancer cases per year and is associated with various premalignant lymphoproliferative diseases, including Hodgkin's lymphoma, gastric cancer, and nasopharyngeal carcinoma [1]. Besides cancer, infectious mononucleosis and multiple sclerosis are linked to EBV [2, 3].

The Replication and transcription activator (Rta) can activate the lytic phase of EBV from its latency and is therefore essential for the EBV life cycle. As a transcription regulator, Rta binds to Rta Response Element (RRE) localized on viral DNA and transactivates a series of lytic genes, including the viral lytic gene PAN [4]. Rta has not been structurally characterized yet, and no direct homologies were identified compared to other known DNA binding or dimerization motifs [5].

Here, we present an interdisciplinary study of Rta function. We characterized the biophysical properties of the DNA binding domain of Rta and its oligomerization, which is crucial for DNA binding. Moreover, we described the structural features of the DNA binding domain of Rta. We determined the binding affinity of Rta towards a specific DNA sequence containing the RRE motif. In parallel with an *in vitro* study, we characterized Rta in a human cancer cell line, where we investigated Rta nuclear localization and its sequestration from nucleoli.

Rta targeting with small molecules presents a new potential approach in the fight against EBV-associated diseases. Thus, a detailed understanding of the Rta structure and oligomeric state is critical for future rational anti-EBV drug design.

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The comparison of LEGO-Lipophosphonoxins' antimicrobial effect on Gram negative and Gram positive bacteria

H. Brzobohatá*[1], M. Dugić[1], T. Dolejšová[1], D. Do Pham[2], V. Mojr[2], G. Mikušová[1], D. Rejman[2], R. Fišer[1]

[1]Department of Genetics and Microbiology, Laboratory of Bacterial Physiology, Faculty of Science, Charles Universtity, Prague, Czech Republic [2]Dominik Rejman Group, Institute of Organic Chemistry and Biochemistry, Czech Academy of Science, Prague, Czech Republic

Lipophosphonoxins (LPPOs) are recently developed synthetic antimicrobials with a modular structure. LEGO-LPPOs represent the new dimeric generation of these molecules. We tried to decipher in rough outline the importance of linker module (LM) and hydrophobic module (HM) properties by characterizing the membrane-disruptive action of selected LEGO-LPPO molecules on artificial membrane systems and in various bacteria. We tested molecules with comparable antimicrobial activity. The studied LEGO-LPPOs differed in the length of their LM in a range of 3-6 carbon atoms while the HM module varied in its hydrophobicity and aromaticity. We observed highly cooperative action of representative LEGO-LPPO in permeabilizing Gram negative (G-) bacteria *Pseudomonas aeruginosa* (Hill number n~8) in contrast to lack of cooperativity in Gram positive (G+) bacteria *Staphylococcus aureus* (n~1). In an artificial membrane system resembling plasmatic membrane of G- bacteria the single-pore conductance was about 10-times higher than in a system mimicking G+ bacteria. This suggests that LEGO-LPPOs form membrane pores of different sizes in the membrane of G+ and G- bacteria. While the character of the LM segment seems to be less critical, the aromatic structure of HM stimulates higher antibacterial activity of LEGO-LPPOs.

Pseudomonas syringae pv. _morsprunorum_ biofilm development by ATR-FTIR spectroscopy and microscopy techniques

J. Budil*[1-2], P. Lišková[1], R. Fišer[1], A. Kromka[2]
[1]*Department of Genetics and Microbiology, Laboratory of Bacterial Physiology, Faculty of Science, Charles University, Praha 2, Czech Republic* [2]*Department of Semiconductors, Diamond Growth Research Group, The Institute of Physics, Praha 6, Czech Republic*

Pseudomonas syringae is a phytopathogenic bacterium that damages harvests of economically important crops. An important part of the *P. syringae* pv. *morsprunorum* (*Psm*) lifecycle is its epiphytic phase on the plant surface mediated by the biofilm growth mode, which improves its survival, increases its resistance to most antibacterial agents, and enables establishing high populations prior to infecting the plant hosts. The biofilm growth further diminishes the effectiveness of commonly used antimicrobial treatments of crops, which is already being reduced by the rising occurrence of naturally resistant strains. Thus improving the understanding of development and dynamics inside the biofilm is an important requirement to improve the treatment methods currently employed to minimize the damage to the crops.

Here we report the development of biofilm grown in a flow-cell chamber by continuously monitoring the bottom-most 1-2 bacterial layers in real time using the non-invasive ATR-FTIR technique with ZnSe reflective crystal substratum, and by direct microscopic visualizations of the biofilm from an adjanced secondary flow-cell chamber with ZnSe and glass substrates, as well as the biofilm from the main ATR-FTIR chamber at selected developmental stages of the biofilm. The influence of nutrition availability, and the presence of antimicrobial agents on the *Psm* biofilm development dynamics on abiotic surface were also compared and explained in terms of ATR-FTIR spectra development and visualized biofilm morphology.

eSPC, an online data-analysis platform for molecular biophysics

O. Burastero*, S. Niebling, A. Struve, M. Garcia-Alai
European Molecular Biology Laboratory, Hamburg, Germany

All biological processes rely on the formation of protein-ligand, protein-peptide and protein-protein complexes. Studying the affinity, kinetics and thermodynamics of binding between these pairs is critical for understanding basic cellular mechanisms. Many different technologies have been designed for this purpose, each based on measuring different signals (fluorescence, heat, thermophoresis, scattering and interference, among others). Evaluation of the data from binding experiments and their fitting is an essential step towards the quantification of binding affinities. Here, user-friendly online tools to analyze biophysical data from steady-state fluorescence spectroscopy, microscale thermophoresis and differential scanning fluorimetry experiments are presented. The modules of the data-analysis platform (https://spc.embl-hamburg.de/) contain classical thermodynamic models and clear user guidelines for the determination of equilibrium dissociation constants (Kd) and thermal unfolding parameters such as melting temperatures (Tm).

Metabolism and DNA repair: clues from the role of human serine hydroxymethyltransferase

F. Di Fonzo*, F.R. Liberati, S. Spizzichino, S. Di Russo, A. Bouzidi, G. Boumis, G. Giardina, A. Paone, S. Rinaldo, F. Cutruzzolà
Department of Biochemical Sciences Rossi Fanelli, University of Rome, La Sapienza, Rome, Italy

Serine Hydroxymethyltransferase (SHMT) is a metabolic enzyme involved in the reversible conversion of serine and tetrahydrofolate, into glycine and N5,N10- Methylenetetrahydrofolate (5,10-CH2-THF). This protein is a pyridoxal 5' phosphate dependent enzyme, whose aggregation state is tetrameric, working as a dimer of obligate dimers.

SHMT's reaction is a part of a complex network of metabolic pathways, one carbon metabolism, that fuels the synthesis of nucleotides, NADPH homeostasis, exploitable by cancer cells to grow and proliferate without control. Given the central role of this protein in sustaining anabolic reactions, SHMTs are overexpressed in several types of tumors, being an interesting target for cancer therapy.

In humans, there are two genes encoding SHMT, a cytosolic (SHMT1) and a mitochondrial (SHMT2) isoform. There is a third isoform SHMT2 alfa, which has the same sequence of SHMT2 but it lacks the mitochondrial import sequence.

Recently, my team has demonstrated a moonlighting activity of this protein, that has a non-canonical nucleic acids binding properties..

In particular, SHMT1 binds its mitochondrial counterpart's transcript 5'UTR, negatively regulating its expression, while the mRNA in turn ribo-regulates the catalytic activity of the enzyme (Guiducci et al., 2019).

Furthermore, SHMT1 translocates into the nucleus during S and G2/M phase or in response to DNA damage, where it forms a protein complex with dihydrofolate reductase and thymidylate synthase for de novo thymidylate biosynthesis in situ (Spizzichino et al.,2021) (MacFarlane et al., 2011).

Therefore, in order to understand how SHMT is involved in DNA repair, both into the nucleus and, as a nucleic acid sensor, in the cytoplasm, and to investigate if the binding to DNA could affect the metabolic activity of the enzyme, we characterized the molecular basis of SHMT1-DNA interaction *in vitro* and *ex vivo*. *In vitro* preliminary data demonstrate that SHMT1 binds preferentially ssDNA with micromolar affinity and that DNA binding affects enzyme activity. In cell studies show how SHMT1 knock out leads to an increased interferon-β expression and a major accumulation of DNA into the nucleus.

What can we learn from a multi-approach biophysical benchmark study aimed at monitoring a biomolecular interaction

C. Di Primo*[1], P. England[2], Y. Nominé[3], T. Schäfer[4], B. Olave[4], I. Rafaniello[4], F. Spiga[5], M. Vegh[5], K. Cottier[5], K. Mercier[6], N. Vollmer[6], C. Frydman[6], M. Albers[7], N. Granqvist[7], R. Bombera[7], J. Kuncova-Kallio[7], M. Murphy[8], P. Page[8], D. Porter[8], K. Fisher[8], S. Berger[9], S. Debrand[9], L. Delarbre[10], M. Lowinski[10], A. Rak[10], C. Quétard[11], H. Wünsche[11], E. Ennifar[3]
[1]University of Bordeaux/INSERM, Pessac, France [2]Institut Pasteur, Paris, France [3]CNRS-University of Strasbourg, Strasbourg, France [4]Polymat- Universidad del Pais Vasco, San Sebastian, Spain [5]Creoptix AG, Wadenswil, Switzerland [6]Horiba, Palaiseau, France [7]BioNavis Ltd, Tampere, France [8]Ametek-Reichert, Depew, United States [9]Servier, Croissy-sur-Seine, France [10]Sanofi R&D, Vitry-Sur-Seine, France [11]Sartorius Stedim FMT S.A.S, Aubagne, France

In fundamental and applied research the characterization of binding parameters of complexes involving biological macromolecules remains a crucial step to understand function, to relate it to the structure, to evaluate whether a specific ligand is of interest for therapeutic purposes, or to validate a molecular probe for biosensing or for more fundamental studies. The determination of the dissociation equilibrium constant, K_D, and if possible the kinetics and the thermodynamic parameters of the binding reaction, is often seen as the Holy Grail for all those requesting the support of a core facility. The last decade has seen the emergence of new manufacturers offering biosensing instruments that suit the needs of researchers. There is now a wide range of instruments that are capable of measuring affinity, kinetic and thermodynamic constants of complexes involving biological macromolecules, with different setups and based on different physical principles. The goals of the present benchmark study was to analyze how different technical setups performed for analyzing a very simple model, the formation of a 10-mer DNA double helix, the stability of which can be easily and finely tuned by temperature, ionic strength or single point mutations. The results show that the measured dissociation constant, K_D, depends to a significant extent on how the DNA formation helix is monitored.

kinITC microcalorimetry and switchSENSE approaches to study interactions between eukaryotic 80S ribosome and viral RNA

E. Schenckbecher[1], G. Bec[1], T. Sakamoto[2], P. Dumas[1], E. Ennifar*[1]
[1]UPR 9002 du CNRS, CNRS, Strasbourg, France [2]Chiba Institute of Technology, Chiba, Japan

Translation initiation, in both eukaryotes and bacteria, requires essential elements such as mRNA, ribosome, initiator tRNA and finally initiation factors. For each domain of life, canonical mechanisms and signals are observed to initiate protein synthesis. However, in some cases other ways of initiation can be used, as for viral mRNAs. Viruses hijack cellular machinery to translate some of their mRNAs through a non-canonical initiation pathway using Internal Ribosome Entry Site (IRES), a highly structured RNAs which can directly recruit the ribosome.

Here we show that electroswitchable DNA nanolevers using the switchSENSE technology [1] and advanced ITC microcalorimetry using the kinITC strategy [2] can be used to study interactions of large macromolecular complexes such as the 96 KDa intergenic IRES from the cricket paralysis virus (CrPV) and the 3.3 MDa eukaryotic yeast ribosome. The comparison of the data derived from the two approaches yielded very similar results, each with its own advantages and limitations.

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Exploring new possibilities of isothermal microcalorimetry: a look at interfaces

P. Garrido*[1-2], M. Bastos[3], A. Velázquez-Campoy[4-5], P. Dumas[6], Á. Piñeiro[1]
[1]Depto. de Física Aplicada, Universidade de Santiago de Compostela, Santiago de Compostela, Spain [2]AFFINImeter-Software 4 Science Developments S.L, Santiago De Compostela, Spain [3]CIQ-UP, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, Porto, Portugal [4]Institute of Biocomputation and Physics of Complex Systems (BIFI), Joint Unit GBsC-CSIC-BIFI, Universidad de Zaragoza, Zaragoza, Spain [5]Department of Biochemistry and Molecular and Cell Biology, Universidad de Zaragoza, Zaragoza, Spain [6]IGBMC, Dept of Integrative Biology, Strasbourg University, Strasbourg, France

Isothermal microcalorimetry has been used in biophysics to measure the heat involved in an interaction process between different biomolecules (binding, for example) or between molecules of the same kind (like self-assembling). The characterization of this heat provides information about the thermodynamics of the event and, therefore, about its stoichiometry, spontaneity, and affinity [1]. Biocalorimetric experiments need no labelling or chemical modification on the studied molecules. They also take place in solution, which may help to reproduce biological conditions. However, some molecular interactions only happen when the target molecule is exposed to polar and non-polar media at the same time, i.e., they can only take place at the interface. Moreover, the molecular adsorption or the denaturation of proteins at fluid interfaces are processes that have not been studied by this kind of calorimetry. In this work, we present the first results of the extension of microcalorimetry to fluid interfaces [2,3].

Through the creation of bubbles or drops in the calorimetric cell, the formation and destruction of interfaces can be studied. In addition, any heat-evolving event that may happen during the growing or stabilization of these interfaces can also be detected. The here-presented new methodology has been developed testing different microcalorimeters, temperatures and systems. We have started from the characterization of the most common fluid-fluid interface (air-water) and continued by adding more complexity to the process until tackling the adsorption of proteins. The growing and delivery of bubbles produce a heat signal that is full of information by itself, with special significance in fundamental studies of interfaces. Additionally, the understanding of this signal and the control of bubble formation has allowed us to extend its applications to determine surface tension from the heat signal or to detect, for the first time, the heat of adsorption of proteins at fluid-fluid interfaces. The methodology is still an ongoing project, and new possibilities are expected for the future.

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The conformational dynamics of the N-terminal tails of a Dps protein from *Deinococcus grandis*

J.P.L. Guerra*[1-2], C. E. Blanchet[3], B. J. C. Vieira[4], A. V. Almeida[1-2], J. C. Waerenborgh[4], N.C. Jones[5], S. V. Hoffmann[5], P. Tavares[1-2], A. S. Pereira[1-2]
[1]UCIBIO - Applied Molecular Biosciences Unit, NOVA School of Science and Technology, Caparica, Portugal [2]Associate Laboratory i4HB - Institute for Health and Bioeconomy, NOVA School of Science and Technology, Caparica, Portugal [3]European Molecular Biology Laboratory, Hamburg Outstation, Hamburg, Germany [4]Centro de Ciências e Tecnologias Nucleares, Insituto Superior Técnico, Universidade de Lisboa, Bobadela, Portugal [5]ISA, Department of Physics and Astronomy, Aarhus University, Aarhus, Denmark

DNA-binding protein from starved cells (Dps), also known as miniferritins, are protein nanocages with a hollow sphere-like structure ~10 nm wide resulting from the assembly of 12 identical monomers with unordered N- and C- terminal tail extensions of variable size. As members of the Ferritin family of proteins, they catalyze the oxidation of ferrous ions using either H_2O_2 or O_2 as co-substrates. The product of this reaction accumulates within the protein in the form of a ferrihydrite mineral core. This activity is designed to protect DNA from oxidative stress, reducing the risk of harmful ROS being formed by Fenton reactions. Strikingly, Dps proteins are also capable of directly shielding DNA through binding, forming both low- and high-order Dps-DNA condensates, adding physical protection to the chemical protection. Whilst it is known that the nature of the DNA-binding properties is related to the N-terminal tail extensions, their intrinsic structural and functional features are still relatively uncharacterized, as well as their striking evolutionary diversity.

We have recently used several biochemical and biophysical techniques to probe the Dps from *Deinococcus grandis*, which possesses an atypically long N-terminal tail, composed of 52 residues, including a metal-binding site. Our data has shown that the conformation of the tails can be modulated by external factors such as the ionic strength of the buffer. A dynamic process in which the tails shift from a smaller compact conformation into an extended solvent-accessible positioning as the ionic strength increases was characterized using Dynamic Light Scattering, Synchrotron-Radiation Circular Dichroism and Small Angle X-ray Scattering.

Moreover, a novel iron-binding site was identified in the tail by monitoring the anaerobic titration of the protein with $^{57}Fe^{2+}$ using Mössbauer Spectroscopy. The spectra reveal a monomeric high-spin ferrous species with parameters distinct from any other ferrous iron-binding sites previously found in miniferritins.

Taken together, the results from this work suggest a physiologically relevant role for the N-terminal tail metal-binding site besides the one previously considered and prompts the search for other tail modulators and their impact on Dps structure and function.

Interaction of an artificial zinc finger protein with toxic metal ions

B. Hajdu*, B. Gyurcsik
Department of Inorganic and Analytical Chemistry, University of Szeged, Szeged, Hungary

Zinc finger proteins are modular specific DNA binders that can recognise almost any DNA sequence [1]. They function as transcription activators inside cells, but they can be turned into specific DNA manipulating agents by attaching an otherwise nonspecific nuclease domain to a zinc finger array [2]. Zinc fingers can only function properly as specific DNA-binders, if the central Zn(II)-ion is coordinated. Therefore, it is essential – especially in the case of a potential nuclease – to determine the zinc-binding stability of the protein. Competition reactions might occur as well inside living organisms, which can demolish the specific DNA binding ability, or might even cause nonspecific DNA degradation. Especially toxic metal ions with soft characteristics (cadmium(II), mercury(II), silver(I)) are supposed to compete with zinc(II).

UV-Vis absorbance, circular dichroism (CD), fluorescence spectroscopic, ESI mass spectrometric (MS), isothermal titration calorimetric (ITC) measurements and electrophoretic gel-mobility shift assays (EMSA) were performed. The application of EDTA competitor was optimized to analyse the zinc(II) binding of a zinc finger nuclease consisting of three finger domains. We also investigated the competition effect of silver(I), cadmium(II) and mercury(II) ions for the zinc finger protein. Further EMSA and CD experiments were performed in the presence of specific or nonspecific DNA sequences to understand the impact of DNA on the competition reactions [3].

Based on our results the zinc finger protein binds the zinc(II) ions strongly and the zinc(II) removal with EDTA does not occur momentarily. The heavy metal ions may partially or completely replace the native metal ion. DNA binding can stabilize the zinc finger protein in some cases.

Acknowledgements: Financial support by the Hungarian National Research, Development and Innovation Office (GINOP-2.3.2-15-2016-00038, K_16/120130, as well as 2019-2.1111-TÉT-2019-00089).

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Macromolecular crowding: experimental characterization of model mimics

J. Hamacek*[1], A. Yethiraj[1-2], V. Thrithamara R[2], F. Piazza[1-3]
[1]University of Orléans / CNRS, Orléans, France [2]Physics & Physical Oceanography, Memorial University, Saint Johns, Canada [3]Physics & Astronomy, University of Florence, Florence, Italy

Artificial macromolecular crowding agents used for simplifying and mimicking a cell-like environment *in vitro* experiments comprise different chemical compounds such as polyethylene glycol, dextran, Ficoll, etc. These macromolecules are ideally monodisperse and do not interact with active components of reaction systems (enzyme, substrates, etc.). Such controlled conditions will allow to gather information on the effect of excluded volume and on the relative enzyme/obstacle size, which is important to address more complex situations closer to natural matrices (a bottom-up approach).

In this contribution we present our recent investigations of aqueous crowding media. It is important to ensure a good homogenization of measured solution in order to avoid/control possible aggregation. A variety of complementary characterization methods is then applied to pinpoint intermolecular interactions not only between crowders themselves, but also their interactions with small molecules (e.g. water, enzyme substrates) and large biomolecules such as proteins. In this context, diffusion coefficients of PNPP (the substrate of alkaline phosphatase) in the presence of Ficoll were measured through PFG-NMR experiments. Complementary data to probe the structural features of this system were measured with spectroscopic methods (FTIR, Raman, CD). The obtained results are discussed in relation with the effects of a complex medium on enzymatic kinetics in crowded environments.

Thermal Liquid Biopsy (TLB): application of differential scanning calorimetry to biological samples for the diagnosis of diseases

S. Hermoso Duran*[1-2], MJ. Domper[1-3], MP. Roncales[1-3], MM. Murillo[3], V. Cros[3], A. Lanas[1-3], A. Velázquez-Campoy[1-2], O. Abian[1-2]

[1]Instituto de Investigación Sanitaria Aragón (IIS Aragón), Zaragoza, Spain [2]Institute of Biocomputation and Physics of Complex Systems (BIFI), Joint Unit GBsC-CSIC-BIFI, Zaragoza, Spain [3]Zaragoza, Spain [4]Hospital Clínico Universitario Lozano Blesa, (HCULB), Servicio Aragonés de Salud, SALUD, Zaragoza, Spain

Background: Differential Scanning Calorimetry (DSC) is a thermoanalytical technique to obtain thermal denaturation profiles of biomolecules and assess their interaction with various metabolites. Thermal liquid biopsy (TLB) is presented as the use of DSC with biological fluid samples (serum/plasma). The comparison of thermograms of biological samples between healthy and pathological individuals has revealed distinctive alterations, helping to validate TLB as a complementary tool for the diagnosis of several diseases.

Methods: Experiments were performed on diluted serum/plasma samples (1:25 in PBS) at a scan rate of 1°C/min, between 10 and 95°C. The thermograms were corrected by baseline and analyzed using software developed in our laboratory implemented in Origin 7 (OriginLab): a multiparametric procedure based on a deconvolution analysis of each thermogram with six individual components where each of them is characterized for three parameters. The 18 primary parameters obtained directly from the deconvolution of the thermogram were combined to define the TLB-score by using a generalized linear model, which represents the probability (a number between 0 and 1 and using a threshold of 0.5) that an individual shows plasmatic alterations in the thermogram and could be associated with the disease.

Results: We have obtained a TLB-score for: lung cancer diagnosis with 91% accuracy rate, 90% sensitivity, 92% specificity[1]; for multiple sclerosis diagnosis with AUC=0.69, 67% accuracy rate, 69% sensitivity, 65% specificity[2].

Conclusions: TLB is a fast, minimally invasive and low-risk technique that can be applied in clinical practice for the diagnosis, screening and periodic follow-up of different pathologies. We are currently working on several projects to expand the application of TLB: diagnosis of ovarian and pancreatic cancer and within the colorectal cancer screening program.

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Novel buffer screen for biomolecular characterization

J. Houser*, J. Kosourová, M. Kubíčková, E. Fujdiarová, M. Wimmerová
CEITEC MU, Masaryk University, Brno, Czech Republic

A biomolecular sample is a cornerstone of biochemical, biophysical, and structural analysis. The quality of such sample, especially protein, determines markedly the quality of subsequent measurements. It was suggested [1] that insufficient sample characterization may cause frequent problems in reproducing the published results. Based on the recommendations by ARBRE and P4EU associations [2], the basic characteristics of the protein sample (identity, purity, homogeneity, stability) should always be checked for improvement of scientific workflows.

The protein characteristics are heavily influenced by the solution nature, however, the determination of a suitable buffer environment for a protein of interest is not an easy task. The requirements of advanced techniques, the demands on the biological material, and the researcher's time needed for buffer optimization, as well as personal inflexibility, lead frequently to the use of sub-optimal buffers. Here, we demonstrate the design of a 48-condition buffer screen that can be used to determine an appropriate environment for downstream studies. By the combination of several techniques (differential scanning fluorimetry, dynamic light scattering, and bio-layer interferometry), we were able to assess the protein stability, homogeneity, and binding activity across the screen with less than half a milligram of protein in 1 day [3]. The application of this screen helps to avoid unsuitable conditions, explain problems observed upon protein analysis and choose the most suitable buffers for further research. The screen is now routinely used in our Biomolecular Interactions and Crystallization Core Facility as a primary screen for buffer optimization and can be introduced also in other labs and facilities.

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Measurements at Biomolecular Interactions and Crystallization Core Facility are supported by the CIISB project of MEYS CR (LM2018127).

Protein:Protein interactions of the aryl hydrocarbon receptor interacting protein (AIP) are affected by N-domain mutations

M. Vella[1], R. Rambo[2], I. Manfield[3], C. Trinh[3], T. Hunter[1], G. Hunter*[1]
[1]Physiology & Biochemistry, University of Malta, Msida, Malta [2]Soft Condensed Matter Village, Diamond Light Source, Didcot, United Kingdom [3]School of Molecular and Cellular Biology, University of Leeds, Leeds, United Kingdom

The aryl hydrocarbon receptor interacting protein (AIP) is a cytoplasmic molecular co-chaperone and tumour suppressor that assists in protein stability and complex formation. Germline mutations in the AIP gene predispose to pituitary tumourigenesis with patients exhibiting an aggressive clinical phenotype. Our studies are focused on the structural and functional characterisation of AIP to investigate whether clinically relevant N-domain mutations affect the ability of the protein to interact with client binding partners. A purification protocol for AIP was successfully devised that maintains the protein in a stable homogeneous state. Similarly, variants of full length AIP harbouring N-domain mutations (R9Q, R16H, V49M and K103R) were purified from *E.coli* to the same level. Circular dichroism spectroscopy showed that the mutations caused no overall disruptive effect in the protein structure or thermal stability. However, through isothermal titration calorimetry and surface plasmon resonance experiments, these mutations lowered, to different extents, the binding affinity of AIP towards two of its binding partners, Hsp90β$^{(530-724)}$ and PDE4A5, also purified in this study. The latter was further demonstrated through a PDE enzymatic assay in which the mutants failed to attenuate the enzymatic activity of PDE to the same degree as the wild type protein. Our study provides clear evidence that AIP N-domain mutations have a significant role in protein-protein interactions and although they may not necessarily contribute directly to pituitary tumourigenesis, the complex interactome of AIP suggests that any observable change in one or more of its binding partners cannot be disregarded as it may have repercussions on other biochemical pathways. Small angle x-ray scattering (SAXS) compared reasonably with full length models predicted by I-TASSER and Alphafold. Currently, the interaction between AIP and Hsp90$^{(530-724)}$ is being further investigated using single-molecule diffracted X-ray tracking (DXT) experiments to investigate the dynamics of AIP-mutants in the absence and presence of Hsp90$^{(530-724)}$ and potentially identify protein-protein binding sites.

Study of the Interaction of Misfolded Proteins with Biomimetic Lipid Bilayer Systems

E. Jankaityte*[1], A. Sakalauskas[2], Z. Toleikis[2], R. Budvytyte[1], V. Smirnovas[2], G. Valincius[1]
[1]Institute of Biochemistry, Life Sciences Center, Vilnius University, Vilnius, Lithuania [2]Institute of Biotechnology, Life Sciences Center, Vilnius University, Vilnius, Lithuania

Protein misfolding is a common cellular event that can occur throughout the lifetime of a cell. Increased accumulation of misfolded proteins in the brain is a hallmark of several neurodegenerative disorders. For example, amyloid beta peptide (Aβ) is associated with Alzheimer's disease (AD). In AD brain, abnormal levels of this naturally occurring peptide clump together to form plaques that accumulate and disrupt functions of neurons [1]. S100A9 protein has amyloid-like properties and can be found in senile plaques together with Aβ. S100A9 is involved in formation of aggregates and inflammatory processes [2].

Intermediate structures of aggregation process are transient and heterogeneous and the mechanism by which amyloids can be neurotoxic has not been fully elucidated. One prevailing hypothesis suggests that toxic effect is accomplished through a membrane disruption mechanism, which leads to loss of synaptic efficiency, neuronal dysfunction, and degeneration [3]. Biomimetic lipid bilayer systems are a useful tool for modelling specific properties of cellular membranes to get more knowledge about their structure and functions. In this work the interaction of S100A9 and Aβ with membrane model systems was studied. Tethered bilayer lipid membranes (tBLMs) and unilamellar liposomes were used as simplified membrane models. The aim of this work was to form tBLMs and liposomes with encapsulated fluorescent dye (calcein) in order to use them in protein-membrane interaction study. Atomic force microscopy (AFM) and dynamic light scattering (DLS) methods were used for characterisation of S100A9 and Aβ. By employing electrochemical impedance spectroscopy (EIS) and fluorescence spectroscopy, we investigate interaction of phospholipid bilayer with S100A9 and Aβ depending on their oligomerisation state.

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Shining Light on Non-chemical Steps in Enzyme Catalysis with Biophysical Approaches

A. Kriznik*[1-2], M. Libiad[3], H. Le Cordier[1], M. Toledano[3], S. Rahuel Clermont[1-2]
[1]IMoPA Enzymologie Moléculaire, Université de Lorraine, Vandoeuvre Les Nancy, France [2]UMS IBSLor, Université de Lorraine, Vandoeuvre les Nancy, France [3]Laboratoire Stress Oxydant et Cancer, Université Paris–Saclay, Gif-Sur-Yvette, France

Hydrogen peroxide H_2O_2 is a neutral, diffusible reactive oxygen species, which has a continuum of cellular effects depending on its concentration. Indeed, at high concentrations, H_2O_2 causes oxidative stress that is toxic to cellular constituents. Conversely, at low concentrations, it acts as a cellular messenger as a redox signal capable of regulating the function of protein targets.

Biological effects of H_2O_2 are transduced by Peroxiredoxins, peroxidases that reduce H_2O_2 by attack of the catalytic Cys on a molecule of H_2O_2 to form a Prx-SOH sulfenic acid intermediate. The regulation of 2-Cys-Prx by hyperoxidation results from the competition between two reactions of the Prx-SOH intermediate, that evolves either towards the formation of an inter-subunit disulfide bridge between the catalytic Cys and the recycling Cys, or to the hyperoxidation by another H_2O_2 molecule, leading to a sulfinic acid hyperoxidized state (Prx-SO_2). In this state, the enzyme is stabilized in a decameric form and acquires a holdase chaperone activity. A model developed on the basis of structural studies proposes that a conformational reorganization step between a Fully Folded (FF) and a Locally Unfolded (LU) forms of Prx plays a decisive role in this competition, by controlling the rate of disulfide bridge formation and/or the reactivity of the sulfenic acid towards H_2O_2.

Exploiting biophysical approaches such Trp fluorescence and CD-based stopped flow, we have identified a kinetically resolved phase that we attribute to a conformation change linked to the FF/LU transition. Using mutants of moderately altered hyperoxidation sensitivities and different peroxide substrates, we observed that hyperoxidation sensitivity is uncoupled from the resolving step kinetics and only depends on the sulfinylation and FF to LU transition rate constants. From both parameters we thus now can predict the hyperoxidation sensitivity index $C_{hyp1\%}$, a result confirmed by in vitro and in vivo sulfinylation patterns.

Dynamics of a Key Conformational Transition in the Mechanism of Peroxiredoxin Sulfinylation. Kriznik A, Libiad M, Le Cordier H, Boukhenouna S, Toledano MB, Rahuel-Clermont S. *ACS Catal*. 2020 Mar 6;10(5):3326-3339.

Structural and functional insights of Hsp90C, the chloroplastic Hsp90 family member

R. La Rocca*, T. Chenuel, C. Bergonzi, P. Meyer
LBMCE, Sorbonne Université, IBPC, Paris, France

The Hsp90 family have been largely studied through the last 30 years, so that now its molecular mechanisms and pathological implications are well known in various species. However, a subset of Hsp90 proteins, namely the stromal Hsp90 proteins, are less described. More specifically, Hsp90C, the main Hsp90 of the chloroplast, is very poorly known despite its crucial role in protein import, which is essential for photosynthesis. Here we provide functional and structural insights of Hsp90C by means of biophysical approaches. First, with ATPase activity assays, we show that Hsp90C has an activity comparable to HtpG, the bacterial homolog. Second, using *in vitro* luciferase refolding assays with the full bacterial refolding machinery, we show that Hsp90C is able to stimulate the renaturation of luciferase induced by DnaK. Interestingly, Hsp90C refolds a significantly higher proportion of luciferase compared to HtpG. Besides, we obtained the first crystallographic structure of the middle domain of Hsp90C. Noticeably, the structure reveals a longer helix located in the end of the domain, which implies that the C-terminal domain may have a different conformation from the other Hsp90 proteins. Further investigation with SEC experiments showed that the Hsp90C dimerization is led by its unique very end of the C-terminal tail among the Hsp90 family. Taken together, our results suggest that Hsp90C is believed to stimulate the refolding of client proteins in the chloroplast and dimerizes through a non-canonical dimerization process.

TRamWAy: Mapping diffusivity and potential energy in large-scale single-particle tracking experiments

F. Laurent*, H. Verdier, M. Duval, A. Serov, C. Vestergaard, J.B. Masson
Decision & Bayesian Computation, Institut Pasteur, Paris, France

Single-molecule localization microscopy allows studying the dynamics of biomolecules in cells and resolving the biophysical properties of the molecules and their environment underlying cellular function. With the continuously growing amount of data produced by individual experiments, the computational cost of quantifying these properties is increasingly becoming the bottleneck of single-molecule analysis. Mining these data requires an integrated and efficient analysis toolbox.

We introduce TRamWAy, a modular Python library that features: (i) a conservative tracking procedure for localization data, (ii) a range of sampling techniques for meshing the spatio-temporal support of the data, (iii) computationally efficient solvers for inverse models, with the option of plugging in user-defined functions and (iv) a collection of analysis tools and a simple web-based interface.

Additionally, the library is made of building blocks that help the user to parallelize and dispatch the analysis onto remote high performance computing clusters, including from Jupyter notebooks, over entire repositories of data files, and regions of interest in each file. The resulting files are automatically retrieved onto the local host.

Example notebooks can be found at https://tramway-tour.readthedocs.io and https://github.com/DecBayComp/TRamWAy.

High Throughput and Multi-Omics based pipeline for Selective Targeting of the *Helicobacter pylori*

R. Maity*[1-2], J. Sancho[1-3]
[1]*Departamento de Bioquímica y Biología Molecular y Celular Área de Bioquímica y Biología Molecular, University of Zaragoza, Zaragoza, Spain* [2]*Aragon Health Research Institute (IIS Aragón), Zaragoza, Spain* [3]*Biocomputation and Complex Systems Physics Institute (BIFI)-Joint Units: BIFI-IQFR (CSIC) and GBsC-CSIC, Zaragoza, Spain*

Antimicrobial-resistant bacteria constitute a global health concern. *Helicobacter pylori(H. pylori)* is a Gram-negative bacterium that infects about half of the human population and is a major cause of peptic ulcer disease and gastric cancer. Increasing resistance to triple and quadruple *H. pylori* eradication therapies poses great challenges and urges the development of novel, ideally narrow spectrum, antimicrobials targeting *H. pylori*. Essential metabolic pathways are a vast promising scenario for antimicrobial development. Flavodoxins are bacterial electron transfer proteins that participate in many metabolic pathways and have been predicted as essential for *H. pylori*. We have conducted a high throughput screening to identify flavodoxin binders from a diverse chemical library of 10,000 compounds. After several rounds of chemical variation and efficacy testing, a family of novel nitrobenzoxadiazol-based antimicrobials has emerged. This family of drugs can be divided into two groups. One group is formed by narrow-spectrum compounds, highly specific for *H. pylori*, but ineffective against enterohepatic Helicobacter species and other Gram-negative or Gram-positive bacteria. The second group includes extended-spectrum antimicrobials additionally targeting Gram-positive bacteria, the Gram-negative *Campylobacter jejuni*, and most of the Helicobacter species, but not affecting other Gram-negative pathogens. In this work, we are describing a Multi-Omics based pipeline for the detection of the mode of action of these drugs and understanding resistance from this bacterium. We will also shortly discuss how this multi-omic pipeline can be used to accelerate cellular biomarker discovery during clinical trials to predict response to therapy, and define novel target pathways.

Design of Hybrid Structure for Bioorthogonal Drug Photoactivation and Photocatalysis

L.F. Mazzei*[1-2], S. H. Mejías[1], A. Martínez[2], L. Salassa[2], A. L. Cortajarena[1]
[1]CICbiomaGUNE, San Sebastian, Spain [2]DIPC, San Sebastian, Spain

Advances in biorthogonal catalysis are creating new opportunities to perform chemoselective transformations in complex biological environments with applications in biomedicine and bioimaging. Recently, riboflavin photocatalysis has been used to activate Pt(IV) anticancer prodrugs *in vitro* under light irradiation. In this work, we propose to expand this strategy by co-immobilization of both photocatalysts and prodrugs, on engineered repeat proteins as templates, and on gold nanoparticles as nanozyme scaffold. These systems are envisioned to have more efficient drug activation and will facilitate controlled cell internalization.

Specifically, we use tetratricopeptide repeat protein as engineered repeat protein with 4-6-8 repeats (CTPR4-CTPR6-CTPR8) and 1.9-nm gold nanoparticles decorated with thiol ligands bearing a TACN (1,4,7-triazacyclononane) headgroup. This contribution describes the conjugation between CTPR4, CTPR6, CTPR8, N3-functionalized riboflavin and Pt(IV) complex by exploiting cysteine (Cys) and lysine (Lys) residues of the protein scaffold, the N3 functional group on the riboflavin and NHS ester-reactive group of the Pt(IV) complex, respectively. Currently, we have achieved a distribution in the number of functionalized riboflavins and platinum complexes linked to the three modular proteins. Light-irradiation studies at 460 nm indicated that riboflavin comjugated to CTPR4, CTPR6 and CTPR8 has increased photostability, compared to free riboflavin, likely due to the ability of the protein scaffold to protect the modified riboflavin from the photodegradation of its ribityl chain. Catalysis studies performed show that riboflavin preserves its photocatalytic activity upon conjugation to the protein. In addition, preliminary cell viability experiments were performed in PANC-1 cancer cell line showing that in dark, Pt(IV) prodrugs linked to the three protein scaffold are not toxic in their inactive form.

On the other hand, using the nano-based system, it was observed that the nanozyme affording a catalytic system capable of activating the Pt(IV) prodrug substrate with an almost identical behavior in terms of catalytic activity of the system without AuNPs, reaching 80% conversion already within 7 min and a plateau at 90% conversion after 30 min of light irradiation with turnover frequency (TOF) of 7.4 min^{-1}.

A fluorescent fusion protein as a structural probe to monitor Aβ-amyloid fibril polymorphism

G. Mohite*[1], M. Lindberg[1], S. Thor[2], S. Nyström[1], P. Hammarström[1]
[1]IFM Chemistry, Linkoping University, Linkoping, Sweden [2]School of Biomedical Sciences, The University of Queensland, Brisbane, Australia

Alzheimer's disease (AD) is the most common neurodegenerative disease worldwide. AD is associated with misfolding and aggregation of Aβ and tau proteins into amyloid fibrils in the brain. Various in vivo and in vitro studies have established the link between Aβ amyloid fibril structural polymorphism and AD progression. We made fusion constructs in which the N-terminal of Aβ1-42 is labeled with mNeon Green (mNG). mNG-Aβ1-42 was expressed in bacteria and purified as per standard protocol. Aggregation kinetics was monitored using the ThT assay, and aggregates were further characterized using transmission electron microscopy (TEM). To study Aβ1-42 amyloid fibril polymorphism in vivo, transgenic Drosophila with gene insertion of UAS-mNG-Aβ1-42, UAS-mNG, were generated. Flies co-expressing both mNG-Aβ1-42 and Aβ1-42 were also made. Hyperspectral imaging shows that mNG-Aβ1-42 gets incorporated in Aβ1-42 fibrils in vitro. Data on flies co-expressing mNG-Aβ1-42 together with Aβ1-42 showed partial attenuation of Aβ1-42 induced toxicity. The X-34 amyloid dye was used to stain the whole brain and cryosections from these flies. We observed mNG positive amyloid aggregates of Aβ1-42 corroborating the in vitro results of incorporation of mNG-Aβ1-42 into Aβ1-42 fibrils in vivo. Spectral data suggest that X-34 and mNG are excellent donor-acceptor pairs for FRET analysis. We used confocal microscopy with FLIM to study aggregates formed in different cell types. FRET analysis of co-aggregated mNG-Aβ1-42 with Aβ1-42 fibrils formed from two distinct subtypes of cells (glia versus neurons) revealed easily quantifiable FRET efficiency between X-34 and mNG. FRET efficiency in glial aggregates compared to neuronal aggregates depended on genotype and age. Our FLIM data suggest that different Aβ1-42 fibril polymorphs are produced in different cell types, corroborating our group's previous results (Jonsson 2018). This new reporter protein allows us to do in-depth (low-resolution) structure analysis of temporal and genetic fibril structural polymorphism in real-time in vivo.

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Platform of Proteomic Identification and Molecular Interactions

G. Ngo*, C. Henriquet, A. Mangé, M. Pugnière
IRCM, INSERM, Montpellier, France

The Platform of Proteomic Identification and Molecular Interactions (PP2I), locating in Montpellier, France, is integrated in a collaborative network of complementary facilities in Proteomics (PPM, http://www.ppm.cnrs.fr/).

At PP2I platform, we offer label-free techniques to measure molecular interactions as well as to determine the stability and aggregation tendency of biomolecules. PP2I is involved in many areas of research including the development of therapeutic antibodies in oncology (Labex MAbImprove, SIRIC Montpellier). PP2I works with many academic and industrial partners.

PP2I presents a long-standing and strong expertise in Surface Plasmon Resonance (SPR), especially on kinetic study of proteins and small molecules screening.[1-3] PP2I is well known for classical antibody characterization, such as affinity and kinetics determination. We are also involved in other studies, such as multispecificity and interaction kinetics with Neonatal receptor (FcRn) and Fcγ Receptors.[4-6] These Fc-Receptors are key partners in the pharmacokinetic and effector functions of therapeutic antibodies.

Besides, one of our current project concerns an innovative preparation of high specific 3D molecularly imprinted polymers (MIPs) using new synthetic hybrid silylated-amino acids (Si-AAs).[7] In general, MIPs are artificial receptors that are designed to recognize and retain specific molecules with high selectivity, strong chemical resistance and low-cost production.[8] Using Quartz Crystal Microbalance with Dissipation (QCM-D) device (recently acquired by PP2I), we study the recognition capacity and specificity of the silylated-hybrid MIPs toward the targeted proteins. In comparison to the classical MIPs, we obtained a significantly increase of the recognition capacity toward the targeted proteins.

Biophysical characterization of interactions between transcription factors FOXO4 and p53

R. Mandal[1-2], K. Kohoutova[1-2], O. Petrvalska[1-2], M. Horvath[1], P. Srb[3], V. Veverka[3], V. Obsilova[2], T. Obsil*[1-2]

[1]Dept. of Physical and Macromolecular Chemistry, Charles University, Faculty of Science, Prague 2, Czech Republic [2]Department of Structural Biology of Signaling Proteins, Institute of Physiology of the Czech Academy of Sciences, Prague, Czech Republic [3]Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Prague, Czech Republic

The transcription factor p53 is a key regulator of apoptosis, senescence and DNA repair, which protects cells against tumorigenesis under various cellular stresses. The functions of p53 are closely intertwined with the activity of Forkhead box O (FOXO) transcription factors. FOXO proteins (FOXO1, FOXO3, FOXO4, and FOXO6) regulate cellular homeostasis, longevity and stress response by modulating diverse cellular functions, including cell cycle, stress resistance, DNA damage repair, apoptosis, tumor resistance and metabolism. Under cellular stress conditions, p53 interacts with FOXO4, thereby inducing cellular senescence by upregulating the transcription of senescence-associated protein p21. However, the structural details of this interaction remain unclear. In this work, we characterize the interaction between p53 and FOXO4 by NMR, chemical cross-linking, and analytical ultracentrifugation. Our results reveal that the interaction between the N-terminal transactivation domain of p53 and the FOXO4 Forkhead DNA-binding domain is essential for the overall stability of the p53:FOXO4 complex. Furthermore, contacts involving the unstructured N-terminal segment of FOXO4, the C-terminal negative regulatory domain of p53 and the DNA-binding domains of both proteins stabilize the complex, whose formation blocks p53 binding to DNA but without affecting the DNA-binding properties of FOXO4 [1]. Therefore, our structural findings may help to understand the intertwined functions of p53 and FOXO4 in cellular homeostasis, longevity, and stress response.

This study was supported by Czech Science Foundation (T.O., grant number 21-02080S) and the Grant Agency of the Charles University (R.M., grant number 1002119). M.H. was supported by Charles University Research Centre program No. UNCE/SCI/014.

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Structural insight into the 14-3-3-dependent regulation of human Nedd4-2 ubiquitin ligase

R. Joshi[1], P. Pohl[1], D. Strachotova[2], P. Herman[2], T. Obsil[3-1], V. Obsilova*[1]
[1]Structural Biology of Signaling Proteins, Institute of Physiology CAS, Praha 4, Czech Republic [2]Institute of Physics, Faculty of Mathematics and Physics, Prague, Czech Republic [3]Department of Physical and Macromolecular Chemistry, Faculty of Science, Charles University, Prague, Czech Republic

Neural precursor cell expressed developmentally down-regulated 4 ligase (Nedd4-2) is an E3 ubiquitin ligase that targets proteins for ubiquitination and endocytosis, thereby regulating numerous ion channels, membrane receptors and tumor suppressors. Nedd4-2 activity is regulated by autoinhibition, calcium binding, oxidative stress, substrate binding, phosphorylation and 14-3-3 protein binding. However, the structural basis of the 14-3-3-dependent Nedd4-2 regulation remains poorly understood. In our previous study, we found that 14-3-3 binding induces a structural rearrangement of Nedd4-2 by inhibiting interactions between its structured domains [1]. Here, we used time-resolved fluorescence intensity and anisotropy decay measurements together with fluorescence quenching and mass spectrometry to further characterize interactions between Nedd4-2 and 14-3-3 proteins. We labeled the individual structured Nedd4-2 domains with the environmentally sensitive extrinsic fluorophore 1,5-IAEDANS which allowed us to monitor the mobility and accessibility of the individual domains of Nedd4-2 upon 14-3-3 protein binding. Our data reveal that the steric hindrance of the WW3 and WW4 domains together with the conformational change in the catalytic domain may be responsible for the 14-3-3 binding-mediated regulation of Nedd4-2 functions. Therefore, 14-3-3-mediated changes in the accessibility and/or mobility of individual WW domains of Nedd4-2 may modulate different dynamic processes of membrane proteins ubiquitination. Overall, our results suggest that steric hindrance of the WW3 and WW4 domains combined with conformational changes in the catalytic domain may account for the 14-3-3 binding-mediated regulation of Nedd4-2 [2].

This study was supported by the Czech Science Foundation (Projects 20-00058S), the Czech Academy of Sciences (Research Projects RVO: 67985823 of the Institute of Physiology)

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A nanoscopic view on the aggregation process of partially labelled peptides solutions

D. Odino*[1], E. Angeli[1], P. Bianchini[2], E. Gatta[1], V. Bazzurro[1], A. Relini[1], A. Diaspro[1-2], C. Canale[1]
[1]Department of Physics, University of Genoa, Genoa, Italy [2]Nanoscopy, CHT Erzelli, Istituto Italiano di Tecnologia, Genoa, Italy

This study investigates the *in vitro* aggregation process of a nonpathological protein, insulin from the bovine pancreas, in the presence of two different fluorescent dyes. We labelled insulin with ATTO 647N (insulin-647) and ATTO 594 (insulin-594), both functionalised with an NHS ester group. We mixed labelled and unlabelled peptides to a final dye-to-protein ratio of 1:20. We induced insulin fibrillation, employing confocal laser scanning microscopy (CLSM) and stimulated emission depletion (STED) microscopy to investigate the distribution of fluorophores within amyloid fibrils. We aimed to determine whether insulin-647 and insulin-594 were recruited together in the protein aggregates. From the analysis of the images, we found Pearson's correlation coefficients [1] of 0.59 ± 0.07 and 0.44 ± 0.11 for the confocal and STED microscope, respectively. These results clearly indicated that a significant number of fibrils accepted a single fluorescent species, suggesting that a fraction of labelled peptides tends to follow only selected aggregation pathways. To confirm these results, fluorescence lifetime microscopy (FLIM) measurements are in progress. In a second set of experiments, on the same samples, we used the correlative STED-AFM technique to verify whether a fraction of unlabelled fibrils was also present. This occurrence was already shown in the aggregation of insulin labelled with a single peptide, ATTO 488-NHS [2]. By analysing the overlay of the STED image with the AFM topography, we obtain an average correlation coefficient [1] of 0.86 ± 0.04 (which represents the fraction of AFM that overlaps the STED image), showing that the great majority of the fibres are fluorescent, and confirming what we deduced from the qualitative view of the images. The approaches proposed in this work can be replicated on other molecular systems to define the best protein labelling methods for studying intermolecular processes.

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Characterization of the *Escherichia coli* bacterial peroxidase

R. Oliveira[1-2], R. Portela[1-2], S. Pauleta*[1-2]
[1]NOVA School of Science and Technology, NOVA University Lisbon, Portugal, Microbial Stress Lab, UCIBIO, Caparica, Portugal [2]NOVA School of Science and Technology, NOVA University Lisbon, Portugal, Associate Laboratory i4HB -Institute for Health and Bioeconomy, Caparica, Portugal

Bacterial peroxidases are divided into two groups: classical and non-classical,[1] with the classical ones harbouring two *c*-type haems (each in a distinct domain) and being dimers, while the non-classical are composed of three domains.

The three-domain bacterial peroxidases have been identified in the genome of pathogenic bacteria, but only a few were isolated and characterized to date.[1] These enzymes have an additional unique feature that is the presence of a N-terminal transmembrane helix, that is proposed to enable the use of the quinone pool as electron donor. These enzymes are involved in the detoxification of hydrogen peroxide in the periplasm of gram-negative bacteria [1] and confer the ability to use H_2O_2 as terminal electron acceptor in the absence of oxygen.[2]

YhjA, a quinol peroxidase from *E. coli*,[3] is proposed to receive electrons from the quinone pool, as it is composed by a C-terminal domain homologous to the classical dihaem bacterial peroxidases, with an additional N-terminal domain, binding one *c*-type haem and a transmembrane helix. The soluble domain of YhjA was already isolated and biochemically characterized, presenting quinol peroxidase activity *in vitro* (millimolar range K_M values) using hydroquinone and menadiol (menaquinol analogue) as electron donors.[3]

YhjA without the transmembrane domain, wild-type and M125A variant (mutation on the proposed axial ligand of the N-terminal haem) were isolated and spectroscopically characterized, and their kinetic parameters using an artificial electron donor were determined and compared to validate the proposed axial ligand of this additional haem and to infer the involvement of this haem in the electron transfer pathway required for catalysis. Inhibition studies were performed using known inhibitors of the peroxidase family (azide and imidazole). The isolation and characterization of the wild-type and M125A full-length YhjA (with the transmembrane domain) are being carried out.

This work was supported by Fundação para a Ciência e Tecnologia by national funds: PTDC/BIA-BQM/29442/2017 (to SRP), and UIDP/04378/2020 and UIDB/04378/2020 to the Applied Molecular Biosciences Unit – UCIBIO.

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A bottom-up multidisciplinary approach to design supramolecular co-assemblies based on amyloid-like peptides and engineered proteins

L. Pérez-Chirinos*[1], I. R. Sasselli[1], A. L. Cortajarena[1-2]
[1]*Biomolecular Nanotechnology, Center for Cooperative Research in Biomaterials (CIC biomaGUNE), Basque Research and Technology Alliance (BRTA), Donostia-San Sebastián, Spain* [2]*Ikerbasque, Basque Foundation for Science, Bilbao, Spain*

Peptide-based supramolecular self-assemblies have been widely studied for applications in nanotechnology due to their inherent self-organization ability. Combining these structures with other molecules opens a whole new perspective for the design of complex functional materials. Here, we aim to incorporate into the self-assembled amyloid fibers a versatile protein with potential applications in optical electronics and nanotechnology. These amyloid fibers will provide to the co-assembled system their characteristic self-organization, driven by π-stacking, hydrogen bonding, and charge. The protein will be used as the versatile functional unit, key for the obtention of functional nanostructures and biomaterials. Coarse-grained molecular dynamics simulations have been employed to screen amyloid sequences with different molecular-level features, self-assembly tendency into amyloid fibers, and co-assembly stability. The selected peptide sequence was used to drive the co-assembly with the designed protein. This protein-fiber monolayer has been characterized experimentally using biophysical techniques. These results validate the simulations performed and assess their morphological features, molecular disposition, and intermolecular interactions with promising results. The synergy of combining the experimental and computational approaches has provided us with a level of understanding of our system that will permit further rational modifications. Hence, we have developed a versatile hybrid material that can be tuned for specific applications in nanotechnology and biomedicine.

EPR gives hints on the functioning of CopI, a periplasmic protein involved in bacterial copper resistance

M. Rossotti*[1], A. Durand[2], G. Gerbaud[1], F. Biaso[1], O. Bornet[3], P. Mansuelle[3], H. Launay[1], S. Ouchane[2], P. Dorlet[1]
[1]BIP UMR 7281, AMU, CNRS, Marseille Cedex 09, France [2]I2BC, CNRS, CEA, Univ. Paris-Sud, Gif-Sur-Yvette, France [3]IMM, CNRS, Marseille Cedex 09, France

CopI is a periplasmic protein of 15 kDa which is induced by high copper concentration and is directly involved in the copper resistance of the purple bacterium *Rubrivivax gelatinosus* [1]. *R. gelatinosus* is deprived of the classical Cu oxidase and CusF export systems, so CopI represents a novel system of Cu bacterial resistance. Its structure and mechanism of action remains to be determined. The sequence of the protein allows identifying three Cu binding regions: a cupredoxin site, a His-rich N-terminal site (not conserved and not required for Cu resistance) and a central His/Met rich region (conserved and required for Cu resistance). In order to get insights into this novel mechanism of copper resistance, we have undertaken biophysical as well as *in vivo* studies. EPR reveals that two Cu(II) binding sites are occupied in the purified protein (Fig. 1): the cupredoxin site, which is a seldom encountered “green” copper site, as well as the N-terminal site with a conventional square planar geometry [2]. I will present the results I have obtained by spectroscopy on the wild-type proteins (native or recombinant *Rg*CopI) as well as specific mutants targeting the Cu binding modules (*Rg*CopIΔDA with a modified N-terminus, *Rg*CopIHMH with His and Met residues of the central His/Met region mutated to Ser and *Rg*CopIT1PC with a modified cupredoxin site; see Fig. 1). A third Cu(II) binding site was identified by titration and shown to be in close proximity to the other two. Our results suggest this site is located in the conserved His/Met rich region and may be a primary Cu(I) binding site. In addition, NMR results show that this region is dynamic. We also studied the potential electron transfer role of the cupredoxin site by using various combinations of Cu(I) and Cu(II) loaded proteins. Based on these results, a potential mechanism will be discussed for this enzyme.

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Towards a label-free biosensor: studying DNA SAMs with a multi-technique investigation

S. Rotondi*[1], S. Dante[2], G. Pinto[1], A. Lagomarsino[1], P. Canepa[1], M. Canepa[1], O. Cavalleri[1]
[1]Department of Physics, University of Genova, Genova, Italy [2]Materials Characterization Facility, Italian Institute of Technology, Genova, Italy

The outbreak of the SARS-Cov-2 pandemic highlighted the need of sensors capable of rapid, selective and highly sensitive detection of biomarkers. The aim is to allow early diagnosis but also to monitor therapeutic treatments, resulting in increasing the quality of life and reducing healthcare costs. To achieve this target, a DNA-based biosensor can be an important candidate for the detection of specific DNA/RNA sequences and for the recognition of macromolecules with the exploitation of DNA/protein conjugates.

We investigated the self-assembly of short sequences of thiolated DNA (22/28 bases) on a gold substrate with a multi-technique approach, analyzing the optical and chemical properties, and molecular surface density of the film. Then we tested the hybridization with specific DNA or RNA sequences, such as SARS-Cov-2 RdRp-Helicase. This characterization is based on SE (Spectroscopic Ellipsometry), AFM (Atomic Force Microscopy), XPS (X-ray Photoemission Spectroscopy) and QCM-D (Quartz Crystal Microbalance with Dissipation).

SE is a non-destructive and ultrasensitive technique through which it is possible to monitor in real-time changes in the optical thickness and observe the DNA absorption at 260 nm, visible at the single monolayer level. As optical thickness is influenced by both refractive index and thickness, the SE experiments were assisted by AFM nanolithography analysis, with which we assessed the thickness of the deposited film. In this way we were able to build an accurate optical model that allowed us to obtain information about the absorption of the single filament and the double helix. The analysis of SE data provided indication of hypochromism in DNA SAMs. Information regarding the surface coverage and the DNA/surface binding was obtained by XPS. With QCM-D, we monitored the deposition and hybridization process in real-time and estimated the molecular density of the film.

One of the advantages of DNA-based biosensors is the possibility to regenerate them by exploiting the denaturation of the double helices through exposure to NaOH, thus allowing repeated use.

Structural insights into the binding mechanism of Doxorubicin with calcium-dependent SORCIN protein by molecular dynamics simulation

V. Saravanan*, S. Bhattacharya
Department of Chemical Engineering, Indian Institute of Technology Bombay, Mumbai, India

Soluble resistance-related calcium-binding protein (SORCIN) is soluble, an acidic, and Penta EF family (PEF) of calcium ions binding oncoprotein. SORCIN plays a vital role in multi-drug resistance (MDR), especially it is overexpressed in various types of cancers, and to diminish endoplasmic reticulum (ER) stress[1]. Typically, the presence of calcium ions on protein acts as a signal in a variety of cellular functions. Hence, this investigation is focused to examine the structural activation between apo and calcium bounded SORCIN dimer and the binding mechanism of doxorubicin with SORCIN dimer by molecular dynamics (MD) simulation. The calcium-bounded SORCIN dimer has got higher structural deviations than the apo-SORCIN. Because calcium ions at EF1, EF2, and EF3 loops induce the overall structural changes between the helices. Particularly, the calcium-binding upon the EF3 (D-E helices) hand induces the large structural reorientation and it may act as an arm dragging the long and rigid D-helix away from the E-helix which is confirmed by the angle between helices calculation. The conformational changes adopted by the calcium-binding let them selectively distinguish and control their targets, thus contributing to the control of several biological processes. Moreover, the addition of a doxorubicin drug between the EF4 and EF5 loop dimer increase the overall structural stability of calcium bounded SORCIN dimer. The binding energy results revealed that doxorubicin interacts with high affinity towards the SORCIN dimer. The addition of doxorubicin reduces the hydrogen bond distances between the EF5 hand of each monomer of SORCIN dimer. Besides, the EF5 hand is responsible for the formation of SORCIN dimer which is exposed by the network correlation analysis. Hence, this research outcome helps to emerge new chemotherapeutic drugs that are potentially capable of reversing the MDR phenotype in cancers.

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Linking L-Arginine and redox sensing in *Pseudomonas aeruginosa* to control c-di-GMP levels and biofilm formation

C. Scribani Rossi*, E. Scarchilli, S. Angeli, F. Cutruzzolà, A. Paiardini, S. Rinaldo
Laboratory affiliated to Istituto Pasteur Italia - Fondazione Cenci Bolognetti – Department of Biochemical Sciences "A. Rossi Fanelli", Sapienza University of Rome, Roma, Italy

The biofilm lifestyle allows bacteria to resist antimicrobial treatments and host defenses. The second messenger c-di-GMP controls biofilm formation and dispersion in response to environmental cues, including NO and N-based nutrients (1). Among nutrients, L-Arginine is associated with chronic infections, biofilm/virulence and antibiotic resistance, being at the crossroad of many metabolic processes and acting as a substrate for NO production by the host immune system (2). We recently found that *P. aeruginosa* RmcA (Redox regulator of c-di-GMP, is a phosphodiesterase able to respond to L-Arginine to control c-di-GMP levels. (3, 4). RmcA is a multidomain membrane protein composed by a periplasmic Venus Fly Trap (VFT) sensory domain, which selectively binds to arginine; the cytoplasmic portion harbours 3 PAS domains, 1 LOV domain (Light, Oxygen and Voltage) and the GGDEF-EAL catalytic tandem.

In this study we show how RmcA controls its phosphodiesterase activity by integrating perception of environmental L-Arginine and intracellular redox potential *via* FAD/$FADH_2$ redox-based switch. Moreover, we found that ligands such as FAD and GTP and/or the substrate itself (c-di-GMP) allosterically regulate the protein both in terms of aggregation state and of turnover. Molecular biophysics and kinetics analysis have been integrated to propose a model of RmcA activation in response to environmental stimuli.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N° 101004806 MOSBRI, Pasteur-PFBMI access provider.

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Reactivity of peroxidases and chlorite dismutases with chlorite and hypochlorite: a spectroscopic investigation of short-lived intermediates trapped by fast freeze-quenching

I. Serra*[1-2], D. Schmidt[3], S. Hofbauer[3], V. Pfanzagl[3], I. García-Rubio[2], P.G. Furtmüller[3], C. Obinger[3], S. Van Doorslaer[1]

[1]Department of Chemistry, BIMEF lab, University of Antwerp, Wilrijk, Antwerp, Belgium [2]Department of Condensed Matter Physics, University of Zaragoza, Zaragoza, Spain [3]Department of Chemistry, Institute of Biochemistry, University of Natural Resources and Life Sciences, Vienna, Austria

Chlorite (ClO_2^-) and hypochlorite (ClO^-) are strong oxidants commonly employed as bleaching agents or disinfectants, however, concerns have been raised around their presence in the environment as pollutants. Chlorite dismutases (Clds) are heme *b*-containing oxidoreductases which possess the unique ability to decompose ClO_2^- into harmless Cl^- and O_2. Investigations into the underlying reaction mechanism thus open ways for potential biotechnological and bioremediation applications [1]. Horseradish peroxidase (HRP), a model enzyme for heme peroxidases, is also known to react with both chlorite and hypochlorite [2]. During the reaction of Clds and HRP with ClO_2^- and ClO^- short-lived intermediates involving transient radicals are formed, making these systems suitable to be studied by electron paramagnetic resonance (EPR) spectroscopy. Given the short time scales of these reactions, a fast freeze-quenching device is needed to trap the transient radical species and collect the frozen sample directly into the EPR tube. In this work we present a comparative EPR investigation of the intermediate states formed during the reaction of HRP, Cld and dye-decolorizing (Dyp) peroxidase with either chlorite or hypochlorite, to identify common and distinct mechanistic features of these closely related enzymatic systems. The results are discussed in the context of the similarities in the active sites of the selected enzymes and the known key residues for their catalytic activities. In addition to that, we present our recent findings [3] on the different binding modes of the substrate-analogue nitrite by chlorite dismutases and we discuss them with regard to the role of the conserved distal arginine which is proposed to be involved in the catalytic mechanism of these enzymes. This work received funding from the European Union's Horizon 2020 research and innovation program (Marie Skłodowska-Curie Grant Agreement n. 813209) and it was supported by the Austrian Science Funds (FWF-project P30979) and the doctoral program BioToP – Biomolecular Technology of proteins (FWF W1224).

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S100A9 protein interaction with biomimetic lipid membranes

R. Tamulyte*[1], Z. Toleikis[2], E. Jankaityte[1], M. Jankunec[1]
[1]Institute of Biochemistry, Life Sciences Center, Vilnius University, Vilnius, Lithuania [2]Institute of Biotechnology, Life Sciences Center, Vilnius University, Vilnius, Lithuania

Pro-inflammatory, calcium-binding protein S100A9 is localized in the cytoplasm of a wide range of cells and regulates several intracellular and extracellular processes. One of them is the participation in the inflammation associated with the pathogenesis of Alzheimer's disease (AD). In Alzheimer's disease, S100A9 serves as a junction between amyloid and neuroinflammatory cascades. Due to its amyloidogenicity, together with β-amyloid S100A9 forms neurotoxic amyloid plaques, which results in neuronal death and memory impairment. The number of studies on the impact of S100A9 in co-aggregation processes with amyloid-like proteins is increasing. However, the interest in the interaction mechanism of protein S100A9 with neuronal cell membranes is still limited.

In this work various biomimetic membrane models, as lipid vesicles in solution and tethered lipid bilayers (tBLMs), were used to examine the binding and interaction between protein and the membrane surface. For this purpose we employed atomic force microscopy (AFM) and fluorescence spectroscopy techniques. Our results indicate that the initial binding and accumulation of S100A9 protein on the lipid membrane surface is lipid phase-sensitive. The most significant loss of integrity is observed in lipid bilayers composed of lipid mixture (brain total lipid extract). We demonstrate that low molecular weight species (monomer, dimer etc.) induce local thinning of the bilayer up to 1 nm. Interaction may result in the insertion of S100A9 into the region of the headgroups, inducing a lateral expansion of lipid molecules that can progress to further bilayer remodelling, such as membrane thinning. Our findings were further supported by thioflavin T fluorescence data which indicates inhibition of S100A9 fibrillation by ordered lipid systems due to potential protein penetration into bilayer. These results might broader the understanding of S100A9 interactions with nerve cell membrane and potentially affect the development of new diagnostic and therapeutic approaches for AD or other related diseases.

Production of stable Siglec-7 in a human cell line for NMR studies

O. Vanek*[1], C. Abreu[1], C. di Carluccio[2], R. Marchetti[2], A. Silipo[2]
[1]*Department of Biochemistry, Faculty of Science, Charles University, Prague, Czech Republic* [2]*Department of Chemical Sciences, University of Naples Federico II, Complesso Universitario Monte Sant'Angelo, Naples, Italy*

Siglec-7 is an inhibitory receptor belonging to the CD33-related Siglec family. It is mainly expressed on innate lymphoid natural killer cells but is also found on T cells, eosinophils, monocytes, and dendritic cells. The extracellular part comprises two C2 and an N-terminal V set Ig domains, preferentially binding α-(2,8)-linked disialylated ligands, generally terminal portions of various gangliosides. As a negative regulator of NK cell-mediated functions, crucial within tumor immunosurveillance, Siglec-7 has recently emerged as a target molecule for cancer immunotherapy. Moreover, the presence of sialylated lipopolysaccharide on certain *Fusobacterium nucleatum* strains, an oncogenic pathogen in different human tissues, may induce the activation of Siglec-7, causing immune suppression that may promote its carcinogenic behavior.

To study the interaction of Siglec-7 with the LPSs isolated from different strains of *F. nucleatum*, we expressed 1) the full extracellular domain fused to the Fc domain of human IgG (Siglec-7_FcHis), 2) the full extracellular domain (Siglec-7_FED), and 3) the N-terminal carbohydrate recognition domain (Siglec-7_CRD) in the human HEK293 cell line, thus keeping the appropriate post-translational modifications of Siglec-7 (disulfide bonds, *N*-glycosylation). The produced proteins were analyzed by SDS-PAGE, nanoDSF, DLS, and AUC. Unexpectedly, the Siglec-7_FED construct is forming a stable non-covalent homodimer in solution. The Siglec-7_FcHis construct, widely used in the literature, is the least stable among the three tested constructs and is prone to aggregation.

Using STD NMR and AUC analyses, we demonstrated the binding of Siglec-7 to the LPS isolated from *F. nucleatum* ATCC 10953 strain (ssp. *polymorphum*) and, interestingly, also to *F. nucleatum* ATCC 51191 LPS, lacking the sialic acid residue in its repeating unit structure. Given the excellent production yield, we aim to prepare a $^{15}N/^{13}C$ fully labeled Siglec-7 for a complete structural description of ligand recognition by NMR. The HEK293 expression system is well suited for immune cell receptor production, and we are open to collaboration.

This work was supported by the MEYS CR (LTC20078) and COST Action CA18103 INNOGLY through the STSM (ID 48689) visit of Cristina di Carluccio, a PhD. student in the group of prof. Alba Silipo, to the group of Dr. Ondřej Vaněk at Charles University.

Detection of subtle changes in biomolecule dynamics : from raw images to interpretable differences

H. Verdier*, F. Laurent, C. Vestergaard, J.B. Masson
Decision and Bayesian computation lab, Institut Pasteur, Paris, France

Photo-activated localization microscopy (PALM) enables high-resolution recording of single proteins trajectories in live cells, providing insights on the small-scale properties of biomolecules environment. A broad spectrum of dynamics have been observed and analysis schemes tailored to specific biological systems have been developed to quantitatively characterize the motion, notably using estimators specific to assumed random walk models. Yet, in most cellular environments, the complex interplay of interactions leads to random walks that do not necessarily match a unique canonical model. We propose a simulation-based inference scheme that allows quantitative and statistical analysis of experimental biomolecule recordings without prior model identification.

The first step of our approach is to associate to each biomolecule trajectory a vector of learnt features. We developed a graph neural network architecture for this purpose, trained on a diversity of simulated trajectories whose properties (including measurement noise) can be adapted to the specificities of the observed system. Features learnt by the neural network circumvent known flaws of conventional estimators when used on short and noisy trajectories such as those recorded in PALM experiments. To demonstrate the relevance of the method, we have shown that it can be leveraged to infer the Hurst exponent of fractional Brownian motion with linear computational complexity. Furthermore, coupling this network to a new approach to Approximate Bayesian Computation, we estimate the full posterior distribution of diffusion parameters.

Taking advantage of this learnt representation of trajectories, we use statistical tests based on the maximum mean discrepancy to assess the significance of differences between experimental observations. This allows us to compare the relative importance of intra- and inter-condition variability, and provides means of interpreting the nature of these differences. We demonstrate our approach by mapping the spatial evolution of the alpha-synuclein dynamics, highlighting the diversity of dynamics occurring inside synapses – without specifying models. Thanks to its very permissive assumptions, this automated pipeline paves the way to single molecule-based pharmacology. In this perspective, we present a recently released Python package allowing researchers to directly use this workflow on their PALM data, starting from raw images.

Characterization of secondary structure of protein by infrared nanospectroscopy

J. Waeytens*[1-2], J. De Meutter[1], E. Goormaghtigh[1], A. Dazzi[2], V. Raussens[1]
[1]*Structure et Fonction des Membranes Biologiques, Université libre de Bruxelles, Bruxelles, Belgium* [3]*Institut Chimie Physique, Université Paris-Saclay, Orsay, France*

During biological processes, protein conformation can change. A better understanding of those biological processes required a description of these conformational changes. Protein secondary structure is commonly studied by circular dichroism (CD) and Fourier transform infrared spectroscopy (FTIR). These spectroscopic techniques are versatile and can monitor those changes; they can be adapted to wide experimental conditions (variation of pH, ligand biding, buffer, metallic ions …) and are non-destructive. In FTIR and in particular in attenuated total reflection (ATR) only a small amount of protein (~100 ng) is needed to obtain good quality spectrum.

ATR-FTIR is very useful for homogeneous sample. An amyloid fiber protein is on the contrary an ensemble of constantly evolving complex structure. ATR-FTIR can only provide an average spectrum even if different species are present. To overcome this limit, optical microscopy can be coupled with FTIR and spectra unfortunately with a resolution of only a few micrometers can be obtain. More recently a technique coupling atomic force microscopy and infrared spectroscopy was developed (AFM-IR). It has a resolution of few nanometers and is therefore able to record spectra of single biomolecule. This technique will improve our knowledge on protein aggregation and other biological processes.

So far, very little is known on the ability of AFM-IR to predict secondary structure of protein. Comparison between FTIR and AFM-IR were reported with good consistency between the two methods, but it was done for a limited number of proteins and using only curve fitting analysis method. We study a protein library of 38-well characterized proteins and developed a model of prediction for the secondary structure. This was done by comparing ascending stepwise linear regression (ASLR) and partial least square (PLS). Even if small differences are observed between FTIR and AFM-IR spectrum, our models of prediction have similar errors for AFM-IR and FTIR around 6 % for a-helix and b-sheet.

Role of zinc-binding sites in Tau aggregation

D. Yatoui*[1], P.O. Tsvetkov[1], R. La Rocca[1], D. Allegro[1], G. Ferracci[1], D. Byrne[2], F. Devred[1]
[1]Aix-Marseille Univ ,CNRS, INP, Institute of neurophysiopathol, Faculté des Sciences Médicales et Paramédicales, Marseille, France [2]Institut de Microbiologie de la Méditerannée, CNRS,FR3479, Aix-Marseille Université, 13402, Marseille, France

Tau is a microtubule associated protein that binds tubulin and promotes the formation and stabilization of the microtubule network. Tau is naturally disordered prone-to-aggregate protein. It forms intraneuronal aggregates in several neurodegenerative diseases including Alzheimer disease (AD). Despite of the importance of tau aggregation in the development of pathogenesis of these diseases the molecular mechanisms of tau aggregation remain unclear. Recently, we reported that zinc interaction with tau protein can trigger its aggregation (Roman *et al.* 2018). Then using NMR, we identified three zinc binding sites located in the N-terminal part, repeat region and the C-terminal part of tau (La Rocca *et al.* 2022).

Here we characterized zinc binding to each of three sites using isothermal titration calorimetry (ITC) and determined the impact of each site on aggregation using turbidimetry and dynamic light scattering (DLS) assays. First, applying ITC to different tau mutants wherein two of three sites are inactivated we were able to confirm the presence of three zinc binding sites on tau and determine the thermodynamic parameters of binding of zinc to these sites. We found a high-affinity zinc binding site located in the repeat region of tau and two N- and C-terminus binding sites with a lower binding constant for zinc. Second, our turbidity results showed that tau aggregation necessitates zinc binding to the high affinity site in the R2R3 region, and that binding of zinc to the low affinity site in the C-terminal part of tau also plays a crucial part, while the N-terminal zinc binding site would not be implicated in the aggregation process.

Regarding the role of zinc ions in the aggregation of proteins in neurodegenerative diseases, these findings bring new insights to the understanding of the aggregation mechanism of tau protein induced by zinc.

Keywords: Tau protein; aggregation; zinc.

Sensitive Circular Dichroism Analysis of Therapeutic Antibodies

M. Textor[1], L. Cole[1], L. Bowsher[2], O. Durrant[2], P. Mc Colgan[3], C. Trippett[3], M. Epe*[1]
[1]Applied Photophysics Limited, Surry, United Kingdom [2]UCB, Slough, United Kingdom [3]CPI, Darlington, United Kingdom

The characterization of a biopharmaceutical along its development from early candidates to a marketed drug product relies on a toolset of orthogonal biophysical methods, including technologies that monitor Higher Order Structure (HOS) as a critical quality attribute. Available analytical techniques differ in the characteristics they probe and, thus, are sensitive to different changes in drug product properties arising from forced degradation conditions. Here we show a collaborative case study on monoclonal antibody products that involved both CD spectroscopy to determine HOS comparability and other methodologies for analysis of other aspects of the protein to illustrate how these complement each other and, together, provide a comprehensive picture about the effects of various stress conditions. By making use of automated liquid handling for high reproducibility and high-sensitivity detection, minor spectral changes in both the near- and far-UV CD were assessed.

Statistical data validation ultimately eliminated subjectivity of visual data interpretation and, together with results from other techniques, supported an informed decision on further time investment for down-stream characterization of the characterized biotherapeutics.

58P Company posters

Quantify and characterize any protein interaction – even in complex backgrounds, even with challenging targets

A. Spice*, I. Watt, R. Worth, A. Isley, M. Piziorska, A. Halski, A. Morgunov, S. Devenish, S. Fiedler
Fluidic Analytics Limited, Cambridge, United Kingdom

Microfluidic Diffusional Sizing (MDS) on the Fluidity One-M measures affinity, concentration, stoichiometry, and size in a single experiment of a wide range of biomolecules. Assays can be performed in purified backgrounds (buffer, detergent, high salt), crude mixtures (cell lysate or cell-culture supernatant), or clinical samples (plasma, serum, saliva). MDS generates a binding curve in <25 min, is maintenance-free, compatible with multichannel pipettes, and requires 60–80 µL of sample per K_D.

Accurate and reliable quantitation of AAV serotypes using novel AAVX biosensors and the Gator® system

S. Pinhal*[1], M. Palmowski[1], V. Nguyen[2], S. Yang[2], A. Pham[2], I. Chakraborty[2]
[1]I&L Biosystems France SAS, Champagne-Au-Mont-D'or, France [2]Gator Bio, Palo Alto, United States

AAV vectors have become a preferred gene therapy delivery modality. Different AAV serotypes target different organs and tissues. Commonly used serotypes include AAV2, AAV5, AAV8, and AAV9. Accurate quantification of purified AAV particle preparations represents a critical step for clinical applications. Determination of total capsid titer is one of the critical quality attributes, hence, accurate and reproducible quantification of AAV titers is essential for the safe and effective use of AAVs in gene therapy.

Here, we present data on the accuracy, dynamic range, robustness, and inter- and intra-assay variability in quantitation using Gator® Prime and AAVX probes for different serotypes. Furthermore, we compare data with Progen AAV9 ELISA.

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A next-generation label-free MALDI-MS based HTS solution taking advantage of timsTOF technology

S. Dikler[1], C. Brunner*[2], U. Schweiger-Hufnagel[1], A. Fuetterer[1], M. Hamester[1], A. Asperger[1]
[1]Bruker Daltonics, Bremen, Germany [2]Bruker Daltonics SPR, Faellanden, Switzerland

Label-free MALDI mass spectrometry (MALDI-MS) has evolved into an established, highly efficient HTS readout method in support of drug discovery. MALDI-MS enables fast reading rates of up to multiple wells per second and has proven itself to feature an outstanding level of robustness. As a result of this, MALDI-MS was successfully applied to full-deck screening of primary compound libraries comprising a million or even larger number of compounds at unparalleled speed.

We introduce a new technology platform bringing MALDI-MS based HTS to a next level by taking advantage of new and innovative timsTOF technology. timsTOF relies on ultra-high-resolution time of flight mass spectrometry (UHR-TOF) in combination with trapped ion mobility spectrometry (TIMS) as an upfront ultrafast dimension of separation orthogonal to MS. TIMS provides an additional dimension of separation in the gas phase which is significantly faster (typically 100-300 ms per separation cycle) than conventional separation techniques, e.g., liquid chromatography. The MS/MS operation mode enhances significantly assay specificity and risk of interferences by spectral overlap with disturbing sample background.

Beyond quantitative assays, the new platform offers unique potential for further important high-throughput applications in drug discovery, e.g., HTE chemistry monitoring, where fast analysis of multiple physical properties, i.e., accurate mass, true isotope pattern, collisional cross-section and structure-specific MS/MS fragmentation, accelerates identification or confirmation of newly synthesized drug molecules.

Linking Structure and Function – Use of PDB data as baseline for in-solution characterization

E.G.P. Stender, H. Jensen*
Fida Biosystems ApS, Soeborg, Denmark

In biophysics, the detection of molecular size changes is a strong, quantitative source of information about the structure of a protein and its complexes. However, correlating a protein's structure to size measures, like Hydrodynamic radius (Rh), requires consideration of structural features of the protein and its assemblies based on either experimental structures or predicted ones by *e.g.,* AlphaFold.

To establish a strong and reliable size baseline, we have developed the Fidabio PDB (Protein Data Bank) Correlator. In the presented work, we use the Fidabio PDB Correlator to predict the in-solution size, Rh, of bovine β-lactoglobulin, human serum albumin, bovine serum albumin and chicken lysozyme. Subsequently, using Fida 1 equipped with a UV fluorescence detector, the Rh of label-free proteins is experimentally determined and compared with the predicted Rh values.

Using FO-SPR to select for nanobodies in phage display

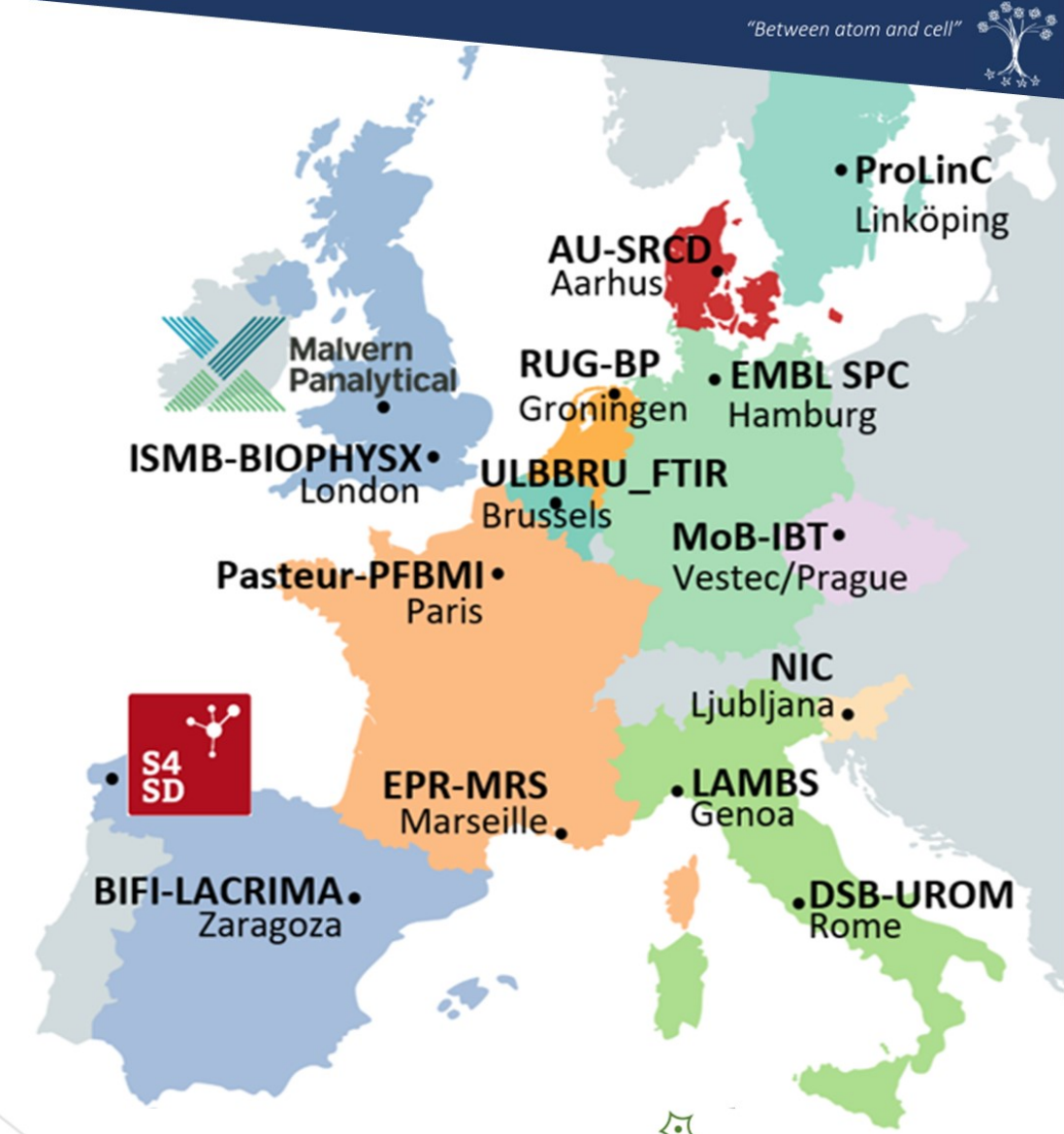
F. Bossard*, F. Delport
FOx Biosystems, Diepenbeek, Belgium

Phage display in conjunction with biopanning is a frequently used strategy in the selection process for nanobodies or other expressed binding proteins with specificity to a target antigen.

Here we present a Fiber Optic-Surface Plasmon Resonance (FO-SPR) based approach that combines both fast kinetic characterization and an efficient selection cycle in a single step. FO-SPR uses microfluidics-free dip-in capture and reading on probes that are pre-functionalized with the target antigen.

This integrated workflow allows to speed-up selection of phage display expressed proteins such as nanobodies or antibodies. As the FO-SPR assay is based on real-time kinetics monitoring rather than on end point characterization such as with ELISA, it presents potential for selection and characterization based on direct and unbiased binding. This method can yield more effective target binders from the clone selection process for therapeutic nanobodies.

AUTHORS & CO-AUTHORS INDEX



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This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101004806