MOSBRI
Molecular-Scale Biophysics
Research Infrastructure

2nd MOSBRI Scientific Conference

5th - 7th June 2023, BIFI, University of Zaragoza, Zaragoza

Book of Abstracts

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Welcome to the University of Zaragoza

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 European countries, coordinated by Institut Pasteur (Paris, France) and funded by the Horizon 2020 programme of the European Commission.

The **2nd MOSBRI Scientific 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

Instituto Universitario de Investigación
Biocomputación y Física de Sistemas Complejos
Universidad Zaragoza

Speakers

Invited Speakers

• Paolo Bianchini (*Genoa, Italy)*

• Maria Gabriela Bruque *(Birmingham, UK)*

• Osvaldo Burastero *(Hamburg, Germany)*

• Pedro Castanheira *(Immunethep, Portugal)*

• Maelenn Chevreuil *(Paris, France)*

• Ana Crnković *(Ljubljana, Slovenia)*

• Francesca Cutruzzola *(Rome, Italy)*

• Irene Díaz-Moreno *(Sevilla, Spain)*

• Felix M. Goñi *(Leioa, Spain)*

• Peter Hinterdorfer *(Linz, Austria)*

• Tristan Kenney *(Toronto, Canada)*

• Rita Rebelo Manuel *(Lisbon, Portugal)*

• Andre Matagne *(Liège, Belgium)*

• Jacek Plewka *(Kraków, Poland)*

• Serena Rinaldo *(Rome, Italy)*

• Javier Sancho *(Zaragoza, Spain)*

• Gideon Schreiber *(Rehovot, Israel)*

• Amanda Eriksson Skog *(Lund, Sweden)*

• Marikken Sundnes *(Trondheim, Norway)*

• Florian Turbant *(Paris, France)*

• Brinda Vallat *(New Jersey, USA)*

• Adrian Velazquez-Campoy *(Zaragoza, Spain)*

• Gijs Wuite *(Amsterdam, the Netherlands)* •

Matja Zalar *(Maribor, Slovenia)*

• Kilian Zuchan *(Marseille, France)*

Scientific Organizing Committee

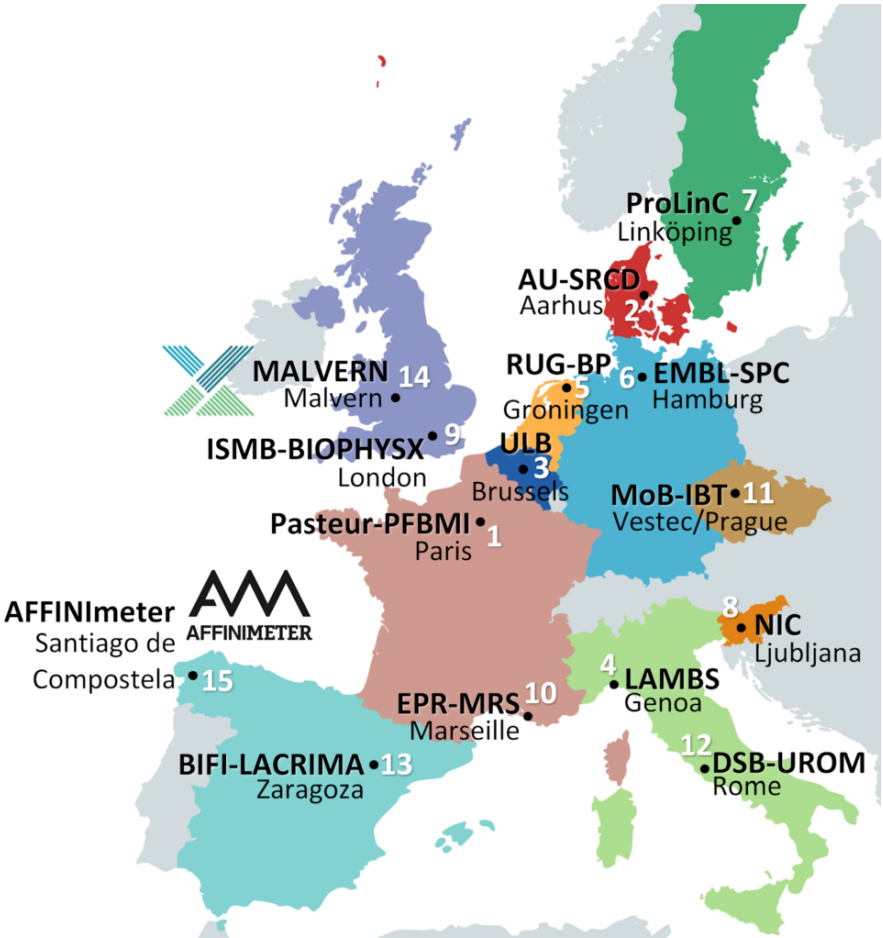
Gregor Anderluh (NIC, Ljubljana, Slovenia)

Francesca Cutruzzola (DSB-UROM, Rome, Italy)

Patrick England (Pasteur-PFBMI, Paris, France)

Javier Sancho (BIFI-LACRIMA, Zaragoza, Spain)

Adrian Velazquez-Campoy (BIFI-LACRIMA, Zaragoza, Spain)



General Information

Once your registration is fully settled, you will be given a badge that you should wear at all times.

Registration fee will give access to the Conference Dinner on Tuesday June 6th, lunches and coffee breaks during the Conference.

The certificate of attendance will be sent to registered participants by email upon request after the conference.

ACCESS TO THE CONFERENCE CENTER

Venue address:

Patio de La Infanta - iberCaja (Salon Rioja)
C/ San Ignacio de Loyola, 16
50008 Zaragoza, Spain

OPENING HOURS OF THE CONFERENCE CENTER - Patio de la Infanta

- Monday June 5th, 2023: 1:00 pm – 7:30 pm
- Tuesday June 6th, 2023: 8:00 am – 8:00 pm
- Wednesday June 7th, 2023: 8:00 am – 5:00 pm

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

ACCESS TO THE CONFERENCE DINNER

Conference Dinner address:

Edificio Paraninfo
Plaza Basilio Paraíso, 4
50005 Zaragoza, Spain

General Information

SCIENTIFIC SESSIONS

Scientific sessions take place in Salon Rioja (Patio de La Infanta).

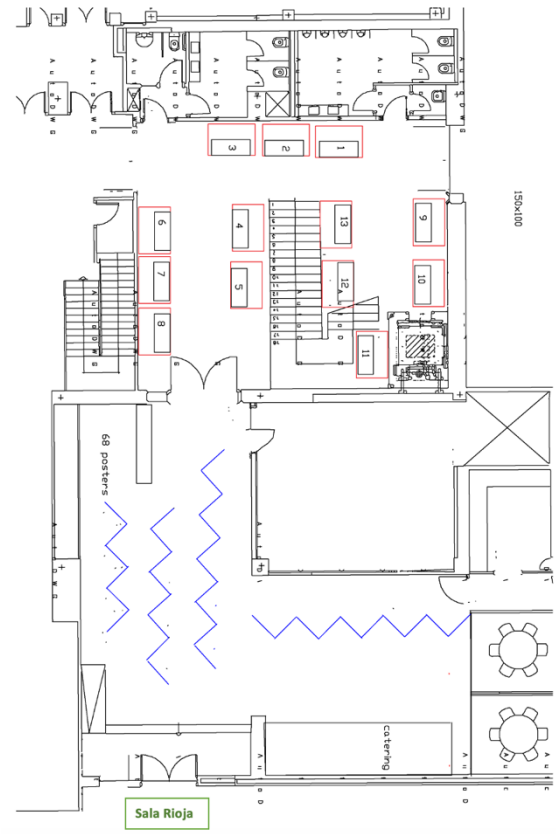
LUNCHES & COFFEE BREAKS

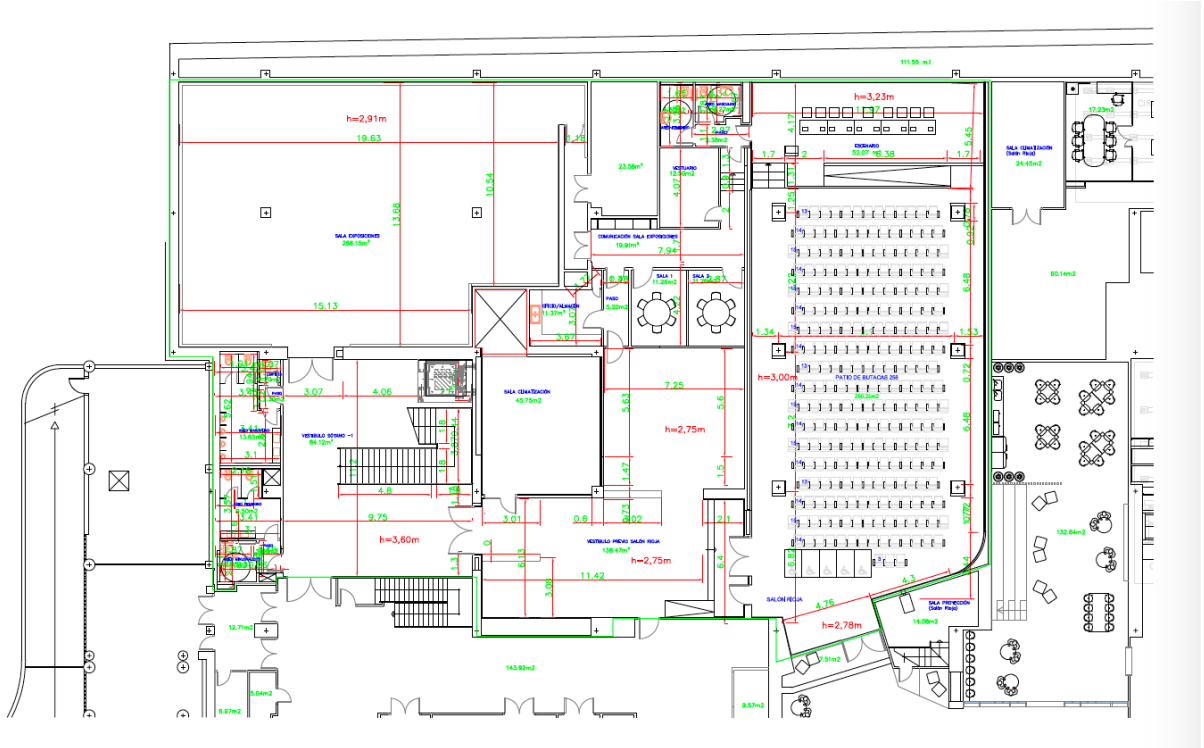
All coffee breaks and lunches will be served in the hall outside Salon Rioja.

Access to lunches is limited to registered participants.

FREE ACCESS WIFI

Map of the Patio de la Infanta





Programme


Instituto Universitario de Investigación
Biocomputación y Física
de Sistemas Complejos
Universidad Zaragoza

Monday 5th June 2023

13:30	Welcome coffee
14:00	Opening/Welcome

SESSION 1: Protein stability, quality control and standards
Chairs: María García (EMBL-SPC), Søren Vrønning Hoffmann (AU-SRCD, Aarhus, DK)

14:15	Functional and structural characterization of Bone Morphogenetic Protein- 2 and the antagonist Noggin | Andre Matagne, University of Liège, Belgium
14:40	How and why do we develop standard proteins? Maelenn Chevreuil, Institut Pasteur, Paris, France
15:05	Circular dichroism as a tool to monitor real-time structural changes in monoclonal antibody manufacture Maria Gabriela Bruque, University of Birmingham, UK
15:20	Elucidating Interactions Between SARS-CoV-2 proteins Jacek Plewka, Jagiellonian University, Poland
15:35	SUPR-DSF - Differential Scanning Fluorimetry for high-throughput protein stability screening | Jemma Roberts, *Applied Photophysics*
15:45	Ultimate DSC: a new tool for protein characterization Yannice Ricci, *Calneos*
15:55	Coffee break

SESSION 2 Redesigning Biomolecules to improve performance
Chairs: Natalia Markova (MALVERN) and Adrián Velázquez-Campoy (BIFI-LACRIMA)

16:30	Tuning protein stability through charged mutations and multivalent excipients Matja Zalar, University of Maribor, Slovenia
16:55	Protein stabilization made simple: new bioinformatics tools for stability design Javier Sancho, University of Zaragoza, Spain
17:20	Characterization of the redox-centres in the electron-confurcating [FeFe]-Hydrogenase (HydABC) of Thermotoga maritima Kilian Zuchan, Aix-Marseille Université, CNRS, France
17:35	Experimental evidence of intrinsic disorder and amyloid formation by the Henipavirus W proteins | Giulia Pesce, Marseille, France
17:50	Specific Extracellular Vesicle detection and isolation in complex samples using FO-SPR | Kris Ver Donck, *Fox Biosystems*
18:00	How to characterize the mode of action and avidity effects of antibodies using switchSENSE® and RT-IC | Amandine Gontier, *Dynamic Biosensors*
18:10	Poster / networking session and cocktail dinner

Tuesday 6th June 2023

08:30	Welcome coffee
SESSION 3 Macromolecular interactions: kinetics and thermodynamics Chairs: Mark Williams (ISMB-BIOPHYSX) and Maria Sunnerhagen (ProLinC)	
09:00	Intrinsically disordered regions in proteins: Implications for physiology and drug discovery | Adrián Velázquez-Campoy, University of Zaragoza, Spain
09:25	Four different biophysical methods and simulations reveal that protein quaternary structures in solution are a mixture of multiple forms Gideon Schreiber, Weizmann Institute of Science, Israel
09:50	Characterization of the interaction of monoclonal antibodies with bacterial GAPDH | Pedro Castanheira, Immunethep, Portugal
10:05	Probing the mechanism of the peroxiredoxin decamer interaction with its reductase sulfiredoxin from the single molecule to the solution scale Hortense Mazon, Nancy, France
10:20	Use of Monolith to measure affinity of antibodies to a trimeric membrane protein | Pierre Soule, *Nanotemper*
10:30	Quantify and characterize any protein interaction – even in complex backgrounds, even with challenging targets | Sebastian Fiedler, *Fluidic Analytics*
10:40	Coffee break
SESSION 4 Architecture of macromolecular assemblies, allostery and protein dynamics Chairs: Pierre DORLET (EPR-MRS) and Bertrand RAYNAL (Pasteur-PFBMI)	
11:15	Role of allostery and oligomerization in tuning one-component systems controlling bacterial biofilm maintenance Serena Rinaldo, Sapienza Università di Roma, Italy
11:40	The role of highly dynamic regions from PP2A inhibitors in orchestrating the binding to respiratory cytochrome c, Irene Díaz-Moreno, Universidad de Sevilla, Spain
12:05	Investigating the Interactions Between Myc and the RNA Polymerase II Component TFIIF | Tristan Kenney, University of Toronto, Canada
12:20	Single-molecule fluorescence applications in protein amyloid aggregation: from basic research to diagnosis | Nunilo Cremades, Zaragoza, Spain
12:35	Fractionation and Characterization of Macromolecules and Nanoparticles: Solutions for Gene Vectors | Nicolas Mignard, *Wyatt*
12:45	Microfluidic Modulation Spectroscopy (MMS): Automated, Highly-Sensitive IR-Based Protein Structural Analysis in situ, Without the Fuss Patrick King, *RedShiftBio*

12:55	Lunch
SESSION 5 Single molecule and time resolved analyses Chairs: Francesca Cutruzzola (DSB-UROM), Wouter H. Roos (RUG-BP)	
14:00	**Protein-protein and protein-nucleic acid interactions in nucleotide metabolism** Francesca Cutruzzola, Sapienza Università di Roma, Italy
14:25	**Single Molecule Manipulation and Imaging of complex DNA-protein interaction** Gijs Wuite, Vrije Universiteit, Amsterdam, the Netherlands
14:50	**Insights into the Catalytic Mechanism of FdhAB from Desulfovibrio vulgaris Hildenborough** Rita Rebelo Manuel, Universidade Nova de Lisboa, Portugal
15:05	**Investigation of lectin binding to SARS-CoV-2 spike glycans using single molecule force spectroscopy** Yoo jin Oh, Linz, Austria
15:20	**Optical tweezers and single molecules: how to visualize and manipulate single biomolecules in real-time** *Vincenzo Mascoli, Lumicks*
15:30	**Mass photometry – an analytical technology for biomolecular characterization** *James Wilkinson, Refeyn*
15:40	Coffee break
SESSION 6 Membranes and condensates Chairs: Gregor Anderluh (NIC) and Felix M. Goñi	
16:15	**Ceramide effects on lipid bilayer dynamics and structure** Felix M. Goñi, Biofísika Institute, Leioa, Spain
16:40	**Modification of pore-forming toxins and their interactions with lipid membranes by directed evolution and insertion of non-canonical amino acids** Ana Crnković, National Institute of Chemistry, Ljubljana, Slovenia
17:05	**Amyloid-Membrane interactions observed with OCD and FTIR** Florian Turbant, CEA and Univ Paris Cité, France
17:20	**Taylor-dispersion induced phase separation (TDIPS) for efficient characterisation of protein condensation** Lars Boyens-Thiele, Copenhagen, Denmark
17:35	**Poster / networking session**
20:00	Congress dinner

Wednesday 7th June 2023

08:30	Welcome coffee
SESSION 7 Molecular bioimaging Chairs: Claudio Canale (LAMBS) and Ornella Cavalleri (University of Genova, Italy)	
09:00	**Binding strategies of SARS-Cov-2 spike variants viewed on the single molecule level** | Peter Hinterdorfer, Johannes Kepler University (JKU) Linz, Austria
09:25	**From super-resolution to correlative microscopy, studying the effects of photosensitizers in cells and pathogens.** Paolo Bianchini, Istituto Italiano di Tecnologia, Genoa, Italy
09:50	**Hyperspectral microscopy and ligand screening of TTR amyloid fibrils** Marikken Sundnes, NTNU, Trondheim, Norway
10:05	**Condensate Formation of Cell Division Proteins FtsZ and SlmA in Physiologically Relevant Glutamate Buffer and Its Time-Dependent Evolution on Lipid Surfaces** Gianfranco Paccione, Madrid, Spain
10:20	**The SPR Pro Series – Information-Rich Assays at Full Flexibility** Cyrill Brunner, *Bruker Daltonics*
10:30	**Pushing the limits of BLI: new biosensor technology for protein and AAV research** | Tobias Zbik, *I&L Biosystems*
10:40	**Group Photo**
10:45	Coffee break
SESSION 8 Computational approaches in biophysics Chairs: Emil Dandanell Agerschou (MoB-IBT) and Juan Sabin (AFFINImeter)	
11:15	**Shining a Light on Dynamic Data: From generation to interpretation** Osvaldo Burastero, EMBL Hamburg, Germany
11:40	**Making integrative structures accessible through PDB-Dev: From single molecules to dynamic macromolecular assemblies** Brinda Vallat, RCSB PDB at Rutgers, The State university of New Jersey, USA
12:05	**The story of KEIF, the assumed appendix that held on for dear life** Amanda Eriksson Skog, Lund University, Sweden
12:20	**Different enzymatic inhibitors trigger different IDO1 conformations that can influence its signalling functions** | Elisa Bianconi, Perugia, Italy
12:35	**Concluding remarks**
	Lunch
14:00	**MOSBRI General assembly** MOSBRI partners only

INVITED TALKS

T1

Functional and structural characterization of Bone Morphogenetic Protein- 2 and the antagonist Noggin

Charly Robert[3], Maxime Gavage[2], Frédéric Kerff[1], Fabrice Bouillenne[1], Marylène Vandevenne[1], Julie Lecomte[2], Patrice Filée[2], André Matagne[1]

[1]Centre for Protein Engineering, University of Liège, Building B6C, Quartier Agora, Allée du 6 Août, 13, 4000 Liège, Belgium.
[2]Biotechnology Department, CER Groupe, Novalis Science Park, Rue de la Science 8, 6900 Aye, Belgium

Presenting author: André Matagne, amatagne@uliege.be

Bone morphogenetic proteins (BMPs) are secreted cytokines belonging to the transforming growth factor-β (TGF-β) superfamily. New therapeutic approaches based on BMP activity, particularly for cartilage and bone repair, have sparked considerable interest; however, a lack of understanding of their interaction pathways and the side effects associated with their use as biopharmaceuticals have dampened initial enthusiasm. Here, we used BMP-2 as a model system to gain further insight into both the relationship between structure and function in BMPs, and the principles that govern affinity for their cognate antagonist Noggin. Both proteins were produced and characterized with the help of complementary biophysical techniques, including optical spectroscopies, mass spectrometry, multi-angle light scattering coupled with size exclusion chromatography, and X-ray crystallography. Additionally, several *in vitro* cell-based assays, including enzymatic measurements, RT-qPCR and matrix staining, demonstrated their biological activity during cell chondrogenic and hypertrophic differentiation. Furthermore, we characterized the simple 1:1 non-covalent interaction between the two ligands (K_D *ca.* 0.4 nM) using bio-layer interferometry and solved the crystal structure of the complex using X-ray diffraction methods. We identified the residues and binding forces involved in the interaction between the two proteins.

T2

How and why do we develop standard proteins?

Maelenn Chevreuil[1], Bertrand Raynal[1], Patrick England[1], Mark A. Williams[2], Thomas A. Jowitt[3]

[1]Institut Pasteur, Université Paris Cité, Plateforme de Biophysique Moléculaire, Paris, France.
[2]Department of Biochemistry and Molecular Biology, University College London, London, United Kingdom
[3]Wellcome Trust Centre for Cell Matrix Research, Faculty of Biology Medicine and Health, University of Manchester, Manchester, England.

Presenting author: Maelenn Chevreuil, maelenn.chevreuil@pasteur.fr

A reliable and rigorous research process relies on both robust data collection and regular assessment of instrument performance as well as of researcher skills. The development of standard samples is one of the necessary tools to perform these assessments. In the frame of the European MOSBRI project, the Institut Pasteur molecular biophysics facility, in collaboration with T. Jowitt (Manchester, UK), is involved in the development of a first set of versatile protein standards that can be used for multiple techniques. To do so, ten recombinant VHH camelid antibody fragments (nanobodies) that bind chicken lysozyme were tested during the last 18 months. So far, one nanobody (B09) has passed all the quality controls and performed well in biophysical assays (hydrodynamic characterizations, structural assays in solution and interaction assays, etc). This presentation aims to highlight the key steps of the process that we set up to match the stringent criteria of selection with the final objective of large usability for biophysical analysis and large-scale distribution through the MOSBRI TNA scheme.

T3

Tuning protein stability through charged mutations and multivalent excipients

Matja Zalar[1]

[1]Faculty of Chemistry and Chemical Technology, University of Maribor.

Presenting author: Matja Zalar, matja.zalar1@um.si

Biopharmaceutic proteins are the fastest growing class of pharmaceutics globally. Despite their versatility and wide applicability, their production poses unique challenges due to their high molecular weight and structural complexity as well as from physical instabilities that lead to loss of quality and efficacy. Protein stability, especially in terms of protein self-association and aggregation, is thus one of the critical attributes for formulation of biopharmaceutical proteins. In particular, we are interested in weak, non-covalent interactions that lead to protein association.

In this talk interplay between structural and colloidal stability of proteins will be introduced and experimental light scattering and nuclear magnetic resonance approaches to characterize protein- protein and protein-excipient interactions will be discussed. Two strategies for tuning proteins stability will be presented (i) introducing additional charge on protein surface by point mutations and (ii) addition of biologically relevant polyvalent ions (ATP) that can protect proteins against thermal aggregation and modulate their phase behaviour.

T4

Protein stabilization made simple: new bioinformatics tools for stability design

Javier Sancho[1,2]

[1]Biocomputation and Complex Systems Physics Institute (BIFI) & Department of Biochemistry and Molecular and Cell Biology, University of Zaragoza, Zaragoza, Spain.
[2]Aragon Health Research Institute (IIS Aragón), Zaragoza, Spain.

Presenting author: Javier Sancho, jsancho@unizar.es

The conformational stability of proteins is typically low and can be greatly altered by solution conditions or single amino acid replacements. The efficient biotechnological/biomedical use of proteins often requires increasing their stability but identification of stabilizing substitutions among the many possible ones remains challenging. We are developing user-friendly bioinformatics tools to design protein stabilization mutations. Our goal is to help both protein scientist and non-expert users who need a quick and efficient guide to stabilize their unstable proteins. Protposer (http://webapps.bifi.es/the-protposer) provides, with the single input of the protein atomic coordinates (e.g., a PDB file), a ranked list of point mutations with the probabilities that they stabilize the protein by at least 0.5 kcal/mol and has a positive predictive value higher than that of existing popular software. Protposer is sufficient to stabilize two-state proteins. However, as will be explained, the rational stabilization of non-two-state proteins is rather more difficult, since many stabilizing mutations implemented in them will only increase the "residual" stability of their partially unfolded conformations. To solve this problem, we are developing ProteinLIPS, a server that identifies the weakest regions of a protein. Those regions are the natural setting for efficient stabilization of non-two-state proteins.

T5

Intrinsically disordered regions in proteins: Implications for physiology and drug discovery

Adrian Velazquez-Campoy[1,2,3], David Ortega-Alarcon[1,2], Ana Jimenez-Alesanco[1,2], Marta Asencio del Rio[1,2], Paula Garcia-Franco[4], Hajar Jeblaoui[3], Sonia Vega[2], Olga Abian[1,2,3]

[1]University of Zaragoza, Department of Biochemistry and Molecular and Cell Biology, Spain
[2]Institute of Biocomputation and Physics of Complex Systems (BIFI), University of Zaragoza, Spain
[3]Institute for Health Research Aragón (IIS Aragon), Zaragoza, Spain
[4]Certest Biotec S.L., Zaragoza, Spain

Presenting author: Adrian Velazquez-Campoy, adrianvc@unizar.es

Disorder is pervasive in Nature. Some proteins are predicted to be entirely disordered (IDPs), whereas others contain disordered sequences, referred to as intrinsically disordered regions (IDRs). The lack of structure provides the plasticity needed to adapt to a changing environment, the ability to interact with multiple biological partners, and the possibility to undergo inter-domain cooperative structural/functional regulation. A wide variety of interaction modes can be observed for disordered proteins: from high to low affinity, from high to low specificity, and from folding-upon-binding to fuzzy complex formation.
In this talk I will comment on two types of proteins: 1) multidomain proteins with a considerable level of structural disorder, exemplified by MeCP2 (methyl-CpG binding protein 2); and zinc-dependent proteins, exemplified by hepatitis C virus NS3 protease from hepatitis C virus and *Bacteroides fragilis* BFT-3 enterotoxin. In both cases, disorder has strong implications on structural and functional aspects with direct consequences in physiology and drug discovery.

T6

Protein quaternary structures in solution are a mixture of multiple form

Shir Marciano[1], Debabrata Dey[1], Dina Listov[1], Sarel J. Fleishman[1], Adar Sonn-Segev[2], Haydyn Mertens[3], Florian Busch[4], Yongseok Kim[4], Sophie R. Harvey[4], Vicki H. Wysocki[4], Gideon Schreiber[1]

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[2] Refeyn Ltd, 1 Electric Avenue, Ferry Hinksey Road, Oxford OX2 0BY, UK.
[3] Hamburg Outstation, European Molecular Biology Laboratory, Notkestrasse 85, Hamburg, 22607, Germany.
[4] Department of Chemistry and Biochemistry and Resource for Native Mass Spectrometry Guided Structural Biology, The Ohio State University, Columbus, OH, 43210, USA.

Presenting author: Gideon Schreiber, gideon.schreiber@weizmann.ac.il

Over half the proteins in the *E. coli* cytoplasm form homo or hetero-oligomeric structures. Experimentally determined structures are often considered in determining a protein's oligomeric state, but static structures miss the dynamic equilibrium between different quaternary forms. The problem is exacerbated in homo-oligomers, where the oligomeric states are challenging to characterize. Here, we re-evaluated the oligomeric state of 17 different bacterial proteins across a broad range of protein concentrations and solutions by native mass-spectrometry (MS), mass photometry (MP), size exclusion chromatography (SEC), and small-angle x-ray scattering (SAXS), finding that most exhibit several oligomeric states. Surprisingly, many proteins did not show mass-action driven equilibrium between the oligomeric states. For approximately half the proteins, the predicted oligomeric forms described in publicly available databases underestimated the complexity of protein quaternary structures in solution. Conversely, AlphaFold Multimer provided an accurate description of the potential multimeric states for most proteins, suggesting that it could help resolve uncertainties on the solution state of many proteins.

T7

Role of allostery and oligomerization in tuning one-component systems controlling bacterial biofilm maintenance

Chiara Scribani Rossi[1], Kelly Eckartt[2], Elisabetta Scarchilli[1], Simone Angeli[1], Adele Di Matteo[3], Alessandro Arcovito[4], Noah Giacon[5], Francesco Fiorentino[5], Dante Rotili[5], Antonello Mai[5], Maelenn Chevreuil[6], Bertrand Raynal[6], Francesca Cutruzzolà[1], Alessandro Paiardini[1], Lars Dietrich[2], Serena Rinaldo[1]

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[2]Department of Biological Sciences – Columbia University, New York, USA.
[3]Istituto di Biologia e Patologia Molecolari, Consiglio Nazionale delle Ricerche, Rome, Italy.
[4]Dipartimento di Scienze Biotecnologiche Di Base, Cliniche Intensivologiche e Perioperatorie Università Cattolica Del Sacro Cuore - Roma, Italy.
[5]Department of Drug Chemistry and Technologies, Sapienza University of Rome, Rome, Italy.
[6]Plate-forme de Biophysique Moléculaire, Institut Pasteur, UMR 3528 CNRS, Paris, France.

Presenting author: Serena Rinaldo, serena.rinaldo@uniroma1.it

Nutrients can control bacterial biofilm, by regulating the intracellular levels of the second messenger c-di-GMP [1]. Many environmental nutrient sensors are one-component transducers, where the periplasmic sensory activity triggers the activation/inhibition of the downstream cytosolic diguanylate cyclase (GGDEF) or phosphodiesterase (EAL) domains, to finally change c-di-GMP levels. One of these transducers is RmcA (Redox modulator of c-di-GMP) from *P. aeruginosa*, able to perceive environmental L-Arginine to decrease intracellular levels of c-di-GMP [2, 3]. RmcA activity prevents colony wrinkling morphology and *in vivo* responds to oxidized phenazines (directly or indirectly as reducing power) [4].
In this study, we demonstrated how RmcA can directly perceive the reducing power, which in turn controls the phosphodiesterase activity of the EAL domain. The redox-dependent control of catalysis involves oligomerization and domain(s) re-organization to finely tune the rate of c-di-GMP hydrolysis (and finally the biofilm architecture).
This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N° 101004806 MOSBRI, Pasteur-PFBMI and DSB-UROM access provider.

1. Rinaldo et al. (2018). FEMS Microbiol Lett. 365(6):fny029.
2. Paiardini et. al. (2018) Proteins 86(10):1088.
3. Mantoni et al. (2018) FEBS J. 285(20):3815.
4. Okegbe et al. (2017) PNAS 114(26):E5236.

T8

The role of highly dynamic regions from PP2A inhibitors in orchestrating the binding to respiratory cytochrome *c*

Miguel A. Casado-Combreras[1], Blanca Jaime-Baños[1], Alejandro Velázquez-Cruz[1], Rosa M. Ríos[2], Adrián Velázquez-Campoy[3], Valérie Belle[4], Laura Corrales-Guerrero[1], Miguel A. De la Rosa[1], Irene Díaz-Moreno[1]

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[2]Centro Andaluz de Biología Molecular y Medicina Regenerativa (CABIMER), University of Seville – CSIC – University Pablo de Olavide, Spain.
[3]Institute of Biocomputation and Physics of Complex Systems (BIFI), Joint Unit GBsC-CSIC-BIFI, Universidad de Zaragoza, Spain.
[4]Centre National de la Recherche Scientifique (CNRS), Aix Marseille University of Marseille, France.

Presenting author: Irene Díaz-Moreno, idiazmoreno@us.es

Intrinsic protein flexibility is of overwhelming relevance for intermolecular recognition and adaptability of highly dynamic ensemble of complexes, and the phenomenon is essential for the understanding of numerous biological processes. These conformational ensembles-encounter complexes-lack a unique organization, which prevents the determination of well-defined high-resolution structures. This is the case for complexes between cytochrome *c* with three well-known protein phosphatase 2A (PP2A) inhibitors: SET/template-activating factor-Iβ (SET/TAF-Iβ) and the Acidic leucine-rich Nuclear Phosphoprotein 32 family (ANP32) members A and B. Soon after DNA lesions, the hemeprotein migrates from the mitochondria into to the nucleus to target flexible regions of PP2A inhibitors, thereby activating the enzyme that dephosphorylates mediators acting during DNA damage response and repairs injured DNA. Whereas the nuclear SET/TAF-Iβ:cytochrome *c* complex is an example of polyconformational and diffuse ensemble, cytochrome *c* induces long-distance allosteric changes in the structured domain of ANP32B upon binding to its disordered low-complexity-acidic region.

T9

Protein-protein and protein-nucleic acid interactions in nucleotide metabolism

Francesca Cutruzzolà[1], S. Spizzichino[1], A. Riva[1], F.R. Liberati[1], F. Di Fonzo[1], A. Tramonti[2], R. Contestabile[1], G. Boumis[1], A. Paiardini[1], G. Giardina[1], A. Paone[1], S. Rinaldo[1]

[1]Department of Biochemical Sciences Rossi Fanelli, Sapienza University, Rome, Italy.
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One-carbon metabolism (OCM) is important for organismal development, cancer cell growth, and immune function (1). OCM, together with the folate cycle, fuels essential precursors for the synthesis of purine and pyrimidine nucleotides, as in de novo thymidylate synthesis. dTMP is synthesized by the combined action of serine hydroxymethyltransferase (SHMT), dihydrofolate reductase (DHFR) and thymidylate synthase (TYMS), with the latter two being targets of widely used chemotherapeutics (antifolates, 5-FU). We have successfully assembled and kinetically characterized the dTMP synthesis complex in vitro, employing tetrameric SHMT1 and a bifunctional chimeric enzyme comprising human TYMS and DHFR. The SHMT1 tetrameric state is required for efficient complex assembly, indicating that this aggregation state is evolutionarily selected in eukaryotes to optimize protein-protein interactions. We have also characterized the intracellular dynamics of the complex by in situ proximity ligation assay, showing that it is also detected in the cytoplasm, where it may play other roles. We are currently characterizing the interaction of these enzymes with nucleic acids, in particular with RNA, to assess the importance of RNA-protein interactions in their activity.

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T10

Single molecule manipulation and imaging of complex DNA-protein interactions

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The genetic information of an organism is encoded in the base pair sequence of its DNA. Many specialized proteins are involved in organizing, preserving and processing the vast amounts of information on the DNA. In order to do this swiftly and correctly these proteins have to move quickly and accurately along and/or around the DNA constantly rearranging it.
In this presentation I will show (Super-resolution) Correlative Tweezers-Fluorescence Microscopy (CTFM), a single-molecule approach capable of visualizing individual DNA-binding proteins on densely covered DNA and in presence of high protein concentrations. Moreover, proteins on DNA can be visualized on multiple DNA strand.
Next, I will show how we can use this instrument for the study of mitotic chromosomes. These structures are highly dynamic and undergo compaction during mitosis to adopt the characteristic "X-shape". Here I introduce a workflow to interrogate the organization of human chromosomes based on optical trapping and fluorescence microscopy. This allows high-resolution force measurements and fluorescence visualization of native metaphase chromosomes to be conducted under tightly controlled experimental conditions. The methods described here open the door to a wide array of investigations into the structure and dynamics of both normal and disease-associated chromosomes.

T11

Ceramide effects on lipid bilayer dynamics and structure

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Ceramides are sphingolipids containing a sphingosine or a related base, to which a fatty acid is linked through an amide bond. When incorporated into a lipid bilayer, ceramides exhibit a number of properties not shared by almost any other membrane lipid: Ceramides (a) are extremely hydrophobic and thus cannot exist in suspension in aqueous media; (b) increase the molecular order (rigidity) of phospholipids in membranes; (c) give rise to lateral phase separation and domain formation in phospholipid bilayers; (d) possess a marked intrinsic negative curvature that facilitates formation of inverted hexagonal phases; (e) make bilayers and cell membranes permeable to small and large (i.e., protein-size) solutes; and (f) promote transmembrane (flip-flop) lipid motion. Unfortunately, there is hardly any link between the physical studies reviewed here and the mass of biological and clinical studies on the effects of ceramides in health and disease.

[Alonso and Goñi, Annu Rev Biophys. 2018; 47:633-654].

T12

Modification of pore-forming toxins and their interactions with lipid membranes by directed evolution and insertion of non-canonical amino acids

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Pore-forming toxins (PFTs) form a heterogeneous group of proteins found in the collection of virulence factors of some pathogenic bacteria or as part of the venom of various animals. Expressed as soluble monomers, these proteins recognize specific receptor molecules on the host membrane, undergo a significant conformational change, and then oligomerize to form a transmembrane pore. Actinoporins, a group of PFTs from sea anemones, are well suited for biotechnological applications such as nanopore biosensing due to their conically shaped, narrow, nanometer-sized channels.

To adapt the newly identified actinoporin from *Orbicella faveolata* for nanopore biosensing, we have developed a series of variants that serve as low-noise, gating-absent nanopores that can be stably inserted into lipid bilayers as well as into polymer bilayers of MinION devices. Through detailed structural analysis, we have identified a single residue that enables the formation of atypical nonameric pores in addition to canonical octamers. Finally, through the use of genetic code expansion techniques, we have introduced a number of non-canonical amino acids into the designed nanopores that expand the number of available side-chain chemistries, enriching the range of potential analytes that can be detected in nanopore biosensing experiments.

T13

Binding strategies of SARS-Cov-2 spike variants viewed on the single molecule level

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Recent waves of COVID-19 correlate with the emergence of the Delta and the Omicron variant. Here, we combine high-speed atomic force microscopy with single molecule recognition force spectroscopy and molecular dynamics simulations to investigate, at single molecule resolution, the interaction dynamics of trimeric Spike with its essential entry receptor ACE2. Spike trimer undergoes rapid conformational changes on surfaces, resulting in arc-like movements of the three receptor binding domains (RBDs). Acting as a highly dynamic molecular caliper, it thereby forms up to three tight bonds through its RBDs with ACE2 expressed on the cell surface. Delta Spike shows rapid binding of all three Spike RBDs with considerably increased bond lifetime when compared to the Wuhan reference strain, thereby significantly amplifying avidity. Intriguingly, Omicron Spike displays less multivalent bindings to ACE2 molecules, yet with a ten times longer bond lifetime than Delta. Delta and Omicron Spike variants enhance and prolong viral attachment to the host, which likely not only increases the rate of viral uptake, but also enhances the resistance of the variants against host-cell detachment by shear forces such as airflow, mucus or blood flow. We uncover distinct binding mechanisms employed by circulating SARS-CoV-2 variants to enhance infectivity and viral transmission.

T14

From super-resolution to correlative microscopy, studying the effects of photosensitizers in cells and pathogens.

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Enveloped viruses, i.e., comprising a phospholipidic membrane encapsulating their genetic material, are a class of pathogens responsible for many diseases, often severe (e.g., COVID-19, AIDS...).
Since the viral envelope has a fundamental role in infectivity[1], it may represent a promising target for broad-spectrum antivirals. We are proposing the use of photosensitizers (PS), molecules that, in the presence of absorbable light, react with O_2 triggering the production of reactive oxygen species, very strong oxidants capable of damaging biological structures, such as membranes.
A specific PS called hypericin (hyp), which is also a fluorophore, exhibits a robust antiviral activity toward enveloped viruses[2]. In a recent article, we demonstrated it to be virucidal against SARS-CoV-2 not only in the presence of light, in accordance with its photodynamic properties, but also in dark conditions, revealing a multimodal mechanism of action[3].
In order to characterize further the effects of hypericin on coronavirus envelopes, we used supported lipid bilayers (SLBs), an excellent model system to simulate biological membranes. We studied their morphology with Atomic Force Microscopy (AFM) and discovered that a substantial rearrangement occurs in the presence of hypericin and light in the dark at higher concentrations; the effect is primarily visible in the more ordered phase, also called lipid rafts.
We aim to address if hyp changes the membrane's mechanical properties (e.g., stiffness and fluidity) using FCS and FLIM in correlation with Force spectroscopy[4].
Since lipid rafts and phosphatidylserine (a lipid on the envelope) are believed to play a crucial role in viral infection and immune escape of SARS-CoV-2, respectively[5,6], we investigate if hypericin multimodal antiviral activity is also related to a preferential interaction with specific lipids, by means of correlative AFM-fluorescence microscopy.

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T15

eSPC, an online data analysis platform for molecular biophysics

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Molecular biophysics experiments provide a detailed understanding of fundamental biological processes at the molecular level. Its insights into biomolecular interactions, macromolecules, and molecular mechanisms are critical for the fields of biotechnology, medicine and many others. The correct evaluation of the biophysical data is a key step in this regard. Here, we present the eSPC platform, a collection of user-friendly online tools for the analysis of microscale thermophoresis (MST), differential scanning fluorimetry (DSF), mass photometry (MP) and dynamic light scattering (DLS) data. The modules are available at spc.embl-hamburg.de and are useful for the determination of binding affinities (DSF and MST) and to assess, at least, protein stability (DLS), sample homogeneity (MP and DLS) and complex formation (MP).

T16

Making integrative structures accessible through PDB-Dev: From single molecules to dynamic macromolecular assemblies

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Structures of many complex biological assemblies are increasingly determined using integrative approaches, in which data from multiple experimental methods are combined. Based on recommendations from the worldwide Protein Data Bank (wwPDB) integrative/hybrid methods task force, a standalone prototype system, called PDB-Dev, has been built for archiving integrative structures and making them publicly available [1]. Data standards and software tools have been developed for collecting, curating, validating, visualizing, archiving, and disseminating integrative structures that span diverse spatiotemporal scales and conformational states. The PDB-Dev system can handle large dynamic macromolecular assemblies and integrative structures that combine experimental restraints with initial structural models computed by deep learning algorithms. Data standards and supporting tools have been extended to capture information regarding conformational dynamics and related kinetic data derived from biophysical methods. Following the FAIR (Findable, Accessible, Interoperable and Reusable) principles, PDB-Dev ensures that the results of integrative structure determination are freely accessible to everyone.

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TNA BENEFICIARIES

TB1

Circular dichroism as a tool to monitor real-time structural changes in monoclonal antibody manufacture

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Therapeutic monoclonal antibodies (mAbs) exhibit a propensity to unfold and aggregate in response to often seemingly trivial changes in process environment during their manufacture; this loss in structure compromises therapeutic efficacy and safety of the drug. Improving our understanding of the impact of processing environments on a given mAb's structure will rely on the development of rapid, sensitive and robust analytical techniques for determining protein structure at all manufacturing stages. Employing traditional circular dichroism (CD) and fluorescence techniques together for routine structural screening represents a significant challenge as such instruments are not configured for use in an industrial setting, where speed and autonomy are paramount. In direct response the University of Birmingham devised a high-throughput low volume capillary-based measurement system combining and automating CD and intrinsic fluorescence (ht-caCD/F) and demonstrated its utility as a real-time monitoring tool for bioprocess development work. The aim of this project is to evaluate the newly developed caCD/F in two guises. First, as a high-throughput screening tool for bioprocess purification and formulation development work, and second, as an in-line detector for monitoring the structural state of mAbs during elution from Protein A capture and polishing chromatography columns.

TB2

Elucidating interactions between SARS-CoV-2 proteins

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SARS-CoV-2 and it impact on humanity urges scientists to maximize the efforts in elucidating complex interactions between its viral proteins responsible for its replication process to deliver effective antiviral treatments. Some of the key proteins we study involves RNA methyltransferases that protects viral RNA from the detection and degradation (nsp14 and nsp16) as well as the main protease Mpro responsible for liberating otherwise inactive non-structural viral proteins from the polyprotein. We show that SARS-CoV-2 methyltransferases can form a heterotrimer complex to encourage the formation of mature capped viral mRNA. Our findings present that nsp14 is amenable to allosteric regulation and may serve as a novel target for therapeutic approaches.

We also consolidate the current state of knowledge on Mpro dimerization state, which is crucial for the processivity of its proteolytic activity. Traditionally, SARS-CoV-2 Mpro is considered to be a dimer, however, our mutagenesis study pins some crucial amino acids responsible for the dimerization process and visualizes the oligomerization states at various protein concentrations determining the dimerization constant.

Both projects benefited from the measurements realized through the MOSBRI, which enabled access to the Mass Photometry equipment and resulted in a scientific collaboration with prof. García EMBL-SPC, Hamburg team.

TB3

Characterization of the redox-centres in the electron-confurcating [FeFe]-hydrogenase (HydABC) of *Thermotoga maritima*

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The electron-confurcating [FeFe]-Hydrogenase (HydABC) of *Thermotoga maritima* coordinates 10 iron-sulfur centres (Fe-S) that connect the hydrogen-converting H-cluster to the bifurcating cofactor, FMN, and to the two electron donors, reduced ferredoxin and NADH. The redox properties of these cofactors are unknown and remain to be determined in order to gain any understanding on the electron-confurcation reaction.
The enzyme with a non-maturated H-cluster as well as the separate flavin-binding subunit HydB, were both heterologously expressed in *E. coli* and subjected to redox-titrations followed by CW-EPR- and UV/Vis-spectroscopy.
These experiments allow the attribution of specific redox potentials for the majority of these redox centres. Four of these centres are [2Fe-2S]-clusters, one of which is coordinated inside a plant-type Fd-like fold whereas the other three are found distributed in all three subunits and coordinated inside a thioredoxin-like fold. Redox titrations monitored by EPR at high (50K) and low (15K) cryogenic temperatures allowed assigning redox midpoint potentials to all [2Fe-2S] centres and approximate redox ranges to most of the remaining [4Fe-4S]-clusters. UV/Vis-monitored redox titrations yielded the midpoint potential of the FMN, which features different characteristics in HydB and HydABC.

This project was funded by the MOSBRI grant 2021-52 allowing for the Continuous wave EPR-measurements.

TB4

Characterization of the interaction of monoclonal antibodies with bacterial GAPDH

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Bacterial glyceraldehyde-3-phosphate dehydrogenases (GAPDH) are cytoplasmic glycolytic enzymes that have been shown to act as virulence factors in Group B Streptococcus by silencing the immune response in the host.
The neutralization of bacterial GAPDH is at the basis of the technologies developed by IMMUNETHEP to fight bacterial infections.
Here we will show the results obtained during a TNA visit to Institut Pasteur, where 3 monoclonal antibodies (mAbs) obtained by hybridoma technology had their interaction with GAPDH kinetically characterized.
The interaction kinetics were measured by Biolayer Interferometry (BLI) and Surface Plasmon Resonance (SPR), and the results have shown that one of the antibodies had poor affinity against bacterial GAPDHs, while recognizing the human protein. The other two mAbs have different interaction mechanisms, with one showing faster association and dissociation, with single digit nanomolar affinities. We have also shown that these two mAbs have different binding stoichiometries and recognize different GAPDH epitopes.
Overall, the TNA visit under the MOSBRI project was of utmost importance for IMMUNETHEP, by allowing access to state-of-the art equipment, and above all for the training and knowledge shared by the PFBMI team at Institut Pasteur.

TB5

Investigating the interactions between Myc and the RNA polymerase II component TFIIF

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The MYC oncoprotein is a master regulator controlling cell growth and is dysregulated in a majority of human cancers. The integral role of MYC in cancer has presented it as an attractive therapeutic target, however, it has not been successfully targeted to date. This is due, in part, to the intrinsically disordered nature of MYC resulting in a lack of deep pockets typical of a drug target. MYC modulates many of its interactions with other proteins through conserved "MYC box regions" (MBs), each of which is thought to be involved in largely unique protein-protein interactions. We have previously demonstrated that the most recently identified MYC box, MB0, is essential for MYC-driven oncogenesis. One validated MB0 interactor is the RNA polymerase II subunit TFIIF. The heterodimeric region of TFIIF interacts with low micromolar affinity to MB0, yet the importance of this interaction has yet to be fully understood. Using an assortment of biophysical techniques, including X-ray crystallography and NMR spectroscopy, we aim to describe an atomic-resolution model of the MB0–TFIIF interaction which will facilitate the development of tool compounds to investigate the MYC-TFIIF complex and will deepen our understanding of how MYC functions as part of the transcriptional machinery.

TB6

Insights into the catalytic mechanism of FdhAB from *Desulfovibrio vulgaris* Hildenborough

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The use of CO_2 as a feedstock for a circular economy is an intense area of research. Formate is an added-value product considered as an alternative liquid fuel [1]. The Formate Dehydrogenase (Fdh) from *Desulfovibrio vulgaris* Hildenborough (FdhAB) is a tungsten containing Fdh that is the main responsible for CO_2 reduction to formate *in vivo* [2]. However, the catalytic mechanism responsible for CO_2 reduction by Fdhs is not fully elucidated yet. This enzyme is an excellent model system for the study of this reaction since it has a simple structure, with only two subunits, it can be handled under aerobic conditions and its CO_2 reduction activity is one of the highest reported [3]. To better understand the catalytic mechanism of CO_2 reduction by FdhAB, we generated enzyme variants with mutations in highly conserved residues at the active site. The effect of the single-point mutations was assessed by kinetic assays and EPR spectroscopy. The results show the critical role of these amino acids for catalysis and confirm that these highly conserved residues are crucial for the understanding of FdhAB catalysis.

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TB7

Amyloid-membrane interactions observed with OCD and FTIR

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Hfq is a bacterial regulator that mediates many aspects of RNA metabolism. Our previous results obtained using biophysical approaches, demonstrated that Hfq C-terminal region (CTR) forms an amyloid structure. Furthermore, we demonstrated that CTR fibrils interact *in vitro* with a model of *E. coli* inner membrane (called EPE) and that the consequence of the interaction is membrane poration. Nevertheless, the specific effect of various lipids found in EPE is unknown. To address this question, orientated Fourier transform infrared spectroscopy (FTIR) and Circular dichroism (CD) have been used to study the conformational changes of the CTR in the presence of lipids, and to determine if there is an insertion in the membrane. We will present herein our FTIR and CD/OCD results indicating that CTR inserts preferentially specific lipids. Our results Will be discussed in the light of a putative role of Hfq in RNA export outside of the bacterial cell.

TB8

Hyperspectral microscopy and ligand screening of TTR amyloid fibrils

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Many neurodegenerative disorders are characterized by amyloidosis, where specific misfolded proteins aggregate into insoluble aggregates known as amyloids, leading to progressive degeneration of neurons. (Araki et al., 2019) A new method for amyloid detection uses novel fluorescent amyloid ligands called Luminescent conjugate oligothiophenes (LCOs), that give a distinct spectral change when bound to amyloid fibrils. (Nilsson et al., 2018) This project has aimed to characterize some of these LCOs and their properties, and assess whether they are suitable to report on amyloids in in-vitro living systems.
In collaboration with MOSBRI, a TNA excursion to ProLinc at Linkoping University was carried out, with the primary objective being screening of suitable LCOs for TTR detection. The emission spectra of eight amyloid ligands in solution and bound to TTR fibrils were measured at excitation wavelengths ranging from 373-535nm using a Tecan plate-reader. X34, HS335, and HS336 were chosen for their large difference in emission intensity between ligands in solution and ligands bound to TTR fibrils. Following this, the binding of X34 and HS336 to insulin fibrils was studied via hyperspectral microscopy. HS336 emphasized the structure of the insulin fibrils clearly, while X34 only gave vague colorization, possibly due to weak binding to insulin fibrils.

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TB9

The story of KEIF, the assumed appendix that held on for dear life

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For the last couple of years, our group have been studying the intrinsically disordered N-terminal of MgtA, one of the Mg^{2+} transporters found in bacteria, referred to as KEIF from the amino acid composition in this region. We are studying KEIF isolated from the MgtA protein complex. Here the story of KEIF will be presented, starting with the physiochemical characterization in solution using both experimental and computational techniques, followed by investigating the adsorption properties. The question has been raised whether KEIF is a passive appendix of the protein complex. However, it has shown signs of the opposite, and we hypothesize that it acts as an anchor of the large MgtA protein to the cell membrane. Our obtained results agree with this hypothesis, and several interesting effects are observed upon adsorption.
A wide variety of techniques have been utilized to innvestigate KEIF, including SAXS, CD/SRCD/OCD, MD simulations, NR, QCM-D, and FT-IR, to only mention a few. This combination of experimental techniques and computational ones allowed us to obtain a deep understanding of the behaviour of this peptide and were only possible to conduct thanks to the large-scale facilities that host these instruments, which we are very grateful for.

SELECTED POSTERS FOR SHORT TALKS

SP1

Experimental evidence of intrinsic disorder and amyloid formation by the Henipavirus W proteins

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The Nipah (NiV) and Hendra (HeV) viruses are zoonotic agents gathered in the *Henipavirus* genus within the *Paramyxoviridae* family. Beyond the P protein, the *Henipavirus* P gene also encodes the V and W proteins which share with P their N-terminal, intrinsically disordered domain (NTD) and possess a unique C-terminal domain (CTD). *Henipavirus* W proteins antagonize interferon (IFN) signaling through NTD-mediated binding to STAT1 and STAT4, and prevent type I IFN expression and production of chemokines. Structural and molecular information on *Henipavirus* W proteins, and in particular on their CTD, is lacking. By combining bioinformatic and biophysical approaches, we showed that the *Henipaviruses* W proteins and their CTD are intrinsically disordered. In addition, fluorimetry assays and negative-staining transmission electron microscopy showed that the W proteins form amyloid-like fibrils. Finally, by confocal and ultramicrotomy experiments the presence of the fibrils was also confirmed in a cellular context, strengthening the hypothesis of their involvement in *Henipavirus* pathogenesis. The present study provides an additional example, among the few reported so far, of a viral protein forming amyloid-like fibrils, therefore significantly contributing to enlarge our currently limited knowledge of viral amyloids.

SP2

Probing the mechanism of the peroxiredoxin decamer interaction with its reductase sulfiredoxin from the single molecule to the solution scale

Hortense Mazon[1], Audrey Beaussart[2], Florent Canonico[1], Jorge Hidalgo[1], Sarah Cianférani[3], Hélène Le Cordier[1], Alexandre Kriznik[1], Sophie Rahuel-Clermont[1]

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Peroxiredoxins from the Prx1 subfamily (Prx) are highly regulated multifunctional decameric proteins involved in oxidative stress response, redox signaling and cell protection. Their redox partner, the small reductase sulfiredoxin (Srx), regulates the switching between Prx cellular functions as an antioxidant and a chaperone. As only static structure of covalent Prx-Srx complexes have been reported, we assessed the non-covalent interaction mechanism and dynamics in solution of *Saccharomyces cerevisiae* Srx with Prx Tsa1 at the decameric level. A combination of multiscale biophysical approaches was used. Native mass spectrometry and atomic force microscopy imaging combined with solution scale techniques (anisotropy fluorescence and dynamic light scattering) showed that Tsa1 decamer can be saturated by ten Srx molecules and that this interaction does not induce Tsa1 decamer dissociation. The single-molecule atomic force microscopy approach using a tip sized to the decamer dimensions, decorated with multiple Srx molecules, allowed resolving up to five native interactions with Tsa1 subunits within a decamer. Combining protein engineering and rapid kinetics demonstrated a two-step mechanism of Srx binding to Tsa1 and the importance of Tsa1 C-terminus flexibility. This combined approach from the solution to the single-molecule level offers promising prospects for understanding oligomeric protein interactions with their partners.

Previously published in: Beaussart A et al (2022) Nanoscale Horizons 7, 515-525.

SP3

Single-molecule fluorescence applications in protein amyloid aggregation: from basic research to diagnosis

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A number of neurodegenerative diseases, including Alzheimer´s and Parkinson´s disease, are associated to protein amyloid aggregation, a process involving the transition from the functional, soluble state of a particular protein into misfolded, toxic oligomers and eventually insoluble fibrils with a hallmark cross-beta structure. Finding molecules with therapeutic or diagnostic potential in neurodegenerative disorders is of utter importance. However, the complexity and heterogeneity of the amyloid conformational landscape, makes amyloid aggregation a tremendously challenging target. Our lab is devoted to the development of novel fluorescence-based tools and applications for the study of the process of protein amyloid aggregation and the design of novel strategies for the diagnosis of some of these devastating diseases. In this presentation, we show some of our recent advances towards these aims.

SP4

Investigation of lectin binding to SARS-CoV-2 spike glycans using single molecule force spectroscopy

Yoo Jin Oh[1], J. F-W Chan[2], D. Hoffmann[3], S. Mereiter[3], D. Canena[1], R. Zhu[1], L. Hain[1], M. Klausberger[4], K-Y. Wuen[2], J. Penninger[3,5], D. Markovitz[6], C. Oostenbrink[7], P. Hinterdorfer[1]

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New SARS-CoV-2 variants are continuously emerging with critical implications for therapies or vaccinations. The 22 N-glycan sites of the Spike protein remain highly conserved among SARS-CoV-2 variants, opening an avenue for robust therapeutic intervention. By using a nanomechanical force-sensing approach, we obtained real-time information about the molecular bonds involved in the binding of carbohydrate-binding proteins, so-called lectins, to viral spike proteins. We determined the binding capacity of a molecularly engineered lectin cloned from banana, BanLec H84T, which was shown to display broad-spectrum antiviral activity against several RNA viruses. Our studies revealed that H84T-BanLec interacts with the Spike protein of the original viral strain, Wuhan-1 and several variants of concern (Delta, Omicron). Based on our force probing technique, dynamic molecular interaction patterns with accurate rupture force and length distributions were depicted. The complex multiple binding features between the dimeric H84T and trimeric spike protein were analysed with respect to the distribution of the glycosylation sites on the spike. Using high speed AFM, we additionally imaged spike proteins complexed with isolated lectin molecules to visualize oligomeric states and complex formation. Our data obtained by AFM techniques elucidate lectin-spike interactions at the single molecule level and uncover candidate receptors involved in spike binding and SARS-CoV-2 infections. The capacity of lectins to block SARS-CoV-2 viral entry holds promise for pan-variant therapeutic interventions.

SP5

Taylor-dispersion induced phase separation (TDIPS) for efficient characterisation of protein condensation

Lars Boyens-Thiele[1], Rasmus Krogh Norrild[1], Thomas Oliver Mason[1], Soumik Ray[1], Alexander Kai Büll[1]

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Protein phase separation or condensation has been proposed to be an important mechanism in biological regulation. Relying on dynamic, weak, and multivalent interactions, liquid-liquid phase separation (LLPS) may explain the *in vivo* occurrence of membrane-less cellular structures, and could have relevance for the development of neurogenerative diseases. We report Taylor dispersion induced phase separation (TDIPS) to observe condensation phenomena in a highly controlled micro-fluidic setup without the need for extrinsic fluorescent labelling of the protein. Notably, the technique employs a convection-diffusion based dilution to effectively exchange buffer/ions in protein solutions to investigate solubility. Implemented on a temperature controlled and commercially available instrument with 96-well plate automated sampling, the technique requires down to 30 nanoliters of sample per measurement, showing clear potential application in large scale screening campaigns and semi-quantitative mapping of the phase behaviour of proteins in solution. Applying TDIPS to the study of the N-terminal domain of Dead-box helicase 4 (Ddx4N1), we probe its NaCl-modulated phase behaviour and highlight a minimum solubility of the protein, not described before. Additionally, we highlight the potential for inhibition studies by addition of a DNA oligonucleotide that inhibits Ddx4N1 phase separation.

SP6

Condensate formation of cell division proteins FtsZ and SlmA in physiologically relevant glutamate buffer and its time-dependent evolution on lipid surfaces

Gianfranco Paccione[1], Miguel Á. Robles-Ramos[1], Carlos Alfonso[1], Marta Sobrinos-Sanguino[1], William Margolin[2], Silvia Zorrilla[1], Begoña Monterroso[1], Germán Rivas[1]

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The *Escherichia coli* division protein FtsZ has been previously shown to form dynamic phase-separated condensates by itself and together with its spatial regulator SlmA under crowding conditions. These GTP-responsive condensates may tune spatiotemporal regulation of FtsZ activity in cell division. Through turbidity measurements and fluorescent microscopy imaging we assessed quantitatively and semi-quantitatively the formation of these biomolecular condensates in physiologically relevant conditions in the presence of glutamate ions and lipid surfaces to better simulate the intracellular environment. Interestingly, the formation of these condensates is enhanced in the presence of glutamate ions when compared to chloride ions in crowded solutions. When incubating these samples on a supported lipid bilayer, condensates accumulated and fused resulting in a time-dependent evolution with an increase in their average size over time. Moreover, through protein capture and GTP response experiments we proved the dynamic properties of these condensates accumulated on the lipid surface. These results yield relevant information for the further reconstruction of the *E. coli* bacterial division system.

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SP7

Different enzymatic inhibitors trigger different IDO1 conformations that can influence its signaling functions

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Indoleamine 2,3-dioxygenase 1 (IDO1) is a heme-containing enzyme that catalyzes the rate-limiting step of kynurenine pathway.1 Cancer cells exploit IDO1 activity to generate an immune tolerogenic microenvironment, indeed, alteration of its expression and activity is located in patients affected by advanced neoplasia.2 Although, many small-molecule inhibitors have been developed to block enzymatic activity, their clinical efficacy remains limited. In addition, IDO1 non-enzymatic functions and its intracellular interaction partners are completely ignored in the drug development. When IDO1 is phosphorylated, it acts as a signaling molecule in plasmacytoid dendritic cells (pDCs), activating genomic effects and then, leading to long-lasting immunosuppression.3 For these reasons, in this work, we investigate the potential ability of IDO1 inhibitors to modulate not only the enzyme catalytic activity but also the signaling pathway. Combining two biophysical techniques, MicroScale Thermophoresis (MST) and Second Harmonic Generation (SHG) analysis, we demonstrate that the binding of enzymatic inhibitors, 4 such as Navoximod, Epacadostat, and Linrodostat, to IDO1 induces different protein conformational patterns and affects the interaction between IDO1 and Src, a tyrosine kinase involved in non-enzymatic functions of the enzyme. These promising data provide a new point of view in the development of IDO1 modulators for the cancer treatment and underline the need of further studies on IDO1 signaling functions.

[1] Mammoli et al. *ChemMedChem* (2021), 16, 2732.
[2] Zhai et al. *Cell Mol Immunol*, (2018), 15, 447.
[3] Iacono et al. *EMBO Reports* (2020), 21, e49756.
[4] Tang et al. *J Hematol Oncol,* (2021), 14, 68.

SPONSORS TALKS

C1

SUPR-DSF - Differential Scanning Fluorimetry for high-throughput protein stability screening

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Differential Scanning Fluorimetry (DSF) is a valuable and widely used technique that monitors protein unfolding by detecting changes in fluorescence as temperature increases. However, the conventional workflow for DSF uses extrinsic dyes that may influence the proteins thermal stability under investigation. This can affect the quality of your data by generating false positives or negatives during screening. The SUPR-DSF system from Protein Stable measures the intrinsic fluorescence of proteins to detect structural changes and avoids using additional dyes. The SUPR-DSF provides fluorescence measurement data directly from 384-well plates with no proprietary consumables. SUPR-DSF reduces operator time and minimizes the risk of errors in multi-step sample preparation while also bringing down the cost of consumables and sample consumption without compromising data quality.
Our Chirascan CD spectrometers contribute to a deeper understanding of biomolecular characteristics, mechanisms, and interactions. Our system can be used to gain insight, detect changes in secondary and tertiary structures, and study folding and unfolding mechanisms during altered physiological parameters.

C2

Ultimate DSC: a new tool for protein characterization

Guillaume Jossens[1], Yannice Ricci[1], Jean-Claude Neyt[1], Mickaël Simond[1]
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Presenting author: Yannice Ricci, contact@calneos.com

In biochemical, biophysical or pharmaceutical research, proteins are an important subject in the development of new drugs or treatments.
The stability parameters of these proteins are necessary for all these developments. It is therefore necessary to know, for example, the denaturation temperatures of the proteins studied, as well as the energy involved in these denaturations (denaturation enthalpy and temperature).
The technology most commonly used to access these thermodynamic parameters is differential scanning calorimetry (DSC). One of the major problems with this technology is the large quantity of protein required to obtain usable results: up to 1 ml per experiment, for proteins that can sometimes be very expensive to produce.
In this context, Calneos has developed the Ultimate DSC, which allows the use of less than 100 µL of sample in extractable crucibles. One of the advantages is the drastic reduction in the amount of sample required to obtain usable thermograms. Another advantage is the elimination of tedious and sometimes unreliable cleaning procedures.
This presentation will focus on the main points to carried out measurements, explain the unique features as Joule effect calibration and real sample temperature measurement, and conclude with results obtained with Lysozyme or RNase in PBS buffer at low concentration and protein quantities.

C3

Specific extracellular vesicle detection and isolation in complex samples using FO-SPR

Kris Ver Donck[1], Filip Delport[1]

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Presenting author: Kris Ver Donck, verdonck.kris@foxbiosystems.com

Reliable detection and quantification of EVs is becoming increasingly desirable due to their potential in disease diagnosis. However, their heterogeneity and the complexity of the matrices in which they are found, together with a lack of reference EV for assay development, and the bias introduced from time-consuming and difficult-to-scale sample purification steps, cause difficulties for many bioanalytical methods.

Fiber-optic surface plasmon resonance (FO-SPR) is a powerful tool that harnesses the performance of surface plasmon resonance (SPR) in an easy-to-use dip-in fiber- optic configuration. Here we describe an automated method for the specific detection, quantification and isolation of extracellular vesicles (EV), a particularly challenging task due to the heterogeneity of EVs and the complexity of the matrices in which they are typically found, such as culture medium or blood plasma.

We are currently expanding this capability with direct isolation from crude liquid biopsies for further analysis of the EV content, which calibrated by the FO-SPR quantification of EV's captured will allow for a fast and reliable detection of biomarkers in patient samples.

C4

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

Agnes Marszal[1], Andreas Kratzert[1], Stefanie Mak[1], Amandine Gontier[1], Nena Matscheko[1], Ulrich Rant[1]

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Kinetic rate analyses are essential for enhancing therapeutic antibody efficacy and safety. Most common antibody targets are transmembrane proteins and their native environment influences binding kinetics. We present two technologies, switchSENSE® and Real-Time Interaction Cytometry (RT-IC). These enable thorough binding kinetics analyses and offer insights into *in vivo* binding modes.

switchSENSE® is an automated fluorescence-based biosensor chip technology employing DNA nanolevers for real-time binding kinetics measurements. We characterized binding kinetics of emicizumab, a therapeutic bispecific antibody, and its two targets, by utilizing specialized Y-shaped DNA nanostructures emulating the platelet surface conditions. The observed avidity effects helped rationalize emicizumab's *in vivo* mode of action.

RT-IC is a technology enabling real-time kinetics measurements directly on living cells. Using flow-permeable polymer cages, single cells are immobilized label-free in a microfluidic channel. We were able to investigate the real-time binding kinetics of anti-PD-1 antibodies with their targets directly on T cells.

In summary, these examples showcase two methods for comprehensively characterizing antibody binding kinetics. switchSENSE® enables an in-depth characterization in a controlled parameter space and RT-IC allows the investigation of interactions in their native environment. These tools contribute to a better understanding of precise binding modes for therapeutic antibodies, advancing the drug discovery process.

C5

Use of Monolith to measure affinity of antibodies to a trimeric membrane protein

Pierre Soule[1]

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As drug discovery researchers pursue ever more challenging targets, there is a need for flexible methodologies for the assessment of binding affinities. The challenges associated with purifying and assessing binding affinity of these targets is complicated by the high sample consumption, immobilization, and assay optimization required of many common affinity measurement tools. Monolith X enables rapid measurement of K_ds between different biological molecules – including proteins, small molecules, nucleic acids – without immobilization, while requiring very little target sample. Learn how spectral shift technology in Monolith X works and see how it helped one group measure the affinity between a large trimeric membrane protein complex and a series of antibodies.

C6

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

Alex Spice[1], Ian Watt[1], Roland Worth[1], Alison Ilsley[1], Adam Halski[1], Alexey Morgunov[1], Sean Devenish[1], Sebastian Fiedler[1]

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Microfluidic Diffusional Sizing (MDS) technology allows researchers to examine the most challenging protein interactions under native conditions and gain insights that were previously unattainable. MDS technology can be accessed on our new Fluidity One-M platform, providing accurate and reliable measurement of interactions based on changes in molecular size. This approach is purification-free, works directly in solution, comes with easy access and simple workflows, and has already advanced discoveries in the fields of infectious disease, oncology, and neurology.

C7

Fractionation and characterization of macromolecules and nanoparticles: solutions for gene vectors

Nicolas Mignard[1]

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Presenting author: Nicolas Mignard, nmignard@wyatt.com

After a brief review of the technologies we are developing, we will concentrate on their applications for gene vector characterization such as size distribution, particle concentration, colloidal stability, lipid concentration, RNA concentration, encapsulation efficiency and size-based payload.

C8

Microfluidic Modulation Spectroscopy (MMS): automated, highly-sensitive IR-based protein structural analysis *in situ*, without the fuss

Patrick King[1], Klaus Mittendorf[1]

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Presenting author: Patrick King, *pking@redshiftbio.com*

Microfluidic Modulation Spectroscopy (MMS) is a powerful approach to the measurement of protein secondary structure, and overcomes many of the measurement limitations of traditional technologies such as CD and FTIR, enabling direct, *in situ*, analysis in even normally difficult backgrounds, fully automated from 24 and 96-well plates using the second-generation Apollo MMS system from RedShiftBio. MMS provides drift-free, background-subtracted, highly sensitive Infra-Red measurements over four decades of concentration, from 0.1 to >200 mg/ml, and is equally capable of measurements in simple, and complex backgrounds such as those containing high-concentration buffers, difficult excipients such as PS80, and organic solvents, enabling direct *in situ* comparisons to be made.

The fully-automated nature of MMS takes care of normally laborious calibration, baselining and cleaning steps in the process of data collection, and enables even a non-expert user to collect very high-quality structural data that is critical for a wide range of applications, such as storage and stability analysis, formulation studies, biosimilarity analysis, investigating the structural aspects of aggregation, and many others!

C9

Optical tweezers and single molecules: how to visualize and manipulate single biomolecules in real-time

Vincenzo Mascoli[1], Fabienne Payen[1], Bärbel Lorenz [1], Jack O'Sullivan[1]

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Imagine you could directly see the location and dynamics of individual proteins binding to a single piece of DNA in real time. What if you could hold a single protein and manipulate its structure to interrogate its conformational landscape? What if you could assemble your biological complex step by step and expose it to different buffer conditions to test your experimental hypotheses?
With the LUMICKS C-Trap, the world's first dynamic single-molecule microscope combining high-resolution optical tweezers, fluorescence microscopy, and advanced microfluidics in a truly integrated system, you can do all of this! We will illustrate how the dynamic single-molecule approach can shed light on a multitude of biological processes: from the mechanism of action of DNA-binding enzymes to protein folding and conformational changes, from molecular condensates dynamics to membrane remodelling.
These experiments show that technological advances in hybrid single-molecule methods can be turned into an easy-to-use and stable instrument enabling control, visualization and manipulation of single molecules in real time. This gives researchers the power to directly prove molecular mechanisms, in ways not previously possible, allowing you to answer mechanistic questions faster.

C10

Mass photometry – an analytical technology for biomolecular characterization

James Wilkinson[1]

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Mass photometry is a bioanalytical single-particle technology that measures the masses of biomolecules in their native states, in solution. The TwoMP mass photometer is an easy-to-use instrument that can measure masses of biomolecules between 30 kDa and 5 MDa, and requires minimal sample for analysis. In this talk, we demonstrate the utility of the TwoMP in a variety of experimental contexts, including quantifying the stoichiometry of biomolecular interactions, monitoring complex assembly, assessing sample purity and more. We also showcase the capabilities of the TwoMPAuto, a mass photometer with automated pipetting that performs measurements autonomously.

C11

The SPR Pro Series – Information-Rich Assays at Full Flexibility

Cyrill Brunner[1], Soumav Nath[2]

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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 conditions (e.g., different pH) and an epitope binning module.
The multiplexing capacities of the instrument enable the performance of information-rich assays at ease with no compromise on throughput. Complex quantification assays, selectivity studies, thermodynamic profiling or off-target investigations can deliver crucial basic information on an interaction. Due to the unique set-up, the instrument supports both small and large assay with an industry-leading throughput of up to 4400 samples per day.
The Sierra SPR Pro platform is a highly flexible system offering broad application possibilities and throughput.

C12

Pushing the limits of BLI: new biosensor technology for protein and AAV research

Tobias Zbik[1], S. Pinhal[1], B. Osborn[2], H. Li[2]

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Presenting author: Tobias Zbik, toz@il-biosystems.de

Biolayer Interferometry (BLI) is an established label-free analysis method that has been used in academics, biotech and pharma for nearly two decades to characterize protein quantity and kinetics. Its rapid and easy applications and the variety of available biosensors make it a flexible and powerful tool with widespread use in research and development.
A new generation of BLI instruments builds upon this foundation and pushes the limits of BLI to the next level with advances in biosensor technology for increased sensitivity, robustness and regenerative capabilities.
This talk will focus on how Gator Bio improves on traditional applications like protein quantitation and kinetic analysis, but also how the new portfolio of unique biosensors opens this application up to research focused on Adeno-associated virus (AAV) and lipid nanoparticles.

POSTERS

P1

The metal binding sites of CopI a periplasmic protein involved in bacterial copper resistance

Melanie Rossotti[1], Anne Durand[2], Diletta Arceri[1], Pascal Mansuelle[3], Olivier Bornet[3], Guillaume Gerbaud[1], Hélène Launay[1], Soufian Ouchane[2], Pierre Dorlet[1]

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[3]Institut de Microbiologie de la Méditerranée (IMM), CNRS, Aix Marseille Université, Marseille, France.

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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 photosynthetic bacterium *Rubrivivax gelatinosus*. Homolog exists in other environmental bacteria but also in human pathogens such as *Vibrio cholerae* or *Pseudomonas aeruginosa*. The 3D structure of CopI has not been determined and its mechanism remains unknown.
We have performed spectroscopic studies on the wild-type protein as well as specific mutants targeting the Cu binding modules from its sequence. Electron and nuclear magnetic resonance experiments show that CopI possesses a green-type cupredoxin site and is the first single domain cupredoxin with at least three Cu sites. The cupredoxin center and a highly conserved His/Met-rich region, which binds preferentially Cu(I), are required for Cu resistance. The non-conserved His-rich N-terminal region is not required for Cu resistance and is a binding site primarily for Cu(II). Moreover, sequential additions of Cu(II) and Cu(I) to the protein indicates its ability to oxidize Cu(I) into Cu(II) via the cupredoxin center. We therefore propose that CopI may have the dual function to detoxify Cu by oxidizing it to its less toxic cupric form and to store Cu(II) in its N-terminal site. The need of other protein partners for CopI will also be presented and discussed.

P2

Protposer: How to improve a currently functioning server

Helena García-Cebollada[1,2], Juan José Galano-Frutos[1,2]; Alfonso López[1,2]; Javier Sancho[1,2,3]

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Presenting author: Helena García-Cebollada, hgarcia@unizar.es

Protposer is a web server for protein stabilization based on the analysis of three-dimensional protein structures, using rational and empirical rules for proposing potentially stabilization. Since its initial beta launch in December 2019 and its publication in May 2022, it has hosted more than 1500 jobs from 250 unique users. Is there anything else we can do to enhance an apparently finished product?
This work focuses on the desired properties of web servers and how can they be improved in a server such as Protposer. Some of those properties are robustness, defined as the capability of maintaining the performance of the server upon external changes; efficiency, that can be associated with the time of calculus; preciseness, both in the theoretical internal calculations and in the final results and functionality, adding new useful capabilities to the server. The modular design of Protposer pairs well with such improvement processes, as each module can individually modified and tested for enhancement, and new modules can be easily added in order to implement a new function. This will be illustrated with some examples of current modular development and the improvement derived from them.

P3

Deciphering the properties of glycosyl inositol phosphoryl ceramides (GIPCs) required for interaction with pore-forming Nep1-like proteins (NLPs)

Andreja Prešern[1], Katerina Naumoska[2,3], Gregor Anderluh[1]

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Necrosis- and ethylene-inducing peptide 1 (Nep1)-like proteins (NLPs) are pore-forming protein effectors produced by many different plant pathogens. They recognize the plant plasma membrane glycolipids glycosyl inositol phosphoryl ceramides (GIPCs) as their receptors. GIPCs are the most abundant plant sphingolipids. They form a heterogeneous group of lipids with different sugar headgroup compositions and are still not commercially available. Therefore, whole GIPC plant tissue extracts have been used so far to study the NLP-GIPCs interaction. The extracts, however, contain a mixture of different GIPC molecules as well as impurities, so more detailed knowledge about the GIPC receptor properties is still lacking. To fill in the missing data, we developed a method to further purify GIPCs from GIPC extracts after their separation by high-performance thin-layer chromatography (HPTLC). We analyzed the GIPC isolates by tandem mass spectroscopy (MS/MS) to determine their composition. The isolates were then used for various interaction studies, i.e., sedimentation assays, TLC blotting followed by immunostaining, and surface plasmon resonance (SPR) analyses. The results collectively show that the composition of the GIPC sugar headgroup is important for the stable binding of NLPs to lipids and membranes.

P4

Combining EPR and NMR spectroscopies to investigate the function and dynamics of XRCC4 protein involved in the non-homologous DNA end joining pathway

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DNA double strand breaks (DSBs) are one of the most dangerous types of DNA damages. In mammals, DSBs are predominantly repaired by the non-homologous DNA end joining (NHEJ) pathway in which several proteins are involved in a large complex. The *X-ray repair cross complementing 4* protein (XRCC4) is part of this complex. XRCC4 is a homodimeric protein whose monomer is formed by a folded head domain, a stalk helical domain and a C-terminal intrinsically disordered region (IDR). Despite several investigations on the folded domain of XRCC4, little is known about the function of its IDR. In this study, we combined EPR and NMR spectroscopy to provide new structural insights about XRCC4. In particular, we used Site directed spin labelling EPR spectroscopy and Paramagnetic Relaxation Enhancement (PRE) NMR experiments to characterize the XRCC4 IDR dynamics and its interactions with different partners. These approaches were extended to the folded head domain to provide a finer understanding of its structural dynamics. Altogether our results demonstrate a high structural flexibility of the XRCC4 IDR and suggest how it could interact with its folded domains to modulate recruitment of multiple partners including DNA during DSB repair by NHEJ.

P5

Impact of SiO_2 surface roughness on the formation and stability of supported lipid bilayers

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Solid supported lipid bilayers (SLBs) are excellent platforms for studying the biophysical properties of cell membranes as well as versatile biomimetic films for biotechnology applications [1]. Among the existing approaches used to form SLBs, vesicle fusion and rupture onto solid supports represents the most commonly employed owing to its straightforward procedure. SLBs are typically formed on atomically flat and very hydrophilic surfaces, overlooking the influence of roughness and topography on membrane formation and organization [2]. As a matter of fact, lipid bilayers in vivo are corrugated at the nanoscale level. Fundamental studies of the effect of surface roughness on SLBs are scarce and restricted to few contributions [3, 4], where nanoroughness has shown to affect lipid mobility by a 5-fold decrease [3] and inhibit domain growth in phase-separated membranes [4].
In this work we study the impact of nanoroughness on the formation of SLBs onto SiO2 surfaces. To this end we use quartz crystal microbalance with dissipation and determine how nanoroughness modifies the activation energy of vesicle fusion, rupture and bilayer spreading. In addition, QCM-D is combined with atomic force microscopy force spectroscopy (AFM-FS) to assess the stability and lateral organization of the formed SLBs [5].

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P6

Spontaneous nanotube formation of Ohmline lipid on solid supports: Role of surface energy and buffer ionic strength

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Among the many assemblies that lipid molecules are able to form, lipid nanotubes (LNTs) are currently one of the most interesting structures, as they can be employed for biotechnological applications such as drug delivery, protein separation and DNA detection.[1,2] Herein we focus on the physical characterization of LNTs formed by a synthetic alkyl-ether lipid called Ohmline.
Developed as an anti-metastatic drug in cancer, Ohmline bases its therapeutic action on changes induced in the mechanical properties of the plasma membrane that prevent the oncoprotein association in several cancer types.[3,4]
In this work we have assessed the role of substrate surface energy, buffer ionic strength and pH on the formation and stability of solid-supported LNTs. For this, we have used quartz crystal microbalance with dissipation monitoring (QCM-D) as a microfluidic adsorption approach to form Ohmline films on SiO_2 and Au surfaces, which also allowed us to acquire data on the main transition temperatures and changes of Ohmline lipid structures upon lipid adsorption. Topographical and nanomechanical characterization of the Ohmline films was assessed through atomic force microscopy measurements. Interestingly, we observed that a very low ionic strength is necessary to form very dynamic LNTs, whose stability is strongly temperature-dependent.

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P7

The battle for silver binding: how the interplay between the SilE, SilF and SilB proteins contributes to the silver efflux pump mechanism

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

P8

AFM reveals the interaction and nanoscale effects imposed by squalamine on *Staphylococcus epidermidis*

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The Gram-positive bacterium *Staphylococcus epidermidis* is responsible for important nosocomial infections. With the continuous emergence of antibiotic-resistant strains, the search for new treatments has been amplified in the last decades. A potential candidate against multidrug-resistant bacteria is squalamine, a natural amino-sterol discovered in sharks. Despite its broad-spectrum efficiency, little is known about squalamine mode of action. Here, we used atomic force microscopy (AFM) imaging to decipher the effect of squalamine on *S. epidermidis* morphology, revealing the peptidoglycan structure at the bacterial surface after the drug action. Single-molecule force spectroscopy with squalamine-decorated tips shows that squalamine binds to the cell surface *via* the spermidine motif. We demonstrated that - although spermidine is sufficient for the initial attachment of squalamine to *S. epidermidis* – the integrity of the molecule needs to be conserved for its antimicrobial action. A deeper analysis of the AFM force-distance signatures suggests the implication of the accumulation-associated protein (Aap), one of the main adhesins of *S. epidermidis*, in the initial binding of squalamine to the bacterial cell wall. This works highlights that AFM -combined with microbiological assays at the bacterial suspension scale- is a valuable approach to better understand the molecular mechanisms behind the efficiency of squalamine antibacterial activity.

P9

Exploring the structural dynamics of SbcCD: a DNA repair nanomachine

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Maintenance of genome integrity relies on the efficient detection and repair of DNA double-strand breaks (DSBs). The Mre11-Rad50 complex serves as an initial responder to DSBs. The bacterial homologs of the Rad50-ATPase and Mre11-nuclease are known as SbcC and SbcD, respectively. Cryo-EM and atomic force microscopy studies captured various distinct configurations of SbcCD, both free and complex with DNA.1,2,3 However, the role of SbcC coiled-coil arms and how they coordinate the active sites of SbcD dimers during DNA repair remains unclear. To answer these questions, we performed a direct assessment of SbcCD structural dynamics using high-speed atomic force microscopy imaging at nanometer spatial and sub-second temporal resolutions. We majorly observed complexes with open coiled-coil arms while persistently connected at the hook region. The addition of ATP triggered the closure of the arms resulting in a ring-shaped configuration via SbcC-ATPase domains. Strikingly, we observed that coiled-coil regions have the ability to intertwine into a compact rod-shaped configuration and can undergo sudden relaxation events, indicating a possible pathway to release elastic energy for triggering large conformational changes. These findings may provide novel insights into the structural dynamics of SbcCD and will be important for interpreting the dynamic imaging data of SbcCD interacting with DNA.

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P10

Biophysical Characterization of LpxC deacetylase from gram-negative bacteria: differences between homologues

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The enzyme LpxC (UDP-3-O-(R-3-hydroxymyristoyl)-N-acetylglucosamine deacetylase) is a zinc-dependent deacetylase. Zinc is required for structural stability and protein functions, but needs to be tightly controlled through different mechanisms). LpxC is broadly conserved in almost all gram-negative bacteria and has no sequence homology with other mammalian proteins. LpxC is required for the synthesis of Lipid A (endotoxin), the membrane anchor of the lipopolysaccharides (LPSs). Lipid A is essential for cell viability and the major lipid component of the outer leaflet of the outer membrane, representing an attractive target for the development of new antibacterial substances.
LpxC deacetylase has been purified and characterized from different gram-negative bacteria in order to compare the structural stability of the protein.
We employed several biophysical techniques such as Differential Scanning Fluorimetry (DSF), Differential Scanning Calorimetry (DSC) and Circular Dichroism (CD), gathering thermal denaturation data in the presence and the absence of zinc.

P11

Biophysical Characterization of the structural role of zinc in HDAC8 for a new pharmacological strategy

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Zinc (Zn) is an essential element required for many proteins structures and catalytic functions involved in cell division, and DNA and protein synthesis, among others. Many metal-dependent proteins undergo considerable conformational changes upon metal association/dissociation, often involving partially or fully unfolded protein states, which Zn-dependent proteins are no exception. Histone deacetylase 8 (HDAC8) is a Zn-dependent lysine deacetylase that belongs to class I HDACs and has certain structural peculiarities that differentiate them from the other HDAC members. HDAC8 structure consists of two molecules packed as a head-to-head dimer. Each molecule binds one Zn 2+ ion and two K + ions. Because HDACs exert a major gene silencing role, HDACs alteration results in impaired acetylation and deacetylation which may cause the onset of numerous disorders including cancer. Therefore, HDACs have been considered as relevant drug targets. Among class I HDACs, overexpression of HDAC8 is found to be highly correlated in breast cancer, gastric carcinoma, and lung cancer, among others. The discovery of a novel HDAC inhibitors as new drugs for transcription therapy and cancer chemoprevention is imperative. In order to achieve it, our main objective is performing a comprehensive biophysical study of the structural role of zinc in HDAC8 for further experimental molecular screening procedures of chemolibraries to identify bioactive compounds which can behave as competitive/allosteric HDAC8 inhibitors.

P12

Chimeric miniproteins targeting the highly conserved heptad repeat 2 (HR2) region of the SARS-CoV-2 spike protein

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COVID-19 is the worst pandemic of the last decades. Several vaccines have been administered globally but immunity decays rapidly and new viral strains evade them. Therefore, new antiviral treatments and vaccines targeting conserved regions of the virus are urgently needed. One potential therapeutic approach against SARS-CoV-2 is to interfere with the fusion mechanism that allows the virus to enter the cell. This mechanism involves the interaction between heptad-repeat regions (HR1 and HR2) of the Spike's S1 subunit to form a 6-helix bundle structure.
We designed, engineered, and biophysically characterized single-chain miniproteins (named CoVS-HR1) that imitate HR1 subdomains in trimeric coiled-coil conformation. Compared to a previous generation of chimeric miniproteins, this second generation showed better structure, stability, and oligomerization properties. We also studied the binding to their complementary HR2-derived peptides and their virus inhibition activity. The N-terminal HR1 subdomain miniproteins mainly determine the binding to HR2 peptides and the inhibition of SARS-CoV-2 virus. In contrast, the C-terminal HR1 subdomain mimetic proteins did not bind to HR2 and did not inhibit the virus. However, binding and inhibition experiments using the complete CoVS-HR1 proteins with different lengths of HR2 peptides showed a clear cooperative distribution of interaction energy along the full HR1-HR2 interface.

P13

Hyperstable gp41-mimetic miniprotein with strong anti-HIV activity

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The discovery of novel protein therapeutics for the treatment of HIV/AIDS has been a major focus of research efforts towards the development of HIV-1 fusion inhibitors. Among these, chimeric proteins mimicking the N-terminal heptad repeat (NHR) of gp41 in a trimeric helical conformation are promising candidates because they bind the C-terminal repeat (CHR) and block conformational changes of the viral glycoprotein gp41, which has a crucial role in the fusion process. However, the inherent instability of the NHR region limits their activity and clinical utility, as it can cause aggregation and inactivation.
In this study, we aimed to enhance the stability of gp41 NHR-mimetic miniproteins by mutating buried polar amino acids to hydrophobic residues. We combined dynamic light scattering, circular dichroism spectroscopy, differential scanning calorimetry and isothermal titration calorimetry to show that the introduction of hydrophobic mutations resulted in a dramatic increase in the thermal stability of the miniprotein and enhanced binding affinity towards its target viral-derived peptide. Moreover, the engineered miniprotein exhibited a higher in vitro inhibitory activity against different HIV-1 strains. Our findings indicate that the conformational stabilization of gp41-mimetic miniproteins through the mutation of buried polar amino acids to hydrophobic residues is a good strategy for improving their utility as HIV-1 fusion inhibitors. These findings have important implications for the development of novel therapeutics for the treatment of HIV/AIDS and other viral infections.

P14

Synthesis of protein-based materials from mini-ferritins and cytochromes

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The development of protein-based materials allows to mimic the existing ones and to expand and modulate the intrinsic natural properties/functions, producing biomaterials with new characteristics and applications.
Ferritins are a family of almost spherical nanocages that comprises the DNA-binding proteins from starved cells (Dps) [1]. As a mini-ferritin, Dps is able to oxidize and store up to 500 iron atoms, and can bind to DNA, for condensation and protection [1]. New ferritin-based materials have the advantage of containing multiple nanocompartments, with enormous potential for biotechnological applications. Long fibres and coral-like structures formed by Dps proteins, with micrometres in size, were synthesized and characterized by several techniques (SDS-PAGE, PAGE, SEC, AFM, and SRCD) [2].
Polymeric assemblies of multihaem *c*-type cytochromes in *Geobacter* bacteria form long extracellular nanowires that are thought to be involved in long-range extracellular electron transfer [3]. Thereby, electric conductive protein nanowires have the potential to be employed in electronic devices, possessing several advantages over traditional materials [4]. The periplasmic trihaem cytochrome PpcA was explored to build semi-artificial wires using click chemistry and showed great potential since it could form chains with at least 14 molecules.

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P15

Disentangling structural morphological and structural heterogeneity in protein self-assembly

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Amyloid aggregates are characterized by a nano- and micro-scale heterogeneity and multiple species may occur possibly resulting in diverse effects on biological systems, both in connection to neurodegenerative diseases and immunogenic risk of protein-drugs.
Using FLIM- phasor approach and Thioflavin T (ThT), we show that it is possible to non-invasively map protein association from the early liquid-liquid phase separation to the formation of micronsized aggregates. ThT is a gold standard fluorescent dye for amyloid studies, its signal is determined by the steric hindrance of the internal rotation of the dye aromatic rings upon interaction with intermolecular □□structures. This last is known to be affected by presence of specific residues, charge or spacing between the β-strands.
Results indicate that ThT fluorescence decay can be described in terms of double exponentials that display a quite general behavior: the shorter lifetime component is attributable to less specific interaction originating from increased environmental viscosity in liquid/gel environment whilst the longer component to specific interactions with intermolecular □-structures. The analysis of the lifetime distribution “trajectory” in the phasor plot provides a graphical method to monitor in real time structural changes during maturation also providing pixel resolution map of the intermolecular architecture of single aggregates.

P16

The structural architecture of an α-synuclein toxic oligomer

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Oligomeric species populated during α-synuclein aggregation are considered key drivers of neurodegeneration in Parkinson's disease. However, their structure and the molecular determinants driving their conversion to fibrils remain elusive. In this work, we determined the symmetry and architecture of α-synuclein oligomers, dissecting the conformational properties of individual chains within these toxic assemblies. We demonstrate that the NAC domain is insufficient to promote oligomer to fibril conversion; instead, this transition is controlled by a short α-synuclein N-terminal motif. A missense mutation causing early-onset Parkinson's disease remodels this N-terminal region conformation, which results in a population of long-lived oligomers less susceptible to disaggregation by the human Hsp70 machinery. Our results provide a structural understanding of oligomer to amyloid conversion and identify targets for therapeutic intervention.

P17

Looking for patterns in TLB according to diagnosis and prognosis in pancreas cancer

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Thermal Liquid Biopsy (TLB) refers to the analysis of serum samples by differential scanning calorimetry, providing a global picture of the proteins and their interactions, showing the difference in heat capacity between serum and reference buffer as a temperature function. Our objectives are: 1/to predict the diagnostic of pancreatic cancer by building a classification TLB-model (n=212 *vs* n=185 control group); 2/to validate the TLB-model in an external cohort of pancreas cancer (n=20); 3/to associate changes in TLB induced by therapy (samples before and after treatment) with prognosis.
The TLB-model was built applying machine learning algorithms. TLB-model showed an area under the ROC curve=0.88, specificity=68% and sensitivity=89%. TLB-model showed different TLB-patterns, that corresponded with the same TLB-patterns obtained when validating it with the external cohort. Patients with both TLB tests positive had a median overall survival expectancy lower (6[5-7] months) than patients with both TLB tests negative or when the second TLB converted to negative (15[12-21] months) (Breslow test: p-value<0.001).
We conclude that: 1/we can obtain different TLB-patterns in pancreas cancer; 2/changes in TLB induced by therapy are correlated with better/worse overall survival. 3/ Further studies are necessary to confirm whether there are different TLB-patterns according to the pathology.

P18

The Sample Preparation & Characterization facility at EMBL Hamburg

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The Sample Preparation & Characterization (SPC) facility of the European Molecular Biology Laboratory (EMBL) is located in Hamburg, Germany, on the DESY Campus. The SPC primarily assists academic and industry researchers in their structural biology studies, while also undertaking innovative biophysics technology development.[1,2] The facility's high-throughput crystallization (HTX) laboratory offers a broad range of crystallization screens for initial hit optimization, and it is located alongside the EMBL beamlines. The users can access the automated crystal harvesting and data processing to streamline crystallization experiments and synchrotron data collection. Additionally, we provide assistance in performing SAXS batch measurements, delivering near-real-time outputs of macromolecular structural parameters and low-resolution solution-state structures. The biophysical platform of SPC employs state-of-the-art technologies to measure biomolecular interactions accurately and determine the stability, shape, and size of different biomolecules and biomolecular assemblies. Starting from initial protein quality control to more sophisticated biophysical approaches, a wide range of services is provided by the SPC. Our online data analysis platform (eSPC), is available at spc.embl-hamburg.de, enabling users to analyze and visualize biophysical data from anywhere in the world.[3] The SPC core facility is part of MOSBRI and a member of other complementary research networks, including iNEXT-Discovery, INSTRUCT-Eric, and HALRIC, that offer researchers from various fields trans-national access to fully-funded methods to support their scientific projects.

[1] Niebling et al. Sci Rep, 2021; 11: 9572.
[2] Niebling et al. Front Mol Biosci, 2022; 9:882288.
[3] Burastero et al. Acta Crystallogr D Struct Biol, 2021; 77, 1241-1250.

P19

Biochemical characterization of the Ferric Uptake Regulator Fur from the strict anaerobe pathogen *Clostridioides difficile* unveils a thiol-based oxidation sensing mechanism

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Clostridioides difficile is a strictly anaerobic pathogen that produces gastrointestinal diseases. Iron is essential for this microorganism, and consequently it has developed several mechanisms to cope with iron limitation into the host. However, as high levels of iron can be lethal for *C. difficile*, iron uptake is tightly controlled at transcriptional level by the ferric uptake regulator Fur. Apart from iron homeostasis, Fur is thought to play key roles in virulence antibiotic resistance and oxidative stress defense in *C. difficile*, although it does not directly regulate oxidative stress or pathogenesis response genes.

In this work we have purified Fur from *C. difficile* and studied the conditions for its binding to the promoter regions of iron-dependent Fur regulated genes. These *in vitro* analyses have shown that *C. difficile* Fur DNA binding activity is specific under reducing conditions and that it is hindered by the presence of metal ions. We have also determined that the activity of this protein is modulated by thioredoxin through a reversible redox mechanism that controls its oligomerization state. Taken together, these results suggest that the activity of Fur from *C. difficile* might undergo redox-dependent apo-regulation under iron deficiency in response to oxidant conditions.

P20

Making biophysical measurements searchable and citable

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Molecular-scale biophysics suffers from a lack of standardized storage data formats for measurements. Especially the metadata describing the experimental system (buffer composition, instrument settings, etc.) tend only to be available in methods and material sections of articles. This makes it time consuming to search for results (e.g., dissociation constants) and the associated measurements performed in the presence or absence of specific experimental conditions (e.g., pH or metal ions). Furthermore, it hampers efforts to re-analyze measurements using new models, as well as employing them in machine learning. Work package 4 (WP4) of MOSBRI is seeking to improve this situation.
WP4 is implementing a database of biophysical data according to the FAIR principles (Findability, Accessibility, Interoperability, and Reusability). The first techniques being focused on are: Micro-Scale Thermophoresis (MST), Surface Plasmon Resonance (SPR) and Bio-Layer Interferometry (BLI). These were chosen based on 1) lack of existing repositories 2) lack of an open standardized (meta)data format, and 3) high number of users.
The efforts revolve around three aspects: 1) defining common metadata elements and structure them into schemas, 2) Creating a repository for deposition of biophysical measurements and results, 3) Ensuring that measurements can be searched, retrieved, and cited in their own right.

P21

Protein HelD – role in bacterial transcription and antibiotic resistance

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HelD, a multidomain helicase-like protein, forms a tight complex with bacterial RNA polymerase. Experimental results accumulated in the recent years reveal details about the role of this protein in bacterial transcription. Our data based on small angle X-ray scattering, crystallography, and Cryo-EM, together with biophysical measurements and transcription assays contribute to the overall structure-function picture. The cryo-EM structures of complexes between the *Mycobacterium smegmatis* RNA polymerase and HelD show HelD simultaneously penetrating into two RNA polymerase channels, thereby removing nucleic acids or other binding partners from these channels [1]. The structures provide insights into the function of HelD in releasing stalled RNAP from DNA. The structural data also point to its protective effect against the antibiotic rifampicin in mycobacteria and possibly also in *Bacillus subtilis* [2]. Here we present the current knowledge about this protein partner of RNA polymerase, including mechanistic insights into its actions to help maintain bacterial transcription.
This work was supported by MEYS (LM2018127, LM2023042) and CSF (23-06295S).

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2. Sudzinová P, et al. What the Hel: recent advances in understanding rifampicin resistance in bacteria. FEMS Microbiol Rev. 2022:fuac051.

P22

Biophysical techniques at Centre of molecular structure of BIOCEV

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The biophysical research facility as a part of the Centre of molecular structure of Institute of Biotechnology provides shared resources of instruments for the determination of size, molecular mass, structure and stability of biomolecules, study of conformational changes and thermodynamics of temperature transitions and characterization of biomolecular interactions. Facility is a member of Instruct-ERIC, Czech Infrastructure for Integrative Structural Biology (CIISB) and Molecular-Scale Biophysics Research Infrastructure (MOSBRI).
Following techniques and instruments are currently available: mass photometry (TwoMP mass photometer) circular dichroism spectroscopy (Chirascan Plus CD spectrometer), spectrophotometry (Specord 50 Plus UV/Vis spectrophotometer), Fourier-transform infrared spectrometry (Vertex 70v spectrometer), fluorescence spectrometry (photoluminescence spectrometer FLS1000), differential scanning fluorescence (Prometheus NT.48), multiangle dynamic light scattering (Zetasizer Ultra), microplate reader (Tecan), differential scanning calorimetry (Microcal VP-DSC), isothermal titration calorimetry (Microcal iTC200 and PEAQ-ITC), microscale thermophoresis (Monolith NT.115 and NT.LabelFree), surface plasmon resonance (ProteOn XPR36) and bio-layer Interferometry (OCTET R8).

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P23

Antibody humanization: development and test of a statistical inference approach

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Antibody humanization is a key step in the preclinical phase for the therapeutic antibodies developed in non-human models. The standard CDR grafting technique, essentially based on point-similarity with human germline sequences, has important drawbacks, often requiring trial-and-error editing of the resulting sequences.
We proposed a method to infer an approximate statistical distribution of human sequences, and define a "humanness score" (MG-score) thereof. After checking its performance by redesigning the framework regions of a few murine sequences, we applied the method in the humanization of a murine antibody against β-1,3 glucans, a vital component of several pathogenic fungi.
Invasive fungal infections mainly affect immunocompromised patients (because of surgery, cancer, etc), and cause over 1.5 million deaths every year. Even if four classes of antifungals are available, the side effects of drugs, as well as fungal acquired or innate resistance, represent major hurdles to be overcome.
The humanized H5K1 antibody has been tested on *C. auris*, one of the most urgent threats, and resulted efficient especially in combination with Caspofungin and Amphotericin B showing an enhancement effect. These results support the interest of our statistical-inference humanization approach, and prompt for further developments for the use of H5K1.

P24

A free web server to automatically fit TSA curves in just two minutes

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The automated data processing provided by the TSA-CRAFT tool [1] enables to reach high-throughput speed analysis of thermal shift assays. While the software is powerful and freely available, it still requires installation process and command line efforts that could be discouraging.
To simplify the procedure, we decided to make it available and easy to use by implementing it with a graphical interface via a web server, enabling a cross-platform usage from any web browsers. We developed a web server embedded version of the TSA-CRAFT tool, enabling a user-friendly graphical interface for formatting and submission of the input file and visualization of the selected thermal denaturation profiles.
We describe a typical case study of buffer condition optimization of the biologically relevant APH(3')-IIb bacterial protein in a 96 deep-well thermal shift assay screening.

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[1] Lee *et al.* (2019) TSA-CRAFT: A free software for automatic and robust thermal shift assay data analysis. *SLAS Discov 24*, 606.

P25

MeCP2 binding partners: Reading epigenetics beyond DNA methylation

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Methyl-CpG binding protein 2 (MeCP2) is a versatile transcriptional regulator consisting of six domains that has been linked to diseases like Rett Syndrome and various types of cancer. MeCP2 is an intrinsically disordered protein (IDP) that recognizes epigenetic marks, such as methylation, and binds to DNA through its methyl-CpG binding domain (MBD). The MBD is sandwiched between two completely disordered domains, the N-terminal domain (NTD) and the intervening domain (ID), which have been demonstrated to impact the protein's structural and functional properties.

We provide a comprehensive thermodynamic analysis of the interaction between MeCP2 and epigenetically modified DNA (with unmethylated, hydroxymethylated, or methylated cytosine) as well as histones (H2A, H2B, H4, and H3K4, K9, K27, K36 trimethylations). We also examine the influence of the remaining domains, such as the transcriptional repressor domain (TRD) and C-terminal domains (CTDα and CTDβ), on the structural stability of MBD and the protein's dsDNA binding ability. Furthermore, we discuss how Rett syndrome-linked mutations impact MeCP2's structure and function, and how these effects vary depending on the molecular context. Our findings shed light on the function of intrinsic disorder in MeCP2 and underscore the importance of taking into account the influence of disordered regions on protein characteristics.

P26

Stabilizing ZapA versus inhibiting SlmA modulate bacterial division FtsZ biomolecular condensates and polymers

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Bacterial division is driven by a contractile ring consisting of a multiprotein complex, which accurately assembles at midcell. For its formation, the polymerization of the GTPase protein FtsZ is required as scaffold for the rest of the proteins involved. The control mechanisms underlying division ring positioning remain largely unknown. However, the nucleoid occlusion protein SlmA and the cross-linking protein ZapA are known to be relevant. FtsZ, SlmA and its DNA sequence have shown to form biomolecular condensates, structures emerging as a mechanism to organize intracellular space, linked to stress tolerance.
We have found that FtsZ-SlmA-SBS condensates are able to recruit ZapA in crowding conditions in bulk and also when encapsulated inside cell-like microfluidics microdroplets. Our combined biophysical and reconstitution approach showed that the ZapA/SlmA ratio controls FtsZ condensate/polymer interconversion through non-competitive binding and that FtsZ polymer stabilization by ZapA is favored under crowding conditions. These findings underline the significance of condensates as concentrated hubs of proteins, which can shed light on cell survival under stress conditions, such as those induced by antibiotic treatment.

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P27

Design and implementation of RNA aptamers for inhibition SHMT1 and SHMT2 metabolic enzymes

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Serine hydroxymethyltransferase (SHMT) is an enzyme that catalyses the reversible conversion of serine and tetrahydrofolate (THF) into glycine and 5,10-methylenetetrahydrofolate (CH2-THF). SHMT plays a pivotal role in the one-carbon metabolism, a complex network fuelling factors fundamental for highly proliferating cells; in fact, as expected SHMT is overexpressed in different tumours and it is considered as a good chemotherapeutic target. We have previously demonstrated that hSHMT1 is a cytosolic moonlighting protein [1] and beyond its enzymatic function, it controls the expression of its mitochondrial counterpart hSHMT2 by binding to the 5'UTR of SHMT2 transcript and interestingly, this bond inhibits at the same time SHMT1 enzymatic activity. Although several small molecules have been tested in the past years, no inhibitor successfully used *in vivo* is currently available [2], suggesting that a completely new approach is needed. Starting from our unpublished results suggesting that also the mitochondrial SHMT2 isoform can bind RNA, we are working towards the design of a new aptamer-based strategy that uses small RNA molecules to specifically target SHMT1 in the cytosol and SHMT2 in the mitochondria. Our preliminary results on the RNA-protein interactions provide evidence that inhibitory RNAs can be successfully employed to target SHMT and possibly other metabolic enzymes known to be RNA-binding proteins.

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[2] Ducker et al. Proceedings of the National Academy of Sciences (2017),114(43), 11404-11409

P28

Biochemical and biophysical characterization of proteins involved in the spatial regulation of the bacterial division ring

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Cytokinesis is a fundamental process in bacteria driven by the formation of a protein ring at midcell. The protein FtsZ self-assembles in filaments serving as scaffold of the ring, whose formation is spatiotemporally regulated by several factors including nucleoid occlusion, the Min system and the Ter linkage. The latter acts as a positive regulator of ring assembly, and consists of the proteins ZapA, ZapB and MatP, a protein that recognizes specific sequences clustered at the Ter macrodomain of the chromosome. The molecular mechanisms underlying the function of ZapB remain largely unknown, in part because of the difficulties associated to its isolation. Starting from a previously described protocol for the purification of His-tagged ZapB we have optimized a procedure for the obtention of the tag-free protein, which was fluorescently labelled for further characterization. Purified ZapB interacts with FtsZ filaments, in the presence of ZapA, a hallmark of its functionality. The three proteins were reconstituted in crowding conditions mimicking the physiological environment of the bacterial cytoplasm in which they perform their function. These reconstitutions are shedding light on the role of ZapB in the spatial regulation of division ring formation.

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P29

Addressing the potential channeling of FMN from *Homo sapiens* Riboflavin kinase to pyridoxine 5'-phosphate oxidase

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Consecutive enzymes in metabolic pathways usually cooperate forming transient complexes named metabolons, where the intermediates are maintained within by substrate channeling, to control the transport and flux of reactants and intermediates. Despite substrate channeling has been widely studied for reactant molecules, information is seldom available for cofactors. Nonetheless, mechanisms of cofactor delivery have been proposed for cofactors such as molybdenum and flavin adenine dinucleotide (FAD). FAD, together with flavin mononucleotide (FMN), ensure the functionality of hundreds of different flavoenzymes in all living beings. Riboflavin kinase (RFK), which catalyzes the biosynthesis of the FMN cofactor, plays a key role in the flavoproteome homeostasis and might operate as a chaperone in the transference of FMN to client apo-proteins, likewise the FAD synthase in the case of FAD. In this context, the possibility of direct assemblage of *Homo sapiens* RFK to the client protein pyridoxine-5'-phosphate oxidase (PNPOx) is here proven, by using experimental techniques as well as docking and molecular dynamics computational approaches.

P30

Erythrocyte adhesion and biomechanics on carotid artery disease patients

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Carotid artery disease (CAD) is characterized by the carotid artery occlusion, impairing blood flow to the brain, due to atherosclerotic plaque formation. High levels of fibrinogen are associated with blood clot formation. Here, we evaluated changes in erythrocyte biomechanical properties, fibrinogen-erythrocyte and erythrocyte-erythrocyte interactions in CAD patients, using atomic force microscopy. Blood samples were collected from CAD patients before prophylactic carotid endarterectomy (CEA), and every 6 months until 36 months after surgery. Results were compared with a control group of healthy blood donors. Higher erythrocyte stiffness was detected on CAD patients before surgery, decreasing 12 months later. Patients had lower fibrinogen-erythrocyte binding forces after surgery, despite higher binding frequency. The force necessary to detach two erythrocytes from CAD patients decreased after surgery. Higher fibrinogen (total and ´ variant) plasma levels were detected in CAD patients before surgery, decreasing after it. Changes on erythrocyte stiffness, erythrocyte adhesion and fibrinogen-erythrocyte binding force were observed during the 36-month follow-up. Erythrocyte adhesion results may eventually be assessed as a cardiovascular risk factor for CAD, to predict and prevent secondary events. Moreover, these results may contribute for the detection of higher probabilities of restenosis events associated with CAD.

P31

A statistical-physics model for codon usage

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The degeneracy of the codon alphabet allows different codons to translate to the same amino acid, and it is well-known that different species show different statistics of codon usage. However, the reasons why a species adopts a particular statistics are not clear, even if protein yield, production speed, and RNA stability are believed to play a role in the choice.
In this context, codon "optimization" involves adjusting the codon sequence for a target protein, to mimic the "natural" choice a given species would make, to produce that protein. However, conventional methods used for codon optimization are often simplistic (e.g., resorting just to the importance of each codon), or phenomenological, using the observed average frequency of codon pairs as input, instead of obtaining it as a result.
Using large databases of human proteins, we propose a statistical-physics model, where¡ the probability of any codon sequence is related to the "interactions" between neighboring codons. We have adjusted the model's parameters to maximize the dataset's probability. We have applied the method to the case of Luciferase, as a simple test protein, optimizing the codon sequence by Simulated Annealing, and comparing the results to those obtained by conventional methods.

P32

MeCP2 as a therapeutic target

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Aberrant DNA hypermethylation is one of the molecular events activating the development of pancreatic ductal adenocarcinoma (PDAC). It has been shown that certain proteins, such as the transcriptional regulator MeCP2, are responsible for reading these marks and pathogenically blocking the expression of certain genes.
MeCP2 could be a promoter in the progression of PDAC due to its markedly different expression in tumor tissues and its ability to induce silencing of interleukin 6 (IL-6), which is responsible for the regulation of the immune response, differentiation, proliferation, cell migration, carcinogenesis, apoptosis; or the regulation of LIN28A expression, an inducer of pluripotency that is responsible for cell development, growth and metabolism.
The aim of this study is to evaluate MeCP2 as a potential therapeutic target against PDAC and to study the antitumor activity of compounds capable of interacting with MeCP2. For this purpose, cytotoxicity assays will be performed in two tumoral cell lines (PANC1 and Mia PaCa2) and in the non-tumoral cell line HPDE. Also, MeCP2 expression will be analyzed in these cell lines and in samples of healthy and tumoral human tissue.
In conclusion, inhibition of MeCP2, an important epigenetic mark reader, could improve the prognosis of pancreatic cancer patients by reversing aberrant transcription patterns.

P33

Unveiling the ability of protein nanocages to interact with plasmid DNA

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Nanocage proteins are composed of a specific number of identical subunits that assemble into a highly symmetrical and spherical hollow structure. DNA-binding proteins from starved cells (Dps) are small multifunctional nanocages that belong to the ferritin family. The first function reported for these proteins was their ability to bind DNA, both circular and linear forms. The interaction is mainly associated to the unordered and flexible N- and C-terminal extensions [10.1016/j.ccr.2021.214187]. *Deinococcus grandis* Dps (DgrDps) exhibits unusual long dynamic N-terminal tails (with 52 residues) that can protrude from the core structure.
Myxococcus xanthus encapsulin (EncA) is a 32 nm-wide protein compartment composed of 180 identical subunits. Encapsulins are protein nanocompartments that have the ability of sequestering small proteins within their inner cavity. The encapsuling shells have a relatively high structural homology and most likely share a common ancestor with the viral capsid of HK97-type viruses [10.1016/j.ccr.2021.214188]. However, their ability to bind DNA was thought to be lost during the evolution process.
In this work we assess the DNA binding ability of DgrDps and EncA using EMSA, AFM and SRCD techniques. The results reveal that both DgrDps and EncA bind DNA, forming large stable aggregates [10.3390/ijms23147829; 10.1002/pro.4567].

P34

Switch your SENSE of studying macromolecular interactions

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

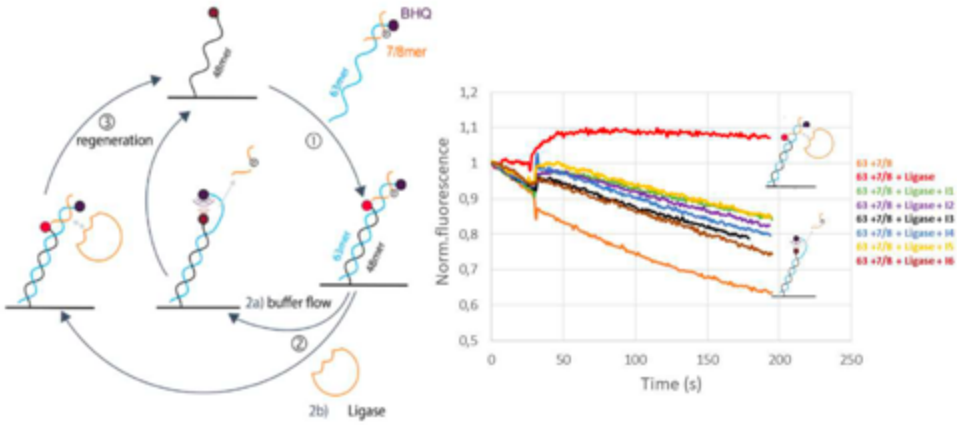

Figure. Scheme for measuring ligation activity (left): ① Hybridization of a DNA of 63 nucleotides with a BHQ (black-hole-quencher) at 5' with two DNAs of 7 and 8 nucleotides, the latter with a phosphate in 5'. ② Flow buffer 10mM TRIS-HCl, 40mM NaCl, 50 µM EDTA, 50 µM EGTA, 1 mM ATP, 10 mM MgCl2 for 5 min a) without and b) with Ligase. ③ Surface regeneration. Example of measurement (right): Control with the 3 DNAs (63+7/8) without Ligase and then with Ligase at 500nM in the presence of 2% DMSO and 6 inhibitors (I1-I6) at 20µM.

P35

Isolation of one-component signal transducers involved in c-di-GMP metabolism

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Signal transduction is a crucial process for bacteria, enabling them to sense changes in their environment and respond accordingly. One area where signal transduction plays a significant role is in the formation of bacterial biofilms, such as those formed by Pseudomonas aeruginosa. These biofilms are known to contribute to antibiotic resistance and evasion of host immune defenses, making them a significant challenge in the treatment of bacterial infections. The intracellular signaling molecule cyclic diguanylate (c-di-GMP) has been identified as a critical regulator of biofilm formation and dispersion in *Pseudomonas* aeruginosa. The level of c-di-GMP within the cell is controlled by membrane proteins with diguanylate cyclase (GGDEF domain) or phosphodiesterase (EAL domain) activity, which synthesize or degrade c-di-GMP, respectively. Our current work focuses on one-component signal transducers whose domains architecture involves a periplasmic nutrient-sensing domain, a transmembrane portion, and a cytoplasmic moiety including the GGDEF and/or EAL domain. Membrane proteins are particularly challenging to isolate and characterize. Here we show novel results on the extraction and purification of such transducers, relevant to biofilm formation and maintenance, comparing the detergents and polymers such as SMA and DIBMA as different methodological strategies.

P36

Mechanistic DNA Properties are key drivers of Nucleosome Unwrapping

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Eukaryotic cells store their genetic information in the cell nucleus, using a condensed protein-DNA polymer known as chromatin. Chromatin's basic unit is the nucleosome, composed of DNA wound around a core of histone proteins. The spatiotemporal organization of chromatin packaging is one of the key regulators of gene expression [1].
Nevertheless, the physicochemical mechanisms are still unknown, as the study of these properties is challenging due to their multiscale nature.
In this study, we focus on nucleosome unwrapping. The study of, as it can provide insight into a key step in chromatin remodelling, the control of gene expression and chromosomal stability, as well as shedding light on how genes are regulated at the molecular level. Our computational methods, which utilize coarse-grain molecular dynamics simulations, provide an efficient tool to study chromatin at the molecular scale, ensuring a balance between computational cost and accuracy [2].
Our results reveal that mechanistic properties of DNA can govern nucleosome unwrapping as well as the effects of histone's PTMs. Our findings also provide detailed information on the molecular interactions that play a role in nucleosome stability and delineate a multi-scale computational framework that can be used to probe the effect of the mechanical properties of DNA into other mechanisms regarding chromatin organization.

[1] Hafner & Boettiger, A. Nat Rev Genet 24, 53-68 (2023).
[2] Farr et al., Nat Commun 12, 2883 (2021).

P37

Pest inhibitors design

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The harmful effect of the commonly used pesticides on pollinators focused our attention on the development of selective inhibitors against pests but not closely related insects. In running research, the identified differences in structures of juvenile hormone epoxide hydrolase from several insects are used for design of the library of potential inhibitors.
We aim to explore a unique approach which is based on intramolecular voids analysis and molecular dynamics simulations (MD) in co-solvents which will guide pharmacophore design. Identified differences and similarities between different insect JHEH will help in the design of new pharmacophores and subsequently species-specific inhibitors.
We aim to carry out extensive studies on selected proteins from ten organisms to best replicate a taxonomic cross-section of species. Targeted proteins are membrane proteins, which presents challenges in both their production and handling. Enzymes from different species will be characterised by methods which provide information about proper folding such as circular dichroism and nanoDSF experiments. The verification of proper folding and information about protein stability and thermal unfolding of the protein will be further explored towards optimisation of conditions required for optional protein crystallization.

The work was supported by the National Science Centre, Poland: UMO-2020/39/B/ST4/03220.
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P38

Designing, developing, and evaluating protein-peptide binding systems for biophysical instrument standardisation

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Tetratricopeptide repeat (TPR) domains are repeating helix-turn-helix motifs that pack together to form a concave surface that can mediate protein-protein and protein-peptide interactions. As part of MOSBRI's effort to develop well-behaved protein systems that can be used as standard samples for method training and instrument evaluation, we have created several TPR domain-based interaction pairs.

Initially, six naturally occurring TPR domains that bind the c-terminal peptide of Hsp90 were produced, and their structure, stability and interaction with the peptide assessed using circular dichroism, dynamic light scattering and isothermal titration calorimetry. The two domains with the highest binding affinity (derived from hPP5 and hSTIP1) were selected for subsequent protein engineering with the aim of improving protein stability and consistency of the solution behaviour. Two design-build-test cycles were carried out creating variants with 2-16 amino acid substitutions from their parent in each cycle.

This has resulted in homogeneous monomeric TPR domain constructs with melting temperatures increased by 20-40°C, and having a wide range of dissociation constants (20 nM to 10 µM). We demonstrate that these TPR domains and the c-terminal sequence are suitable for use in constructing multi-domain fusion proteins that interact in a predictable manner.

P39

Resonant effects in polymer translocation under transversal fields

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Polymer translocation has long been a topic of interest in the field of biological physics given its relevance in both biological (protein and DNA/RNA translocation through membranes) and technological processes (nanopore DNA sequencing, drug delivery) [1,2].
We simulate the translocation of a semiflexible homopolymer through an extended pore, driven by both a constant and a time-dependent end-pulled force, employing a model introduced in previous studies [3]. We investigate the effects of this periodic driving on the translocation times. We find a large minimum region of the mean translocation times as function of the frequency of the force that is typical of the Resonant Activation effect [3], with key differences between the two considered driving regimes. This minimum is present independently of the physical characteristics of the chains and reveals a linear relation between the optimum translocation time and the corresponding period of the driving. We propose an explanation for this relation.

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[2] Zwolak & Di Ventra, Rev. Mod. Phys. 80, 141 (2008).
[3] Fiasconaro et al. Phys. Rev. E 91, 022113 (2015).

P40

MEPSA and MEPSAnd. Minimum Energy Pathway Analysis for energy landscapes over 3-dimensional and n-dimensional surfaces

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Construction of n-dimensional free energy surfaces is now a common technique in computational biophysics, physical chemistry, and materials science. However, interpreting the resulting data can be challenging, as it often requires identifying the minimum energy path connecting two regions of the surface. Our group has developed two open source (GPLv3) tools to simplify the analysis of free energy surfaces from a transition state perspective: MEPSA and MEPSAnd.

MEPSA, introduced in 2015, was the first software to apply a Dijkstra-like algorithm through a user-friendly graphical user interface (GUI) for efficiently identifying minimum energy paths over 3D free energy surfaces (defined by two collective variables and free energy). MEPSAnd, released in 2019, builds on this approach, extending it to surfaces with an arbitrary number of dimensions by abstracting any given surface as graph (regardless of its dimensionality).

MEPSAnd offers numerous advantages over its predecessor. It can handle n-dimensional surfaces, partially sampled or not, automatically provide alternative paths, and help represent complex n-dimensional surfaces and computed paths with graph plots.

In conclusion, MEPSAnd is a powerful and highly flexible GUI-based tool for researchers working with free energy surfaces of any kind.

P41

Assessment of protein stability on cryo EM-Grids *via* DLS

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Standard microbatch plates are used for automated scanning DLS. The sample droplets are kept under paraffin oil to prevent evaporation. In this setup it is possible to perform routine DLS measurements with droplet volumes down to 100 nL. Such small sample volumes can cope with the variety of conditions while ensuring maximum sample efficiency in a formulation process. More recently, routine DLS measurements can be performed by placing a sample on a cryo-EM grid using the same hardware as for automated scanning DLS. This on-grid DLS is the very last analytical step in the single-particle 3D cryo-EM sample preparation process where information can be obtained from a sample prior to freezing. The DLS grid itself isn't used for cryo-EM. It is used as a representative check of the sample's behaviour when it comes into contact with the grid surface. Such on-grid DLS measurements are also carried out under paraffin oil. The response of a sample after contact with a cryo-EM grid is often remarkable. These investigations are made possible by the unique optical arrangement of the DLS optics in the SpectroLight 600, which underlines the versatility of the hardware. This method will help to increase the success rate of future cryo-EM approaches.

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A sample efficient sample qualification method. automated scanning *in plate* DLS

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In plate Dynamic Light Scattering (*in plate* DLS) [1, 2] is a convenient tool for protein size determination and the basis for automated scanning for protein buffer response analysis. DLS itself is a standard non-invasive method that provides information about the particle size and, in particular, the aggregation state of biological macromolecules. The monodispersity of such samples is a prerequisite for almost all subsequent structure determination methods, e.g., crystallisation, cryo-electron microscopy (cryo-EM), small-angle X-ray scattering (SAXS) and nuclear magnetic resonance (NMR). After purification, biological macromolecules, i.e., proteins, often exhibit a degree of aggregation. This can be due to an unsuitable buffer, partially denatured proteins or incomplete folding. With automated scanning DLS, the response of a protein to a large number of different buffers or other additives can be rapidly investigated, even with very small sample volumes (< 0.5 µl). One of the features of DLS is that it is non-invasive, which means that measurements can be repeated virtually indefinitely. Once a formulation has been found, *in plate* DLS can be used to parameterise the formulation in terms of some fundamental criteria, such as maximum concentration, storage time, homogeneity, optimum storage temperature, temperature and chemical stability.

[1] SpectroLight 600 an *in situ* DLS system, see www.xtal-concepts.de
[2] Birch et al. Methods 2018, 147:150-162.

P43

Dissecting complex binding schemes using isothermal titration calorimetry: protein-metal and protein-protein interactions

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Isothermal titration calorimetry (ITC) is a widely used technique to characterize biomolecular equilibria. Often, analysis of the binding isotherms is performed with predefined binding schemes. However, for binding processes involving multiple species concomitantly interacting or competing for the same sites, custom binding models should be used to properly fit the data and obtain a complete picture of the system under analysis. This is the case of metal binding proteins, whose interactions are influenced by metal ions.
In the present work, two multi-component complexes are described. In the first case, the Ni(II)-binding properties of HypA and UreE2, two metallochaperones essential for the maturation of Helicobacter pylori urease, as well as of HypA•UreE2 complex, were investigated using a global fitting strategy. This approach demonstrated that the HypA•UreE2 interaction forms a novel Ni(II)-binding site with a sub-nanomolar affinity, not present in the isolated proteins1. In the second example, the Ca(II)-dependent ability of calmodulin to bridge the circadian clock regulator cryptochrome (dCRY) and the INAD protein of Drosophila melanogaster is demonstrated2. In both cases, ITC was integrated with biochemical and biophysical techniques, such as site-directed mutagenesis, co-immunoprecipitation, NMR, molecular modelling, which provided a complete picture of the system under analysis.

1. Zambelli et al. Metallomics, 2023, doi: 10.1093/mtomcs/mfad003
2 Mazzotta et al. Front Mol Neurosci 2018, doi: 10.3389/fnmol.2018.00280

P44

Phosphorylation and lipid binding of the intrinsically disordered region of NDRG1, a possible target for lung cancer therapy

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NDRG1 (N-myc downstream regulated gene) is a human protein involved in cell growth and differentiation, lipid biosynthesis, stress responses, cancer development among other functions. It contains a N-terminal α/β hydrolase domain and a C-terminal, 83 residues long, intrinsically disordered region (IDR, NDRG1*C).[1] The latter is characterized by a three-times repeated sequence of ten residues, binds nickel[2] and lipids, and is functionally regulated by phosphorylation. Previous functional and cellular studies demonstrated that this IDR is required for NDRG1 function in cell and in vivo.[3]In the present work, the effects of NDRG1*C phosphorylation and lipid binding on protein folding and interactions are investigated through biophysical techniques. The polypeptide was expressed and purified from *Escherichia coli* both in the unmodified and phosphorylated form. Experiments of isothermal titration calorimetry, light scattering and circular dichroism were carried out to establish the impact of post-translational modification on its metal-binding activity, as well as secondary and quaternary structure. Preliminary NMR data on the phosphorylated protein indicated the position of the phosphorylated residues and confirmed that the protein remains disordered in the phosphorylated form. Moreover, the interaction of NDRG1*C with lipids was followed by FT-IR spectroscopy. This work beneficed of TNA support from MOSBRI.

1 Mustonen V. et al., FEBS J. 2021, doi: 10.1111/febs.15660.
2 Beniamino Y. et al., Biomolecules 2023, doi: 10.3390/biom12091272
3 You G. et al., Cells 2022, doi: 10.3390/cells11091581

P45

Multifaceted effects of small molecule inhibitors on the aggregation mechanism of Huntingtin Exon 1

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Huntington's disease (HD) is a neurodegenerative disorder caused by the expansion of the polyglutamine (polyQ) segment in the exon 1 of the huntingtin (HttEx1) protein. This expansion of the polyQ domain leads to protein misfolding and the formation of β-sheet-rich fibrillar aggregates. The major challenge is to understand the mechanism of amyloid formation and their toxicity. Here, we study how a polyphenol modulates the HttEx1 aggregation mechanism at sub-stoichiometric ratios. A notable delay of protein aggregation was observed even at sub-stoichiometric ratios of curcumin relative to the HttEx1 protein. Mechanistically, extension of the lag phase suggests an impact on the nucleation process. Fibrils formed in presence of curcumin were also less toxic to cultured neuronal cells. The reduced-toxicity fibrils featured specific structural and morphological differences. Here, we propose a molecular model for the impact of the inhibitor on the aggregation mechanism, impacting the nucleation process and redirecting it toward a distinct aggregated state with modified toxic properties. This integrated multidisciplinary approach provides a novel perspective on the ability of small molecule inhibitors to modulate the misfolding landscape of huntingtin exon 1, with potential implications for future treatment strategies.

P46

Mitochondrial membrane fusion using Martini and Alphafold

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Mitochondria are organelles found in most eukaryotic cells that play a critical role in producing energy for the cell. Through the formation of dynamic fusion and fusion networks, they are able to regulate cellular processes such as apoptosis and the regulation of cellular energy levels. Mitochondrial fusion occurs when two or more mitochondria join together to form a single, larger mitochondrion. This process is mediated by a group of membrane proteins including mitofusin 1 and 2 (Mfn1 and Mfn2) and optic atrophy 1 (OPA1). Although the proteins involved in this process are mainly identified, the underlying mechanism of mitochondrial fusion are not completely understood.
In this work we propose the use of Alphafold [1] and MARTINI [2] as tools to unveil the mitochondrial fusion process as pictured by molecular dynamic simulations. Essential information will be obtained from the protein-protein interactions and their remodeling effect on the embedding lipid bilayers. Also, specific lipid-protein interactions are demonstrated to be fundamental for triggering the fusion process.

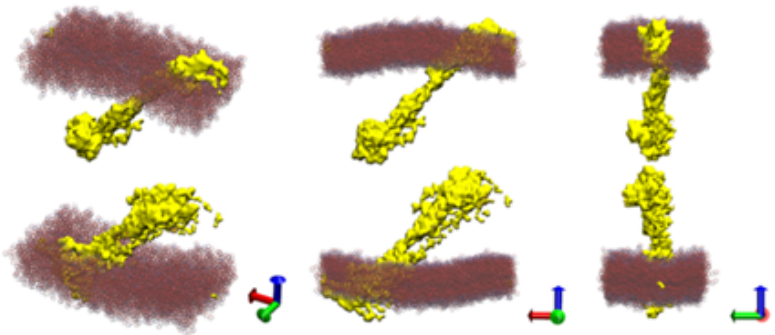

Figure 1. Molecular Dynamics simulation (using MARTINI3 framework) of human Mitofusin 1.

This work is being financially supported by the Comunidad de Madrid and Universidad Complutense through the PR-27/21 program (PR-27/21-030 reference).

[1] Jumper et al. Nature, 2021, 596, 583-589.
[2] Siewert et al. J Phys Chem B, 2007, 111, 27, 7812-7824.

P47

Alkaline phosphatase-hydrogel platform: from enzymatic characterization to biotechnological applications

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Our study was conducted on incorporating and characterizing the alkaline phosphatase (ALP) enzyme into a three-dimensional polymeric network through an eco-friendly protocol to produce transparent polyacrylamide hydrogels. Various methods for immobilizing ALP in the hydrogel were explored, and the properties of the entrapped enzyme were compared with those in solution. The conformation and stability were analyzed by monitoring changes in the protein intrinsic fluorescence as a function of temperature, to investigate the unfolding/folding process within the hydrogel, which is closely related to enzymatic activity. Our findings revealed that the immobilized enzyme retained its activity and exhibited slightly enhanced thermal stability. Furthermore, it could be stored at room temperature as a xerogel without losing its properties. The xerogel could be conveniently handled and divided into small, readily transportable fragments that were swollen in a substrate solution to conduct colorimetric enzyme assays. As a demonstration, we assessed the device's ability to rapidly and affordably detect ALP inhibitors, such as phosphate ion. Given the ALP@AETA xerogel's excellent stability and ALP's versatility in various fields, including environmental protection and therapeutics, we believe that this research is highly valuable in promoting the development of new devices for protein delivery and sensing.

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P48

MurG membrane-bound proteins do domains

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Bacterial membranes have distinct components and are exposed to different environments, such as temperature variations, compared to eukaryotic cells. Microdomains of increased fluidity, known as Regions of Increased Fluidity (RIFs), have been observed in bacterial membranes and may act as platforms for the assembly of proteins. However, the exact mechanisms governing the organization of proteins in bacterial membranes are still not fully understood [1,2]. It is possible that protein assembly may not always rely on lipid segregation or pre-existing lipid domains, and other factors, such as electrostatic interactions between proteins, protein concentration, and lateral pressure, may be important.
This study used High-Speed Atomic Force Microscopy (HS-AFM) [3] to investigate the partitioning of MurG glycosyltransferases from Gram positive and Gram-negative bacteria into phospholipid bilayers. Surprisingly, the MurG proteins were found to partition the membrane without requiring any preliminary lipid-induced partitioning. This study reveals important information about the membrane organisation of MurG proteins in phospholipid bilayers, providing insight into how these proteins help structure the bacterial membrane.

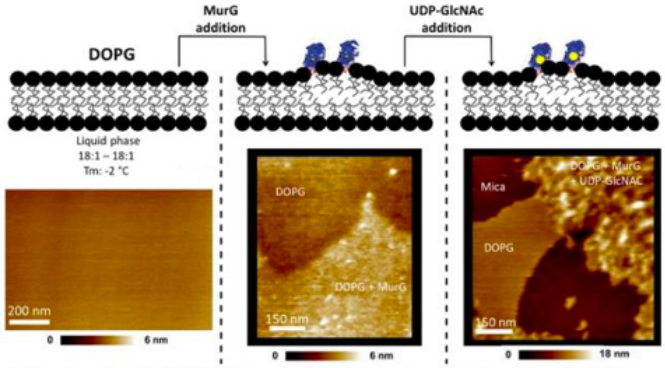

Fig. 1: **MurG creates partitioning in DOPG bilayers**. Representative image of DOPG supported bilayer before (left), after addition of MurG protein (middle) and after addition of MurG 's substrate: UDP-GlcNAc (right).

[1] Jiang et al. Commun Biol 2, 316 (2019).
[2] Carey et al. *Biophys Rev* 14, 111-143 (2022).
[3] Zuttion et al. *Nat Commun* 11, 6312 (2020).

P49

Structural insights into xanthine oxidoreductase chain formation

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Xanthine oxidoreductase (XOR) is a molybdoflavin enzyme which occurs in two forms; xanthine dehydrogenase (XDH, EC 1.17.1.4) and xanthine oxidase (XO, EC 1.17.3.2). In humans, it is a 293 kDa homodimer which catalyses consecutive hydroxylation steps of purine degradation. XO produces hydrogen peroxide and superoxide, both of which are reactive oxygen species (ROS) which can interact with various biomolecules producing adverse reactions. XOR can also produce nitric oxide, a cardiovascular protective molecule. XOR-produced ROS may provide protection against infection, while at the same time can also lead to inflammation, oncogenesis, brain injury and stroke. Consequently, presence of XOR in blood can be used as an indication of disease and can act as a biomarker for a number of conditions including oxidative stress and cardiovascular disease.

Recent findings in our lab showed that XOR can also form chains at different conditions, particularly at physiological conditions. This is interesting as the dimer and chain form might have different enzymatic activities, implying that chain formation is involved in activity regulation. XOR chain formation is an intriguing route to investigate since XOR was shown to bind and concentrate on endothelial membranes, leading to an elevation of ROS levels in blood.

P50

Pancreatic lipase in non-conventional media: deep eutectic solvents and hydrogels

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Water is the natural environment for biomolecules. However, the incorporation of cells or proteins in non-natural media offers interesting advantages for the development of new biotechnological applications. Among these non-conventional media are supercritical fluids, ionic liquids, deep eutectic solvents (DES) or confined environments, where biomolecules are retained in nanosized pores. Understanding the structure, thermal stability and function of biomolecules in these environments is essential to optimize their potential applications. Focusing on the latter two environments: DES and confined media, specifically acrylamide-based hydrogels, in this work the fluorescence properties of the enzyme pancreatic lipase were studied, and its activity was explored by colorimetric assays. The results show that, at room temperature, the protein conformation as well as its activity is preserved once dissolved in either of these media. However, while the thermal unfolding of the protein is similar in water and inside the hydrogel, it follows a complex behavior in the DES and the most remarkable finding is that at high temperatures there is a kinetic stabilization, so that the protein is not completely denatured. In addition, the enzyme partially refolds when it returns to room temperature, which does not occur in aqueous solution.

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P51

Unraveling antibacterial mode of action: The power of OMICs and protein solubility alteration

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The issue of antimicrobial resistance poses a severe threat to the global community, causing an estimated 5 million deaths each year. It is imperative to develop new antibiotics that target "Priority pathogens," such as Helicobacter pylori, and improve socioeconomic indicators, international cooperation, vaccines, and diagnostics. While the need to identify the mode of action may be debated, comprehending drug mechanisms at the molecular level can significantly benefit future drug development. Recent advancements in OMICs techniques can offer insights into drug-induced changes in cellular processes. However, filtering and integrating large-scale omics data for bacterial systems remain a significant challenge. We have used a long-standing knowledge that, when heated, proteins denature and generally become insoluble and interaction with small molecule can induce changes in solubility. The Proteome Integral Solubility Alteration method was employed in this study to filter differential expression data from proteomics and transcriptomics to better understand drug targets and off-target interactions. The study aimed to identify the most affected pathways and validate them through functional assays to obtain a comprehensive understanding of the cellular response to drugs.

P52

Improving Langmuir monolayer studies of anticancer drug interactions using real cell membranes and Molecular Dynamics simulations

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In anticancer drug developments, it is crucial to characterize the interactions between the anticancer drugs and cell membranes. Efforts have focused on mimicking cell membranes using lipidic Langmuir films. Although this provides valuable information at a molecular level, the film and real membrane compositions slightly differ, and data at the atomic level is limited.
Here, stable Langmuir films were created using real membranes extracted from human breast adenocarcinoma cells (line MCF-7). This novel strategy maintains all the components of the membrane and rearranges them in a half membrane. Doxorubicin anticancer drug was then introduced into the subphase while recording the changes in surface pressure to observe its effect on the membrane films. Also, the morphology was analyzed using microBAM and AFM imaging. In addition, atomic scale information about the interactions was obtained using Molecular Dynamics simulations. Healthy and tumor cell membranes were modeled with different lipid monolayers on a water slab, and curcumin was parametrized and added as an anticancer agent.
All these results were compared to those obtained in Langmuir experiments of lipidic monolayers equivalent to the ones used in simulations, where the presence of curcumin caused a greater destabilization of the tumor model membrane.

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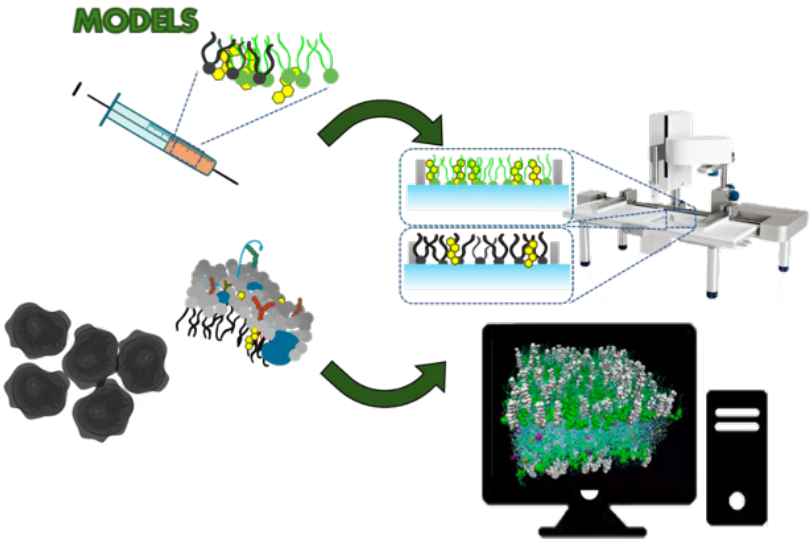
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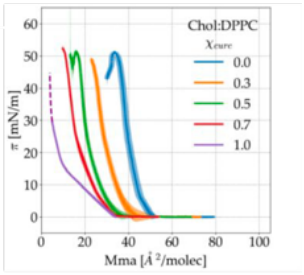
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χ_{Chol}
0.0
0.3
0.5
0.7
1.0
π [mN/m]
Mma [Å²/molec]
0
10
20
30
40
50
60
0
20
40
60
80
100

P53

Control of phenylalanine blood levels in *PKU* patients

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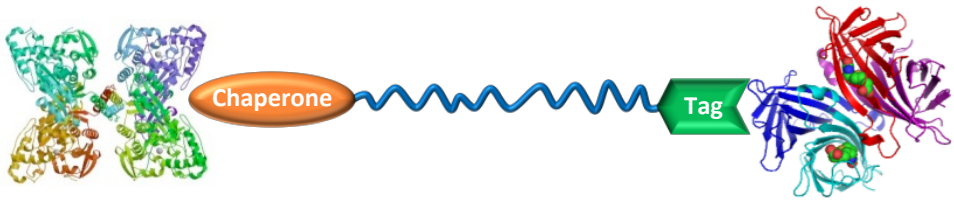
Human phenylalanine hydroxylase (PAH) is a metabolic enzyme that catalyzes the L-phenylalanine (Phe) hydroxylation to L-tyrosine in liver. Decreased enzymatic activity in PAH lead to a rise Phe blood levels which end in a rare metabolic disease that it is called *phenylketonuria* (PKU). [a,b] Furthermore, this increase of Phe in blood result toxic to the brain due to it cause mental retardation and psychological problems. To avoid these problems, an early diagnosis with a neonatal screening in newborns by heel prick within a few hours of birth and following a low phenylalanine diet are essential to avoid developing serious physiological problems. At this moment, the control of Phe levels in blood is carry out by fluorescence spectroscopy, HPLC and mass spectrometry which are expensive and lengthy analytical techniques. In this work, we design a colorimetric PKU familiar test to control in patients the Phe levels in blood at home. For this purpose, we focus on the competition between the amino acid and pharmacological chaperones by binding to the PAH active site. With this aim, we developed a "*sandwich complex*" based on a bifunctional molecule which can interact at the same time with PAH and a tag-binding protein, which allows the control of Phe levels.



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P54

The DNA 3'-end resection by archaeal Cdc45

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Eukaryotic Cdc45 is key factor for the formation of the CMG (**C**dc45-**M**CM-**G**ins) macrocomplex during DNA replication. Despite it does not show nuclease activity, it is evolutionary related to RecJ exonucleases (RecJs). Bacterial RecJs catalyse resection of DNA 5'-ends breaking the DNA-bond on the 3´-O of the sugar, they do not show Cdc45-like behaviour and their role has been recently suggested in Base Excision Repair (BER). Conversely, Archaeal RecJs show a broader panorama working in both or either 5'→3' and 3'→5' directions and some of them are also involved in the assembly of the RMG (**R**ecJ-**M**CM-**G**ins). At present no structural information at the basis of the DNA 3'-end resection are available so that the role of Archaeal Cdc45 at the replication fork remains a mystery. Here we present a complete biochemical characterization of the RecJ from *Methanothermobacter thermoautotroficum*, a unidirectional Mn^{2+} dependant 3'-end exonuclease ancestor of Cdc45 (hereafter Mth Cdc45) that acts both on DNA and RNA. By combining X-Ray crystallography and Molecular Dynamics (MD) simulations, we show that Mth Cdc45 exists in the opened and closed states and that they can shift from one to the other in a *screwing-pincher like* dynamics during the enzymatic function. The high-resolution structures in complex with dCMP and ssDNA ligands and the simulated catalytic mechanism provide the first structural evidences of the DNA 3'-end resection.

P55

Design and synthesis of FMN derivatives for covalent binding to variants of *Anabaena* apoflavodoxin

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Flavoproteins are electron-transfer proteins, involved in vital metabolic transformations, which have great biotechnological relevance and play essential roles in small organisms and humans. More specifically, flavodoxins, a kind of flavoproteins, contain a non-covalently bound flavin mononucleotide molecule as redox component.
Flavodoxins are subject to dissociation equilibrium and, depending on the solution conditions, the cofactor can dissociate, which can lead to destabilization of the protein and, as a result, irreversible loss of its catalytic activity. In order to obtain a rational increase of flavoproteins´ conformational stability, we have been working on getting variants of the Anabaena flavodoxin bearing covalently bound catalytically active FMN. For that purpose we have designed and synthesized some FMN derivatives which contain electrophilic groups.
We have performed a computational study to confirm which compounds are suitable to establish these covalent links with new flavodoxin variants, which have also been designed, expressed and purified, maintaining the native orientation of the isoalloxazine moiety relative to the apoprotein.
We have carried out experiments to find the best conditions to achieve such covalent binding of FMN derivatives through nucleophilic residues strategically located near the active center; and currently, with the aim of obtaining better results, new FMN-derivatives are being synthesized.

P56

Protein stabilization: industrial and biomedical use

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For studying the function and the processes in which a protein is involved, it is usually desirable to determine its stability. This is of utmost importance when these proteins are going to be used for industrial or biomedical applications. Among all the different ways of measuring stability, we will focus on thermal denaturation (thermostability) in order to characterize proteins.
Furthermore, proteins have the ability to maintain their function even if they are modified in certain regions. Thanks to this feature, it is possible to increase protein stability (e.g., thermal stability) without changing their function by using site directed mutagenesis. This means that by changing some residues the protein could be more stable at higher temperatures. This concept is used by Protposer, a server that proposes stabilizing mutations for any protein sent to it as a PDB file.
Using this server, different stabilizing mutations have been predicted for 6 proteins of industrial and biomedical interest. The best proposed mutations have been clustered into different mutants and introduced by site-directed mutagenesis. Finally, we expect to obtain mutants with higher thermostability and, therefore, with higher usability.

P57

Redox regulation of Kv7 channels through EF3 hand of calmodulin

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Neuronal KV7 channels, important regulators of cell excitability, are among the most sensitive proteins to reactive oxygen species. The S2S3 linker of the voltage sensor was reported as a site mediating redox modulation of the channels. Recent structural insights reveal potential interactions between this linker and the Ca2+ -binding loop of the third EF-hand of calmodulin (CaM). We found that precluding Ca2+ binding to the EF3 hand, but not to EF1, EF2 or EF4 hands, abolishes oxidation-induced enhancement of KV7.4 currents. Monitoring FRET we observed that S2S3 peptides cause a reversal of the signal in the presence of Ca2+, but have no effect in the absence of this cation or if the peptide is oxidized. The capacity of loading EF3 with Ca2+ is essential for this reversal of the FRET signal, whereas the consequences of obliterating Ca2+ binding to EF1, EF2 or EF4 are negligible. Furthermore, we show that EF3 is necessary and sufficient to translate Ca2+ signals to reorient the channel. Our data is consistent with the proposal that oxidation of cysteine residues in the S2S3 loop relieves KV7 channels from a constitutive inhibition imposed by interactions between the EF3 hand of CaM which is sufficient for this signaling.

P58

A biophysical approach to study an orphan disease: the case of CblC, a rare disorder of vitamin B12 intracellular metabolism

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The CblC disease is an inborn disorder of the vitamin B12 (cobalamin, Cbl) metabolism and the affected children manifest devastating symptoms involving vision, growth, and learning. The illness is caused by mutations in the gene codifying for MMACHC, a protein that transports and transforms the different Cbl forms. Although the crystal structure of the wild-type (WT) protein is available, a systematic study on the effect of each specific mutation on the resulting protein is still lacking.
Here we present data on the biophysical characterization of WT MMACHC, and two variants resulting from pathological mutations found in CblC patients. By using a biophysical approach including spectroscopy, Light and Small X-Ray Angle Scattering, Molecular Dynamics, we investigated protein structure/stability and ability to bind and transform Cbl. Moreover, we evaluated whether non-specific stabilizers (osmolytes) could restore functionality in MMACHC mutants.
Overall, our results reveal how a biophysical approach can offer new insights in the study of CblC mutations' specific effect and help prospecting new routes for the CblC treatment.

P59

Molecular interactions and forces that make proteins to fold: A complete energetic picture of protein stability from MD simulations

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In a previous work we demonstrated that enthalpy and heat capacity changes associated with protein folding reactions can be calculated, within experimental error, using molecular dynamics (MD) simulations of native protein structures and their corresponding unfolded ensembles. By extensively analyzing MD simulations of four model proteins (CI2, barnase, SNase, and apoflavodoxin), we dissected the energy contributions to protein stability made by the molecular players (polypeptide and solvent molecules) and the physical interactions involved (electrostatic, van der Waals, and bonded interactions) in their folding reaction. We observed that the folding energetics of these proteins follow the same quantitative pattern despite their differences in length, isoelectric point, and fold class. This pattern indicates that compared to the unfolded ensemble, the native conformations are enthalpically stabilized by comparable contributions from protein-protein and solvent-solvent interactions, and they are almost equally destabilized by interactions between protein and solvent molecules. On the other side, in terms of physical interactions, the native conformations are stabilized by van der Waals and Coulomb interactions, while destabilized by conformational strain resulting from bonded interactions. The sign of the heat capacity change is determined by interactions between protein and solvent molecules or, alternatively, by Coulomb interactions, across the four proteins analyzed. If a proper breakdown and treatment of the protein energetics have been done by the simulation program and the force field used in this work, our modeling offer, for the first time, a comprehensive quantitative depiction of protein stability. These findings have the potential to enhance our ability to exert rational control in protein design endeavors.

P60

Decrypting the role of AIF dimerization in AIF:CHCHD4 interaction and mitochondrial homeostasis

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The human apoptosis-inducing factor (AIF) is a flavoenzyme that exerts an important role in mitochondrial redox metabolism through its interaction with the chaperone CHCHD4 (coiled-coil-helix-coiled-coil-helix-domain containing 4)[1] in the mitochondrial intermembrane space (IMS). While CHCHD4 controls the importation and oxidative folding of subunits from respiratory complexes, AIF would act by regulating its proper localization[1-2]. AIF-CHCHD4 interaction has been described as NADH-dependent, being favored by reduced and presumably dimeric AIF[1]. In IMS, AIF exists in a monomer-dimer equilibrium shifted towards dimer by NADH oxidation, stabilization of a long-life $FADH^-/NAD^-$ charge transfer complex (CTC) and conformational reorganization[3].

We investigate the AIF's dimerization role in its physiological interaction with CHCHD4 and its contribution in mitochondrial AIF's NADH oxidase activity. For that, we performed the biophysical characterization of two mutants: the H454A variant, which affects the AIF's active site, leading to a dimer conformation in absence of NADH[4]; and the E413A/R422A/R430A variant - unable to stabilize the dimer in the presence of NADH[3]. To analyze the contribution of mutations in AIF:CHCHD4 interaction, we evaluated their effects in AIF redox activity, structural stability/conformation, CTC stabilization, and CHCHD4 interaction. These studies are key to understand the molecular basis of AIF redox activity in healthy cells.

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P61

Highly accessible OpenSPR System for kinetic analysis of molecular interactions

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P62

Myhre syndrome SMAD4 variants stabilize complex formation with other SMAD transcription factors

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SMAD4 is a key transcription factor in TGF-beta signaling and its alteration affects the normal functioning of many biological processes. Reports in the bibliography have identified potential gain-of-function mutations in the SMAD4 protein, in addition to the more common loss-of-function mutations found in cancer and other diseases. The reported gain-of-function variants, R496C and I500M/T/V, are associated with Myhre (MyS) and Werner syndrome (WS). These are two rare diseases associated either with defects in embryonic development and body maturation (MyS) or producing an accelerated aging phenotype (WS). Patients with MyS also have autistic-like behavior, skeletal abnormalities and changes in the structure and function of the heart. Using single-molecule biophysics and structural biology, we have shown how R496C and I500M/T/V confer an enhanced ability to interact with R-SMADs, increasing their affinity and complex half-life. We acknowledge the MOSBRI Transnational Access (TNA) grant (MOSBRI_2022_97) for the characterisation of SMAD4 mutations using SPR, mass photometry, nanoDSF and DLS/SLS at the ProLinC facility at Linkoping University (Linkoping, Sweden) and SPC facility at the EMBL Hamburg (Hamburg, Germany).

P63

Using the capacity of combined multi-detection coupled with Size Exclusion Chromatography and advanced analysis to gain macromolecules-structural information in solution. A joint research activity in the MOSBRI infrastructure

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Size exclusion chromatography is a well-established technique, and over the last 20 years, different online detectors have been developed to gather information on the separated macromolecules. The most popular detectors that have been associated with this technique are UV spectrophotometers, refractometers, static light scattering detectors, and, to a lesser extent, viscometers and dynamic light scattering detectors. The main objective of the manufacturers developing light scattering detectors, in association with UV and refractive index detectors, was to build an instrument that can measure the concentration and molar mass of the macromolecules present in the sample. Additional information such as hydrodynamic radius, and intrinsic viscosity of macromolecules has been obtained by incorporating viscometers and dynamic light scattering. However, there has been no integrated analysis of the different measured variables to decipher more information about the macromolecules, such as hydration, size, shape, or even ab-initio modeling.
During this joint research activity, we are combining all the previously mentioned detectors into a single instrument. Simultaneously, we are developing an analysis process that will enable us to obtain information on mass, concentration, hydration, shape, and size in a single measurement. Furthermore, we will develop an integrated ab-initio modeling process that will allow us to generate a shape envelope of the hydrated molecule. An example using BSA will be presented.

P64

Understanding the role of the hexameric conformation in the bacterial transcriptional factor RcsB

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The transcriptional factor RcsB is a response regulator protein conserved in the Enterobacteriacea family. It forms part of the Rcs system which senses cell envelope stress to achieve adaptation and survival that comprises the outer membrane sensor protein RcsF coupled to the inner membrane sensor protein IgaA, which is also coupled with the inner membrane phosphorelay proteins RcsD and RcsC[1]. Upon the activation of the phosphorelay proteins, RcsB becomes phosphorylated acquiring the activated conformation to regulate gene transcription affecting different functions such as repression of motility and production of an extracellular capsule. However, non-phosphorylated RcsB also shows gene transcription regulation. In the last years, thanks to our previous structural work we were able to obtain a dimerization conformation stabilized by phosphorylation that was able to bind a specific DNA box in a base readout mode[2,3]. However, we could also trap an alternative dimeric conformation of RcsB, that formed hexamers just in the crystals, pointing to a possible conformational dynamism involved in function[2]. Now, we have produced a mutational analysis in RcsB that has allowed us to stabilize the hexameric conformation, according to SEC-MALS analysis, which seems to bind DNA in an alternative mode taking into account base and shape readout.

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P65

Phasor-FLIM for a direct investigation of Transportan 10 interactions with model membranes

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Transportan 10 (TP10), a short and positive charged peptide, belonging to the family of the cell penetrating peptides has gained increasing attention for its antimicrobial and anticancer activity but also for its applications in drug delivery as it is able to translocate therapeutic molecules in cellular environment. Due to the complexity of the phenomena involved in cellular uptake and following processes, which strongly depend on the membrane lipid composition, structural details of the peptide (e.g., charge, hydrophobicity, steric hindrance) and environmental conditions, it is not easy to understand the general rules governing them. Here, we combine spectroscopic techniques and fluorescence lifetime imaging microscopy (FLIM) to investigate (i) the fate of the TP10 in the presence of model membranes, analyzing its conformational changes occurring at membrane interface and distinguishing peptide adsorption from insertion into the lipid bilayer (ii) the changes of the fluidity of the membrane and the formation of pores into the latter induced by TP10 interaction. In addition, thanks to the use of the environment sensitive fluorescence dyes, Laurdan and di-4-ANEPPDHQ, and of the phasor approach to analyze FLIM data, we were able to monitor in real time fine events at different depths of phospholipid bilayers.

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Characterization of Therapeutic Antibodies using Digital SPR

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Alto is the world's first SPR instrument to integrate digital microfluidics (DMF) with nanotechnology-based biosensors. With simultaneous detection across 16-channels, Alto provides high-throughput analysis of up to 48 unique targets, while further streamlining user workflows with automated sample dilutions, disposable fluidics, and sample requirements reduced by up to 200X. In collaboration with Sino Biological, a global leader in research reagents, we demonstrate how Alto is an ideal platform for therapeutics development through crude library screening, binding kinetics characterization, and epitope binning of Sino Biological's influenza products.

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Drug discovery for inhibiting *Bacteroides fragilis* toxin isoform 3: from a chemical screening to an animal model

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Bacteroides fragilis is identified as the leading anaerobe in bloodstream infections and intra-abdominal abscesses. Several studies have demonstrated that enterotoxigenic strains (ETBF) of *B. fragilis* may arise and elicit diarrhea, anaerobic bacteremia, inflammatory bowel disease, and colorectal cancer. ETBF's only recognized specific virulence factor is a zinc-dependent metallopeptidase called *B. fragilis* toxin (BFT), which damages the intestinal mucosa and triggers disease-related signaling mechanisms.

We have focused on one of the naturally occurring BFT isoforms, BFT-3, and managed to repurpose several approved drugs as BFT-3 inhibitors through a combination of biophysical, biochemical, structural, and cellular techniques. In contrast to canonical inhibitors, which target the active site of mature enzymes, these effectors bind to a distal allosteric site in the proBFT-3 zymogen structure, which stabilizes a partially unstructured, zinc-free enzyme conformation by shifting a zinc dependent disorder-to-order equilibrium. This yields proBTF-3 incompetent for autoactivation, thus ablating hydrolytic activity of the mature toxin. Once inhibitors against BFT-3 have been identified *in vitro*, bacterial growth inhibition assays have been carried out with strains of *B. fragilis*, as well as studies in an infection animal model (*Galleria mellonella* larvae), confirming that these compounds have an inhibitory effect in *in vivo* models limiting ETBF pathogenicity.

LIST OF AUTHORS

T Invited talk
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