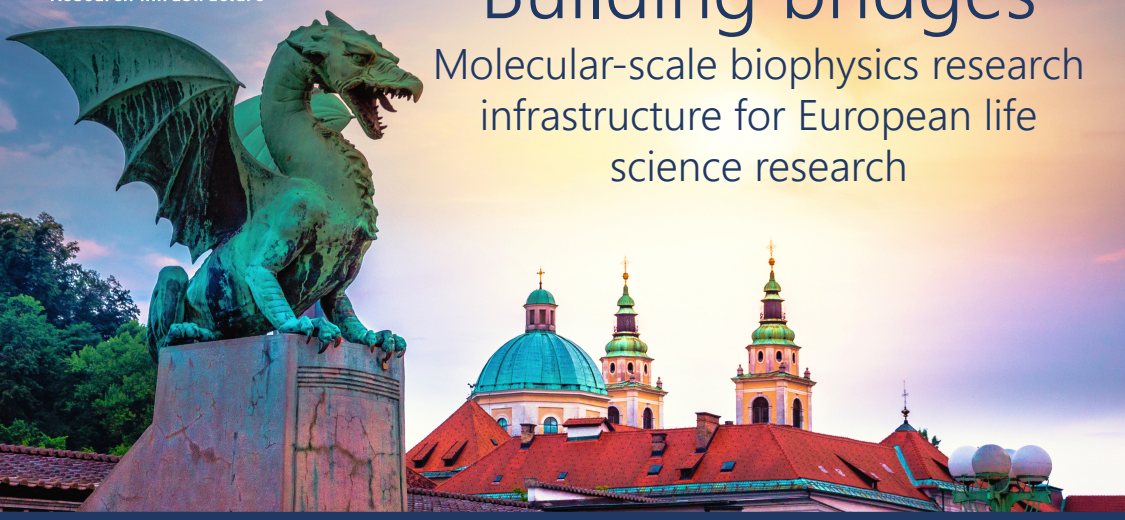
MOSBRI
Molecular-Scale Biophysics Research Infrastructure

Building bridges

Molecular-scale biophysics research infrastructure for European life science research



3rd MOSBRI Scientific Conference

10th - 13th June 2024, Ljubljana, Slovenia

Programme & Abstracts

NATIONAL INSTITUTE OF CHEMISTRY

www.mosbri.eu

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Posters

Abstracts

Included in the electronic version.

Visit the conference website

3rd MOSBRI Scientific Conference Programme & Abstracts
Ljubljana, 10th – 13th June 2024
Book of abstracts

Organizer: National Institute of Chemistry, Ljubljana, Slovenia.
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(updated 17/6-24 to reflect the actual programme)

WELCOME

Molecular-scale biophysics focuses on the architecture, dynamics and interactions of the molecules of life: proteins, DNA, RNA, polysaccharides, lipids, by measuring their physical properties. It is fundamental to our understanding of how living organisms function, and for our ability to manipulate the function of these molecules, notably for therapeutic purposes. **MOSBRI** aims to address the urgent need for a truly integrated and multi-method approach to modern biophysics. To this end, **MOSBRI** conferences are central to the exchange of ideas and technological approaches by experts in various fields of biophysics.

The third **MOSBRI** conference aims to bring together a larger community of researchers from the fields of structural biology, biochemistry, synthetic biology and biophysics. The conference will show how an interdisciplinary and integrated network can tackle an unusually wide variety of life science research questions. It will include plenary lectures, presentations by invited renowned experts, **MOSBRI** partners, short oral presentations, company presentations, as well as presentations by scientists who have already benefited from the free trans-national access (TNA) opportunities that **MOSBRI** provides. We will make a special effort to facilitate presentations by early career scientists.

The conference thematically follows on from the first two conferences, but is complemented by Emerging Approaches in Biophysics (co-organised by the Association of resources for Biophysical Research in Europe, ARBRE), Integrative Structural Biology (organised by Instruct-ERIC) and Molecular Bioimaging (organised by the Slovenian Biophysical Society).

We welcome you to Ljubljana, to spend a few days discussing exciting topics connected to biophysical research!

Prof. dr Gregor Anderluh
Chair of the scientific committee

Dr Ana Crnković
Chair of the organizing committee

Scientific Organizing Committee

Gregor Anderluh, chair
Francesca Cutruzzolà
Kristina Djinovic Carugo
Jan Dohnálek
Patrick England
Søren Vrønning Hoffmann
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Mirijam Kozorog
Aljoša Marinko
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Organizing Institution

National Institute of Chemistry
Hajdrihova 19, p.p. 660
SI-1001 Ljubljana
Slovenia

MOSBRI
Molecular-Scale Biophysics Research Infrastructure

NATIONAL INSTITUTE OF CHEMISTRY

SESSION TOPICS

The topics of the sessions at the conference are:

Macromolecular interactions, kinetics and dynamics

Emerging approaches in biophysics – co-organised by the Association of Resources for Biophysical Research in Europe, ARBRE

Integrative structural biology – organised by Instruct Eric

Computational biophysics and artificial intelligence applications

Molecular bioimaging – organised by the Slovenian Biophysical Society

Macromolecular design, stability and quality control

PRIZES

At the **MOSBRI** 2024 conference, prizes will be awarded for the three best posters and the three best oral presentations, as well as two prizes for the best presentation of research conducted as part of **MOSBRI** TNA.

Each prize is endowed with €200 and are supported by ARBRE, Applied Photophysics, PicoQuant and MOSBRI. The prizes awarded by ARBRE also include an annual ARBRE membership for the year 2024.

ARBRE
Association of Resources for Biophysical Research in Europe

AppliedPhotophysics

MOSBRI
Molecular-Scale Biophysics Research Infrastructure

PicoQuant

SPONSORS

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REDSHIFTBio
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Bronze Sponsors

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delivered by VWR™

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Other Sponsors

ARBRE
Association of Resources for Biophysical Research in Europe

CHEMASS
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Ljubljana Tourism

NATIONAL INSTITUTE OF CHEMISTRY

omega

PicoQuant

Sanolabor

SLOVENSKO BIOKEMIJSKO DRUŠTVO

Links to sponsor websites can be found on the MOSBRI conference website.

LIST OF SPEAKERS

Plenary speakers

Roman Jerala, National Institute of Chemistry, SL
Lynne J. Regan, University of Edinburgh, UK
Harald Schwalbe, Johann Wolfgang Goethe-University, DE; Instruct-ERIC
Andrej Šali, University of California at San Francisco, USA

Invited speakers

Maria Garcia Alai, EMBL Hamburg, DE
Claudio Canale, University of Genova, IT
Isabella Caterina Felli, University of Florence, IT
Stefano Gianni, Sapienza University Rome, IT
Sara Garcia Linares, Complutense University, ES
Giovanni Maglia, University of Groningen, NL
Vitalii Mudryi, Max Planck Institute for Multidsciplinary Studies, Göttingen, DE
Laura Orellana, Karolinska Institutet, SE
Marjetka Podobnik, National Institute of Chemistry, SL
Mathias Rief, Technical University München, DE
Wouter Roos, University of Groningen, NL
Juan Sabin, AFFINImeter, ES
Bohdan Schneider, Institute of Biotechnology of Czech Academy of Sciences, CZ
Eva Sevcsik, Technical University Vienna, AT
Erdinc Sezgin, Karolinska Institutet, SE
Maria Sunnerhagen, Linkoping University, SE
Sharon Wolf, Weizmann Institute, IL

MOSBRI TNA beneficiary talks

Ten **MOSBRI** TNA beneficiaries will present their projects at the **MOSBRI** 2024 conference in Ljubljana.

Federico Ballabio, Universitá di Milano, IT
Hugo Fraga, FMUP/I3S, PT
Ana Carvalho, NOVA School of Science and Technology, PT
Toni Kurt Träger, Martin-Luther-University Halle-Wittenberg, DE
Salvatore Adinolfi, University of Torino, IT
Maria Raquel Pacheco, NOVA School of Science and Technology, PT
Matthew Walker, University of Glasgow, UK
Paolo Di Gianvincenzo, CIC biomaGUNE, ES
Karim Ben Ali Gacem, I2BC CEA Saclay, FR
Wouter Versantvoort, Heinrich-Heine-Universität Düsseldorf, DE/Radboud University, NL

Company presentations

The following companies will be giving a presentation at the conference.

Aberrior Instruments
Applied Photophysics
Bruker
Calneos
Cytiva Europe
Depixus
Dynamic Biosensors
I&L Biosystems
Lek Farmacevtska družba
Nanotemper Technologies
Nicoya Lifesciences
Refeyn
ThermoFisher
Waters, Wyatt Technology

Oral presentations

There are another 20 presentations, which were chosen based on submitted abstracts.

CONFERENCE VENUE

The venue of the conference is the Grand Union Hotel.

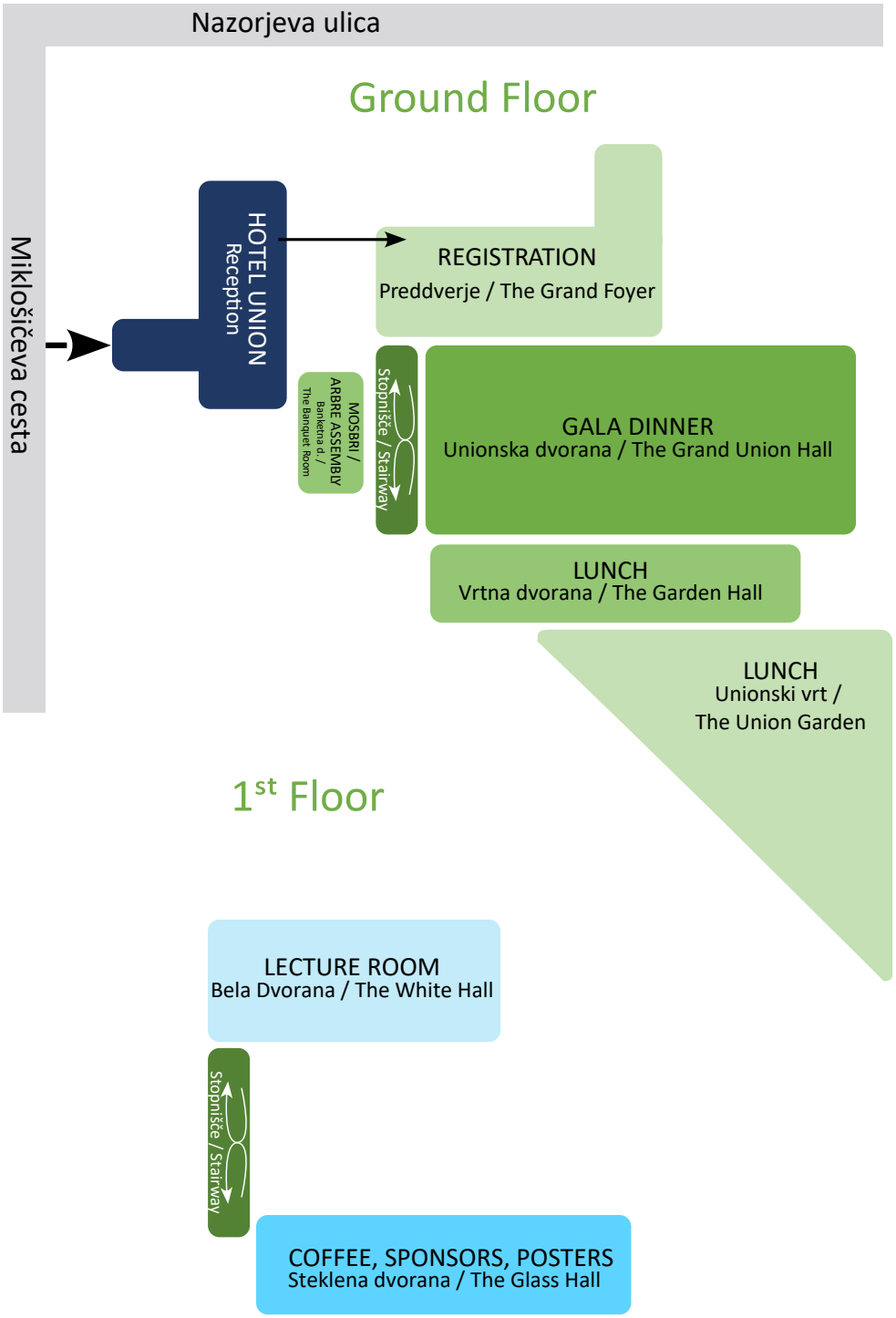
Miklošičeva cesta 1
1000 Ljubljana,
Slovenia

Scientific sessions will take place in the White Hall on the 1st floor of the hotel.

EXHIBITORS

The following companies have stands at the **MOSBRI** 2024 conference. The exhibition takes place in the Glass Hall on the 1st floor. The location of the hall is indicated in the drawing on the opposite page.

Aberrior Instruments
Applied Photophysics
Bruker
Calneos
Cytiva Europe
Depixus
Dynamic Biosensors
I&L Biosystems
Lek Farmacevtska družba
Nanotemper Technologies
Nicoya Lifesciences
Refeyn
ThermoFisher
Waters, Wyatt Technology


Nazorjeva ulica
Miklošičeva cesta
Ground Floor
HOTEL UNION
Reception
REGISTRATION
Preddverje / The Grand Foyer
MOSBRI / ARBRE ASSEMBLY
Banketna d. / The Banquet Room
Stopnišče / Stairway
GALA DINNER
Unionska dvorana / The Grand Union Hall
LUNCH
Vrtna dvorana / The Garden Hall
LUNCH
Unionski vrt / The Union Garden
1st Floor
LECTURE ROOM
Bela Dvorana / The White Hall
Stopnišče / Stairway
COFFEE, SPONSORS, POSTERS
Steklena dvorana / The Glass Hall

Welcome reception

A welcome reception will be held with the poster session on Monday the 10th of June in the Glass hall.

Lunch and coffee breaks

Lunch will take place in the Garden Hall on the ground floor. Coffee breaks will be on the 1st floor.

Poster sessions

There will be two poster sessions during the conference, these will be held in the Glass Hall on the 1st floor. Posters should be put up on the assigned board (numbers are indicated in the list of posters) before the first poster session and can remain there for the duration of the conference. All posters should be removed before the end of the conference.
Presenters with odd-numbered posters should be by their posters during the session on Monday and even-numbered posters on Tuesday (all posters can remain up for both days).

Wi-Fi access

There will be free wi-fi access available throughout the conference, details of which will be given to participants at the conference.

Badges

The badges given to participants during registration should be worn at all times.

Conference dinner

The conference dinner will take place at 19:30 on the 12th of June in the Grand Union Hall on the ground floor of the conference venue.

Questions?

Contact **MOSBRI2024@mosbri.eu.**

Social activities

A guided tour of Ljubljana Botanical Gardens has been arranged to take place at 19:00 on Tuesday 11th of June.

The University Botanic Gardens, Ljubljana, is the oldest Slovene cultural, scientific and educational institution. It was founded in 1810, the time of the Illyrian Provinces, as a garden of native flora and a section of the Central School (École Centrale). It was planned by Franc Hladnik, who was also its first director. It is a certified botanical garden by the Botanic Gardens Conservation International and is also a recipient of the Conservation practitioner certificate. In its collection, it has over 5,700 different plant species from different parts of the world. It also has the largest seed bank of natural plant species in Slovenia and one of the largest seed banks in Central Eastern Europe. There are currently 18,517 units in it. Its main activity is in-situ and ex-situ protection of plant species. It is also a member of the European Botanic Gardens Consortium ENSCONET seed bank network.

Anyone interested in joining the tour should sign up via email to:
MOSBRI2024@mosbri.eu
No ticket is needed and the tour is free.

Participants should meet at the Gardens at 19:00 (location 25 on the map on the next page); it is a ~25 minute walk from the conference venue.

MAP OF THE AREA

National Institut of Chemistry, Hajdrihova ulica 19, SI - 1000 Ljubljana
46° 2' 33" N, 14° 29' 37" E

PUBLIC PLACES AND BUILDINGS

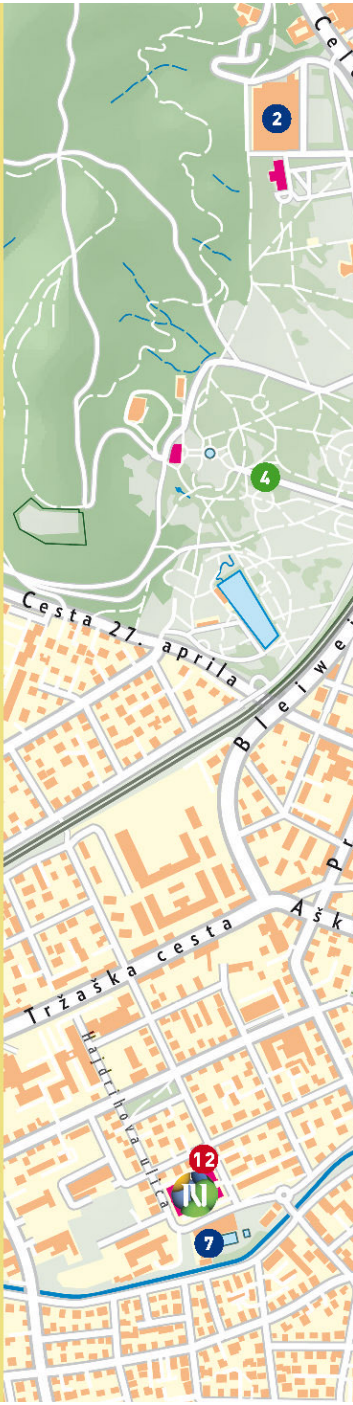
1. Exhibition and Convention Centre
2. Tivoli Hall
3. Bus and Railway Station
4. Tivoli Swimming Pool
5. Ilirija Swimming Pool
6. University Medical Center - Emergency block
7. Kolezija Swimming Pool

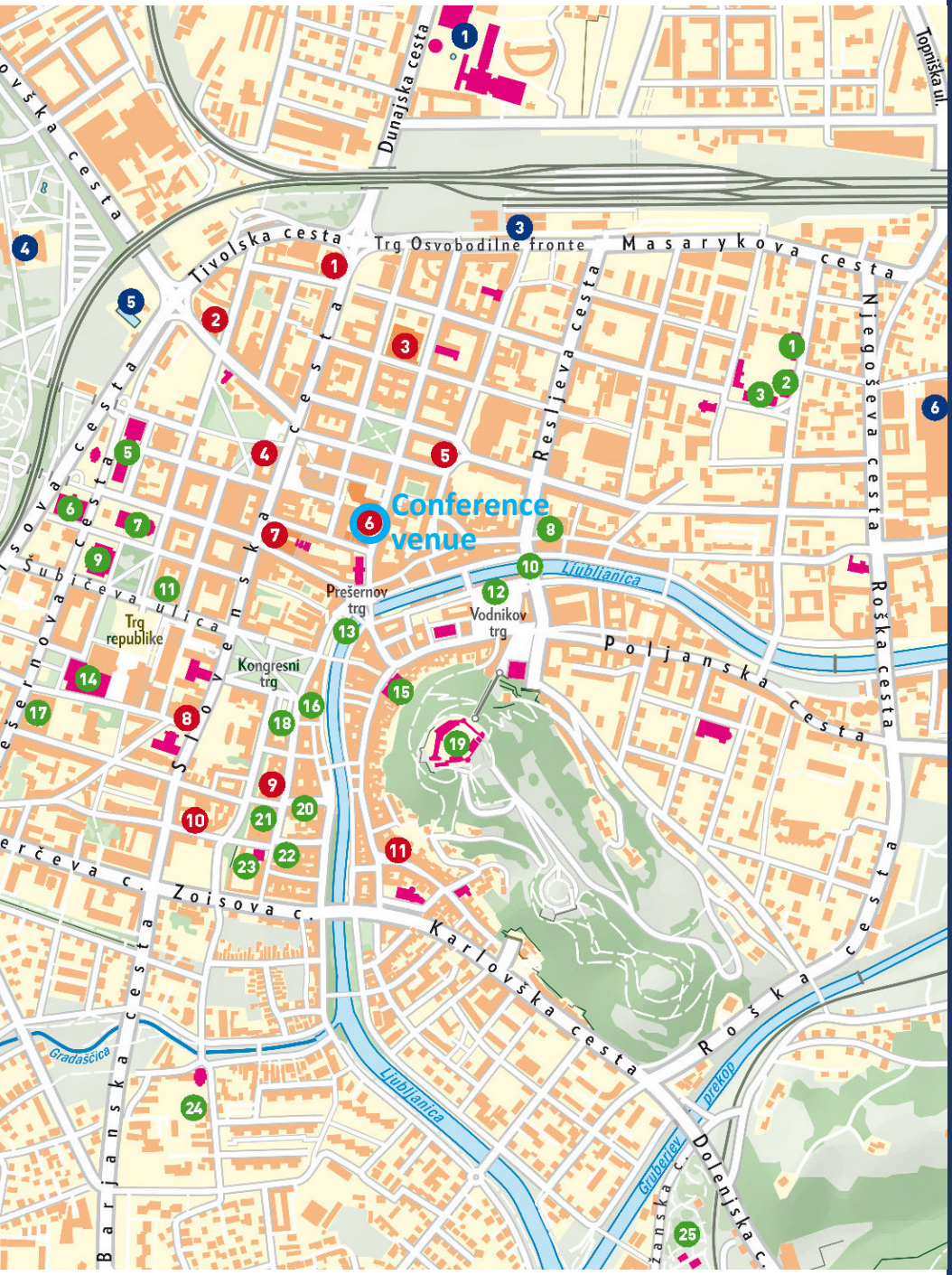
LANDMARKS, MUSEUMS AND IMPORTANT BUILDINGS

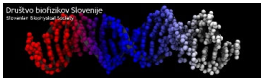
1. Museum of Contemporary Art
2. National Museum of Slovenia
3. Slovene Ethnographic Museum
4. Tivoli Park
5. National Gallery
6. Museum of Modern Art
7. Opera and Ballet
8. House of Experiments
9. National Museum of Slovenia and Slovenian Museum of Natural History
10. Dragon Bridge
11. Parliament
12. Central Market
13. Triple Bridge
14. Cultural and Congress Centre
15. Town Hall and Old Ljubljana
16. Slovenian Philharmonic
17. Presidential Palace
18. University of Ljubljana
19. Ljubljana Castle
20. Slovenian Academy of Sciences and Arts
21. National and University Library
22. City Museum of Ljubljana
23. Križanke Outdoor Theatre
24. Plečnik House
25. Botanical Garden

HOTELS

1. InterContinental Ljubljana
2. Hotel Lev
3. Central Hotel
4. Urban Hotel
5. City Hotel Ljubljana
6. Grand Hotel Union & uHOTEL
7. Best Western Premier Hotel Slon
8. Hotel Cubo
9. Antiq Palace
10. Hotel Mrak
11. Hotel Galleria
12. Vila Teslova




Conference venue
Dunajska cesta
Tivolska cesta
Trg Osvobodilne fronte
Masarykova cesta
Resljeva cesta
Njegoševa cesta
Topniška ul.
Slovenska cesta
Šubičeva ulica
Trg republike
Kongresni trg
Prešernov trg
Vodnikov trg
Ljubljanica
Poljanska cesta
Roška cesta
Zoisova c.
Karlovska cesta
Barjanska cesta
Gradaščica
prekop
Gruberjev
Dolenjska c.
Žanska c.

Monday 10.06.2024	Tuesday 11.06.2024	Wednesday 12.06.2024	Thursday 13.06.2024
	Plenary lecture Macromolecular interactions, kinetics and dynamics	Plenary lecture Integrative Structural Biology	Emerging approaches in biophysics ARBRE Association of Resources for Biophysical Research in Europe
	Coffee break	Coffee break	Coffee break
	MOSBRI Molecular-Scale Biophysics Research Infrastructure	instruct ERIC	MOSBRI Molecular-Scale Biophysics Research Infrastructure
			Closing plenary lecture
			Close of the meeting
Coffee	Lunch	Lunch	Lunch
Opening of the meeting Opening plenary lecture Macromolecular design, stability and quality control	Computational biophysics and artificial intelligence applications	Molecular Bioimaging 	
Coffee break	Coffee break	Coffee break	
MOSBRI Molecular-Scale Biophysics Research Infrastructure	MOSBRI Molecular-Scale Biophysics Research Infrastructure		
		MOSBRI general assembly (open to all)	
Poster session	Poster session		
Welcome reception	ARBRE general assembly	Conference dinner	

MONDAY 10TH JUNE 2024

13:00 Registration, welcome coffee

14:00 **Welcome address**

14:15 **Synthetic biology for the design of modular protein assemblies and mammalian cell regulation** — *Opening plenary* — T1
Roman Jerala, National Institute of Chemistry, SI *Chair: Gregor Anderluh*

Session 1: Macromolecular design, stability and quality control
Chairs: Gregor Anderluh, Javier Sancho

15:00 **Unveiling the molecular biophysics of the oncoprotein MYC and its interactions** — *Invited presentation* — T2
Maria Sunnerhagen, Linköping University, SE

15:25 **Characterization of new Dps-based protein structures by SRCD** — *TNA Beneficiary* — T3
Raquel Pacheco, NOVA School of Science and Technology, PT

15:40 **Understanding macromolecular structural features in solution by combining size exclusion chromatography, multi array detectors and integrated analysis** — *Oral presentation* — T4
Bertrand Raynal, Institut Pasteur, FR

15:55 Coffee break

16:25 **High-throughput light scattering as a powerful tool for molecular characterization, interactions, and quality control** — *Company* — T5
Malgorzata Łopaciuk, Waters, Wyatt Technology, DE

16:35 **Advancing biomolecular interaction analysis with GatorBio biolayer interferometry technology** — *Company* — T6
Jeroen Schoorl, I&L Biosystems, DE

16:45 **Novel strategy for mAb developability assessment: focus on the Fab with vibrational spectroscopy** — *TNA Beneficiary* — T7
Karim Ben Ali Gacem, I2BC CEA Saclay, FR

17:00 **Tracking the steps of a completely *de novo* designed random protein walker** — *Oral presentation* — T9
Ajasja Ljubetič, National Institute of Chemistry, SI

17:15 **Poly-affibodies and their binding to membrane receptors: synthesis and biophysical characterization** — *Oral presentation* — T10
Josef Hamacek, CNRS, FR

17:30 **Design of nanoreactors for plastic depolymerization based on pore-forming toxins** — *Invited presentation* — T11
Sara García-Linares, Complutense University of Madrid, ES

17:55 Poster / networking session and welcome reception (Glass Hall)

TUESDAY 11TH JUNE 2024 a.m.

08:00 Welcome coffee

08:30 **Live Paint – a versatile new method for super-resolution imaging in live cells** — *Plenary* — **T12**
Lynne Regan, University of Edinburgh, UK — *Chair: Iztok Urbančič*

Session 2: Macromolecular interactions, kinetics and dynamics
Chairs: Maria Sunnerhagen, Serena Rinaldo

09:15 **The ABC of oligomannose-6 binding to FimH *E. coli* adhesin** — *Oral presentation* — **T13**
Julie Bouckaert, UGSF, UMR 8576 CNRS and University of Lille, FR

09:30 **NLPs from fungi *Moniliopthora perniciosa* and oomycete *Pythium aphanidermatum* display pore-forming activity on GIPC-rich membranes** | Nika Žibrat, National Institute of Chemistry, SI — *Oral presentation* — **T14**

09:45 **Exploring the role of the N-terminal tails dynamics of a Dps protein nanocage on plasmid DNA binding** — *TNA Beneficiary* — **T15**
Ana J. Carvalho, NOVA School of Science and Technology, PT

10:00 Coffee break

10:30 **Comprehensive characterization of molecular interactions from small molecules to cells using switchSENSE® and RT-IC** — *Company* — **T17**
Stéphane Pinhal, Dynamic Biosensors, DE

10:40 **Kinetic mechanism and deteminants of EF-P recruitment to translating ribosomes** — *Invited presentation* — **T18**
Vitalii Mudryi, Max Planck Institute for Multidisciplinary Sciences, DE

11:05 **Impact of redox alterations on IDO1 inhibitors binding properties** — *Oral presentation* — **T19**
Elisa Bianconi, University of Perugia, IT

11:20 **Biochemical and biophysical characterization of *Mycobacterium tuberculosis* SseA, a thiosulfate:cyanide sulfurtransferase and its interaction network** | Salvatore Adinolfi, University of Torino, IT — *TNA Beneficiary* — **T20**

11:35 **Monolith X: a tool for molecular-scale biophysical measurements** — *Company* — **T21**
Quentin Canelle, Nanotemper Technologies, DE

11:45 **Folding of multi-domain proteins – folding intermediates, hidden kinetic traps and cryptic functional features** — *Invited presentation* — **T22**
Stefano Gianni, Sapienza University of Rome, IT

12:10 Lunch

TUESDAY 11TH JUNE 2024 p.m.

Session 3: Computational biophysics and artificial intelligence applications
Chairs: Jan Dohnálek, Jan Stransky

14:00 **Computational biophysics meets medicine – from biomolecular simulations to animal models** — *Invited presentation* **T23**
Laura Orellana, Karolinska Institutet, SE

14:25 **Annotation and validation of nucleic acid structures: increasing the quality and interoperability of the data** — *Invited presentation* **T24**
Bohdan Schneider, Institute of Biotechnology of the Czech Academy of Sciences, Czech Republic

14:50 **Accurate and efficient SAXS/SANS implementation including solvation layer effects suitable for molecular simulations** — *TNA Beneficiary* **T25**
Federico Ballabio, Universitá di Milano, IT

15:05 **Probing protein-induced local membrane deformation: a small-angle scattering and computational study** — *Oral presentation* **T26**
Enrico Federico Semeraro, University of Graz, AT

15:20 Coffee break

15:50 **Identification of ClpC1 inhibitors using an in silico approach** — *TNA Beneficiary* **T27**
Hugo Fraga, FMUP/I3S, PT

16:05 **Orange and Quasar: interactive data exploration for spectroscopy** — *Oral presentation* **T28**
Marko Toplak, University of Ljubljana, SI

16:20 **Molecular Biophysics Database of raw data – MBDB** — *Oral Presentation* **T29**
Jan Dohnálek, Institute of Biotechnology of the Czech Academy of Sciences, CZ

16:35 **Computational tools to get the most out of biophysical data** — *Invited presentation* **T30**
Juan Sabín, AFFINImeter, ES

17:00 Poster/networking session (until 19:00 in the Glass Hall)

17:30: ARBRE Assembly until 19:00. Open to all.
(The Banquet Room, ground floor)

WEDNESDAY 12TH JUNE 2024 a.m.

08:00 Welcome coffee

08:30 **Instruct-ERIC- pan-European research infrastructure for structural biology. Examples for integrated structural biology studies** | Harald Schwalbe, Goethe-University, DE — *Plenary* — T31

Chair: Kristina Djinovic Carugo

Session 4: Integrative structural biology
Chairs: Kristina Djinovic Carugo, Marjetka Podobnik

09:15 **Intrinsically disordered proteins studied by NMR spectroscopy: insights from ^{13}C direct detection**
Isabella Felli, University of Florence, IT — *Invited presentation* — T32

09:40 **Progress in the integrative analysis of the pyruvate dehydrogenase complex metabolon**
Toni Träger, Martin-Luther-University Halle-Wittenberg, DE — *TNA Beneficiary* — T33

09:55 **Spectroscopic insights into the mechanism of hydrazine synthase**
Wouter Versantvoort, Heinrich-Heine-Universität Düsseldorf, DE — *TNA Beneficiary* — T34

10:10 **Orthogonal spectroscopy-systems for the analysis of protein stability and structural changes**
Markus Epe, Applied Photophysics, UK — *Company* — T35

10:20 Coffee break

10:50 **Deciphering the intricate hierarchy: subtleties in clathrin heavy chain binding boxes provide selectivity among adaptor proteins of budding yeast** | Maria Garcia Alai, EMBL, DE — *Invited presentation* — T36

11:15 **A closer look at interactions between actinoporins and lipids: insights through Cryo-EM and molecular dynamics simulations**
Gašper Šolinc, National institute of Chemistry, SI — *Oral presentation* — T37

11:30 **Integrative structural biology to decipher the mechanism of host cell intoxication by the CyaA toxin**
Alexandre Chenal, Pasteur Institute, FR — *Oral presentation* — T38

11:45 **Integrative structural biology on cytokine-receptor assemblies for fundamental and translational science**
Savvas Savvides, Ghent University, BE — *Oral presentation* — T39

12:00 **Pushing the boundaries of cryo-EM throughput and resolution at 200kV**
Itziar Serna Martin, ThermoFisher, NL — *Company* — T40

12:10 **Cryo-STEM tomography: a bridge between molecular and cellular morphologies**
Sharon Wolf, Weizmann Institute of Science, IL — *Invited presentation* — T41

12:35 Lunch

WEDNESDAY 12TH JUNE 2024 p.m.

Session 5: Molecular bioimaging
Chairs: Iztok Urbančič, Eva Sevcsik

14:00 **Small variations in protein structure induce selectivity in the aggregation process of insulin**
Claudio Canale, University of Genoa, IT
Invited presentation T42

14:25 **Alterations in unfolding of FRET-labelled fibronectin in the extracellular matrix of NIH/3T3 fibroblasts and MDA-MD-231 breast cancer cells** | Matthew Walker, University of Glasgow, UK
TNA Beneficiary T43

14:40 **Microscopes and antarctic fish: how developing high-resolution microscopy for 0°C imaging sheds a new life on cold-adapted systems** | Anne-Pia Marty, University of Cambridge, UK
Oral presentation T44

14:55 **Measuring biophysical properties of cells and nanoscale bioparticles in health and disease**
Erdinc Sezgin, Karolinska Institutet, SE
Invited presentation T45

15:20 **Spatial requirements for T-cell receptor triggering probed via a DNA origami-based biointerface**
Eva Sevcsik, Technischen Universität Wien, AT
Invited presentation T46

15:45 Coffee break

16:15 **Current trends and developments of biophysics in Africa and the actions organized by Society of Africa Biophysical Societies (SABS)** | Philip Amuyunzu Mang'are, Masinde Muliro University of Science and Technology, KE *Chair: Juan Sabin*
Networking oral presentation T47

Session 6: Company presentations
Chair: Kristina Eleršič Filipič

16:30 **MINFLUX tracking for real-time observation of biological processes at molecular resolution**
Ulf Matti, Aberrior Instruments, DE
Company T48

16:40 **The Triceratops SPR #64 – tap into a new dimension with next generation multiplexing SPR** | Cyrill Bruner, Bruker, CH
Company T49

16:50 **Alto & OpenSPR: Nicoya's label-free solutions for biologics research** | Dina Niculaes, Nicoya Lifesciences, CA
Company T50

17:00 **BiacoreTM 1 series – Next generation one needle SPR system from Cytiva** | Florian Koelle, Cytiva Europe, DE
Company T51

17:10 **Recent advances in biomolecular characterization by mass photometry** | Matthias Langhorst, Refeyn, DE
Company T52

17:20 **Sandoz Slovenia**
Tamara Cvijić, Lek Farmacevtska družba, SI
Company T53

17:30 **MOSBRI General Assembly** (The White Hall, 1st floor). Open to all.

19:30 **Congress Dinner** (The Grand Union Hall, ground floor)

THURSDAY 13TH JUNE 2024 a.m.

08:00 Welcome coffee

Session 7: Emerging approaches in biophysics
Chairs: Patrick England, Josef Houser

08:30 **Single-molecule nanopore proteomics: from enzyme dynamics to protein sequencing**
Giovanni Maglia, University of Groningen, NL
Invited presentation **T54**

08:55 **Preparation of non-covalent self-assembled nanoparticles for nanomedicine**
Paolo Di Gianvincenzo, CIC biomaGUNE, ES
TNA Beneficiary **T55**

09:10 **MAGNA™: a novel single molecule approach for the real-time analysis of biomolecular interactions using magnetic force spectroscopy**
Pascale Beurdeley, Depixus, FR
Company **T56**

09:20 **Forces in chaperone-mediated protein unfolding and cell adhesion**
Matthias Rief, Technische Universität München, DE
Invited presentation **T57**

09:45 **Infrared nanospectroscopy a new tool in biophysics**
Jehan Waeytens, Université Libre de Bruxelles, BE
Oral presentation **T58**

10:00 Coffee break

10:30 **Site-directed spin labelling and electron paramagnetic resonance for the study of the human flavoprotein Cytochrome P450 reductase**
Maxime Bizet, CNRS, FR
Oral presentation **T59**

10:45 **First step toward the development of an optical biosensor based on vibrational spectroscopy for the detection of airborne pathogens**
Tiziana Mancini, University of Rome La Sapienza, IT
Oral presentation **T60**

11:00 **Ultimate DSC, a new tool for protein characterization**
Yannice Ricci, Calneos, FR
Company **T61**

11:10 **Nanoscale dynamics of biomolecular interactions: from viruses to antibiotics**
Wouter Roos, University of Groningen, NL
Invited presentation **T62**

11:35 **From integrative structural biology to cell biology**
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Synthetic biology for the design of modular protein assemblies and mammalian cell regulation

Roman Jerala[1,2]

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The principles of synthetic biology can be used to engineer biological systems to design biomolecules and biological systems to achieve new interesting properties and understand the function of natural systems. Modularity can be applied to design protein architectures unknown in nature, as well as for the regulation of protein function and biological processes in mammalian cells. Coiled-coil (CC) modules have been used to design new protein folds, based on the assembly of the polypeptide into a polyhedral scaffold composed of CC modules. Recently we have determined the 3D structure of CCPO-based trigons and a tetrahedron. CC modular design facilitates the introduction of chemical regulation of the assembly. On the other hand, a combination of tunable weak interacting CC modules can be used to design liquid-liquid protein condensates from a single or several types of polypeptide chains and their regulation. The combination of CC-dimers with split proteases enabled the introduction of the rapid regulation of selected cellular processes in mammalian cells. Designable coiled-coil dimers have been used for allosteric regulation of several diverse proteins, called INSRTR, which is based on the insertion of a coiled-coil-forming peptide into the selected protein. The formation of a heterodimer disrupts the protein function or triggers its activation, in the case of the fused autoinhibitory CC segment. This platform enables the construction of ON/OFF protein switches, regulation by small molecules, and logic functions with rapid response in mammalian cells. INSRTR was demonstrated on more than 10 proteins with diverse functions including enzymes, signaling mediators, DNA binders/transcriptional regulators, fluorescent protein, and antibodies implemented as a sensing domain of anticancer chimeric antigen receptors on T cells, offering extraordinary potentials for regulation of biological systems and therapeutic applications.

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Unveiling the molecular biophysics of the oncoprotein MYC and its interactions

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The oncoprotein MYC is an intrinsically disordered protein (IDP) which in aggressive tumors becomes a transcriptional superactivator driving oncogenesis. Impairing MYC activity in model systems leads to complete tumor regression. In this talk, I will present work from my group on the conformational ensembles of this protein and its interactions. To further understand the dynamic aspects of MYC interactions, we use an integrated structural biology approach (NMR, crystallography, HDX, Bio-ID, biophysics, SAXS and SANS, molecular modelling) in close collaboration with cell biologists. Our aim is to shed light on how MYC regulates critical biological events through its disordered interactions with key target proteins, to identify cases where it is possible to pharmaceutically interfere.

Characterization of new Dps-based protein structures by SRCD

M. Raquel Pacheco[1], Tomás Calmeiro[2], Nykola C. Jones[3], Søren V. Hoffmann[3], Elvira Fortunato[2], Pedro Tavares[1], Alice S. Pereira[1]

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Life's intricate machinery hinges on complex biomolecules, with proteins taking center stage due to their remarkable versatility. The development of supramolecular protein structures allows us to mimic the natural ones offering exciting possibilities to expand and modulate protein functions, as well as to produce biomaterials with new characteristics and applications.

Ferritins are a family of almost spherical nanocages, widespread in nature, that comprises the DNA-binding proteins from starved cells (Dps) [1]. These protein nanocages, essential for prokaryotes survival, are made of 12 identical subunits and have an external diameter of ~9 nm and ~4.5 nm of internal diameter. They can store up to 500 iron atoms within their hollow cavity, playing a dual role of iron storage and reactive oxygen species detoxifier [1]. Ferritin's unique properties as a hollow, spherical nanocage with a biocompatible protein shell make them ideal building blocks for novel biomaterials. They can be engineered to create differentiated compartments that allow the incorporation of functional molecules. This compartmentalization enables the design of new biomaterial with tailored properties with enormous biotechnological potential.

Our laboratory has been developing high molecular weight Dps-based polymeric structures for the past few years [2]. These structures were synthesized through thiol-ene coupling, a class of click chemistry reactions, using small organic compounds, such as 1,2-bis(allyloxy) ethane, as linkers between the protein molecules. We utilize multiple techniques to gain a comprehensive understanding of their structure and properties. Besides polyacrylamide gel electrophoresis (SDS-PAGE and PAGE) and size exclusion chromatography (SEC), the structure and morphology of these biomaterials were achieved through atomic force microscopy (AFM). Synchrotron radiation circular dichroism spectroscopy (SRCD) provided invaluable information about the secondary structure and thermal stability of these new Dps-based biomaterials. A detailed analysis of the SRCD data will be presented.

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Understanding macromolecular structural features in solution by combining size exclusion chromatography, multi array detectors and integrated analysis

Bertrand Raynal[1], Maelenn Chevreuil[1], John Stenson[2], Natalia Markova[2], Patrick England[1]

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Size exclusion chromatography (SEC) is a well-established technique, and over the last 20 years, different online detectors have been developed to gather information on SEC-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 dynamic light scattering detectors and viscometers. However, there has been no integrated analysis of the different measured variables to decipher additional information about the macromolecules, such as hydration, size, shape, or even ab-initio modeling.

In the frame of 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 are developing an integrated ab-initio modeling algorithm that will allow us to generate a shape envelope of the hydrated molecule.
This approach can be applied to different type of macromolecules such as proteins, carbohydrates, polymers or DNA giving information on the arrangement of the molecule in solution and will soon be offered to the TNA applicants of the Pasteur-PFBMI MOSBRI site.

High-throughput light scattering as a powerful tool for molecular characterization, interactions, and quality control

Malgorzata Łopaciuk

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Light scattering measurement is a powerful tool in macromolecular research. Dynamic (DLS) and static light scattering (SLS) can be used not only for the quality control of macromolecules by determining their size, polydispersity, and molar mass.

Properly designed light scattering experiment may be also used to define e.g. thermal and colloidal stability, aggregation kinetics, or conformational changes of macromolecules of interest. As the conventional, cuvette-based DLS/SLS measurement is relatively slow and laborious, the use of these methods can be limited.

With a revolutionary DynaPro™ Plate Reader by Waters | Wyatt Technology, DLS/SLS measurements went at last into high-throughput. Now, e.g. aggregation characteristics, protein crystallization conditions, or formulation screening can be determined quickly and precisely.

Advancing biomolecular interaction analysis with GatorBio biolayer interferometry technology

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Biolayer Interferometry (BLI) has evolved as an essential technique for understanding biomolecular dynamics and interactions with high sensitivity and real-time kinetics. The new GatorBio BLI technology builds upon this foundation, providing a significant advancement in this domain, offering unprecedented precision, versatility, and efficiency.

During this presentation we will address the core principles and unique capabilities of the GatorBio BLI platform. We will delve into the multiple applications of this technology, spanning from gene therapy, AAV, nanoparticles, small molecule characterization, protein-protein interactions, epitope mapping, and more. Furthermore, we will explore recent innovations and improvements of the GatorBio BLI platform, highlighting its enhanced functionality and broadening its impact in various research fields.

Novel strategy for mAb developability assessment: focus on the Fab with vibrational spectroscopy

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Assessing the developability of therapeutic monoclonal antibodies (mAbs) is a critical early stage in drug discovery and development. This process involves assessing various properties of candidate mAbs to ensure their potential as successful therapeutic products. mAbs with poor stability or high immunogenicity may encounter developmental challenges and are less likely to succeed therapeutically. Developability studies typically incorporate a combination of *in vitro* and *in vivo* experiments, computational modeling, and analytical techniques to comprehensively assess the candidate mAb's properties. In this study, I studied novel biophysical approaches to evaluate mAb developability, focusing on the antigen-binding fragment (Fab), which is particularly susceptible to degradation. I compared data from multiple techniques including differential scanning fluorometry, dynamic light scattering, Raman, and FTIR spectroscopies. My results demonstrate that with minimal material (10mg), we can conduct a thorough developability study, highlighting stability fingerprints to characterize the effects of various stresses (thermal, pH, aging) and that vibrational approaches bring useful information. My work offers new valuable insights to optimize the success of mAbs through preclinical and clinical development stages.

Remarkable structural plasticity and tunability of the potyviral coat protein

T8

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This talk did not take place.

Tracking the steps of a completely *de novo* designed random protein walker

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Powered protein walkers such as kinesin, dynein or myosin are responsible for most movements within the cell and for the transport of crucial cargo. Deep learning methods have greatly increased experimental success rates for *de novo* design of single chain proteins[1]; however large dynamic protein mechanisms have not yet been designed. I will present the design and characterization of a random protein walker that can diffuse along micro-meter long fibers. This represents a scaffold for future powered molecular robots.

The requirements for such a system are threefold: a track, attachment points and a walker/roller scaffold. I will briefly present reversible heterodimers that I developed to serve as attachment points. These heterodimers behave well as monomers, have a range of affinities and fast binding/exchange kinetics in solution.

Next, I will show how I rigidly fused heterodimers and designed helical repeat (DHR) proteins to de-novo designed fibers to form a track for the walker. This turned out to be the hardest part of the project. I will present the experimental 3D structure of the fiber solved by Cryo-EM.

Finally, I will outline the different walker scaffolds I have designed and present several experimental 3D structures. The scaffolds have a varying number of feet which change their mobility. I will present trajectories obtained from single molecule TIRF microscopy experiments.

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Poly-affibodies and their binding to membrane receptors: synthesis and biophysical characterization

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Affibody molecules (AfB) are small three-helix bundle protein scaffolds (~ 7 kDa) acting as robust non-immunoglobulin affinity ligands capable of binding to a wide range of proteins [1]. The optimized AfBs can be thus used as efficient tools for molecular recognition in bioimaging, diagnostic and therapeutic applications. AfBs were initially developed for targeting the human epidermal growth factor membrane receptors (EGFR, HER2) that are often overexpressed in different carcinomas. AfBs attracted a considerable attention for their ability to bind these tumor markers with nanomolar affinities.

In this context, we have started to develop multivalent molecular platforms for combining several AfBs within the same macromolecule. These constructs exhibit several advantages including high affinity and avidity. In this work, the AfB conjugates (mono-AfB and two di-AfBs) were obtained through a covalent coupling via bispecific linkers based on flexible PEG chains.2 Advantageously, we have also incorporated a fluorescent motif into the anchoring linker to facilitate optical detection. The synthesized compounds were fully characterized with NMR, mass spectrometry and circular dichroism. In preliminary studies, the interactions of the linker with bovine serum albumin, DNA and RNA were studied and quantified. The binding affinities of AfB constructs to purified HER2 receptors were then determined with suitable biophysical techniques such as fluorescence anisotropy and biolayer interferometry. Flow cytometry was performed to validate the di-AfBs binding to surface receptors of HER2-overexpressing cells.

This work was supported by the Ligue contre le cancer and the CNRS.

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Design of nanoreactors for plastic depolymerization based on pore-forming toxins

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Petroleum-based plastics are durable and accumulate in all ecological niches. Approximately 80% of waste objects are made of macroplastics and most of them end up being dumped into the oceans [1]. PET (polyethylene terephthalate) is the main component in many synthetic fibers and water bottles, and it comprises at least 5% of the total identified plastic particles, accounting for 14.4% of total plastic waste [2]. Our work focuses on improving the biotechnological processes of PET degradation by designing more efficient enzymes [3]. For this purpose, biocatalytic nanopores are designed based on the homo-octamer of a sea anemone toxin that forms pores in biological membranes. This pore is assembled into nanodiscs, forming individual and water-soluble particles. A computational modeling approach based on protein structure is used, followed by a specific assembly method in the form of the aforementioned nanopores. At only 40 °C temperature and neutral pH, this biocatalytic nanoreactor efficiently hydrolyzes bis(2-hydroxyethyl)-terephthalate (BHET). It is also capable of degrading PET nanoparticles from a plastic bottle and other commercial versions of PET used to manufacture plastic products. The products of these reactions are soluble BHET dimer, BHET itself and mono-(2-hydroxyethyl)-terephthalic acid (MHET). The results obtained so far already constitute a proof-of-concept for building biodegradable pore-based catalytic nanoreactors that could drive new developments in nanobiotechnology, here exemplified by efficient systems to decompose PET at levels of the best-performing known engineering PETases [4] and, moreover, at relatively low temperatures.

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Live Paint – a versatile new method for super-resolution imaging in live cells

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Single molecule localization methods allow one to perform super-resolution fluorescence imaging below the diffraction limit. Many methods have been developed to enable such imagining. Here I present Live Paint – a method we have developed that can be used in living cells: I will show data from yeast and mammalian cells as exemplars. The method is versatile, easy to implement and widely applicable. In addition to not requiring fixing and cell-permeabilisation, this method also has the advantage of enabling multi-colour measurements, and facilitating long acquisition times, resulting in higher resolution data.

The *ABC* of oligomannose-6 binding to FimH *E. coli* adhesin

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The FimH type-1 fimbrial adhesin allows pathogenic *Escherichia coli* to adhere to glycoproteins in the epithelial linings of human bladder and intestinal tract, by using multiple fimbriae simultaneously. Pauci- and high-mannose type *N*-glycans are natural FimH receptors on those glycoproteins. Oligomannose-3 and -5 bind with the highest affinity to FimH by using the same Manα1,3Man branch [1]. Oligomannose-6 is generated from oligomannose-5 in the next step of the biogenesis of high-mannose *N*-glycans, by the transfer of a mannose in α1,2-linkage onto this branch. Using serial crystallography and by measuring the kinetics of binding, we demonstrate that shielding the high-affinity epitope drives the binding of multiple FimH molecules [2].

First, we profiled FimH glycan binding on a microarray containing paucimannosidic *N*-glycans and in a FimH LEctPROFILE® assay. To make the transition to oligomannose-6, we measured the kinetics of FimH binding using paucimannosidic *N*-glycans, glycoproteins and all four α-dimannosides conjugated to bovine serum albumin. Equimolar mixed interfaces of the dimannosides present in oligomannose-6 and molecular dynamics simulations suggest a positive cooperativity in the bivalent binding of Manα1,3Manα1 and Manα1,6Manα1 dimannosides.

The binding of core α1,6-fucosylated oligomannose-3 in the co-crystals of FimH is monovalent, but interestingly the GlcNAc1 – Fuc moiety retains highly flexibility. In co-crystals with oligomannose-6, two FimH bacterial adhesins bind the Manα1,3Manα1 and Manα1,6Manα1 endings of the second trimannose core (A-4'-B). This cooperative switch towards bivalent binding appears sustainable beyond a molar excess of oligomannose-6. Our findings provide important novel structural insights for the design of multivalent FimH antagonists that bind with positive cooperativity [3].

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NLPs from fungi *Moniliopthora perniciosa* and oomycete *Pythium aphanidermatum* display pore-forming activity on GIPC-rich membranes

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Necrosis- and ethylene-inducing 1-like proteins (NLPs) constitute a superfamily of proteins found in diverse phyla of plant-associated microorganisms. Numerous NLPs are cytotoxic and facilitate infections in a wide range of crops. The evidence of NLPs being toxic to both monocot and dicot plants is ambiguous [1-3]. The target for interaction of NLPs with the plant plasma membrane is a plant sphingolipid glycosyl inositol phosphoceramide (GIPC) [3]. Studies of NLP_{Pya} from oomycete *Phytium aphanidermatum* highlighted a unique mode of membrane damage. Upon binding, the protein oligomerizes on the membrane, which is followed by the formation of transient heterogeneous pores that are permeable to small molecules [4]. So far, other NLPs have not been studied for their plant plasma membrane binding and mechanism of damage and respective pore formation.

We studied MpNEP2, NLP from the fungus *Moniliopthora perniciosa*, for its pore-forming ability. A microfluidic system [5] was utilized to study the binding and probe release from GIPC-containing liposomes of both MpNEP2 and NLP_{Pya}. Either monocot or dicot GIPC were extracted from plant tissue and incorporated into giant unilamellar vesicles membrane. Like NLP_{Pya}, also MpNEP2 binds and forms pores on dicot-containing liposome membranes. Both NLPs exhibit pore-forming activity on dicots and also on monocot GIPC-containing liposomes. Kinetics of binding to the membrane and release of fluorescent probe from the vesicles indicate that both NLPs similarly affect liposomes. We suggest that NLPs can exhibit pore-forming activity on model lipid membranes containing lipids extracted from monocot or dicot plant membranes.

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Exploring the role of the N-terminal tails dynamics of a Dps protein nanocage on plasmid DNA binding

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Protein nanocages have recently gathered transdisciplinary research potential due to their wide array of structural and functional properties. These multimeric protein systems are found throughout all domains of Life and present several biological roles and technological capabilities. One of such proteins is Dps (DNA-binding proteins from starved cells), a member of the Ferritin family present only in prokaryotes. Dps present a cube-like structure of twelve monomers (consisting of four-helix bundles), with an outer diameter of approximately 10 nm and a total molecular mass varying between 220 and 250 kDa [1]. Besides the ferroxidation and iron management properties typical of Ferritin systems, these proteins also present the unique ability of DNA binding and protection, for which the structurally disordered N-terminal tail is a key component [2].

Dps protein from *Deinococcus grandis* (here designated DgrDps) distinguishes itself from its counterparts with an elongated N-terminal tail composed of ~ 50 residues (instead of the 10 to 20 residues found in most of the Dps homologues currently described), possessing a novel metal binding site located closely to the spherical cage of the protein. The N-terminal tails of the monomers can adopt different conformations, varying between a closed conformation (with the tails compacted against the protein core structure) and an extended, star-shaped conformation, with the N-terminal extensions fully exposed to the solvent. Incubation of the apo-DgrDps with various divalent metal ions, namely Zn^{2+}, shifts the extended conformation to the closed one due to the binding of the metal to the N-terminal metal binding site [3].

We are currently studying the formation of DgrDps-DNA complexes using supercoiled plasmid DNA (pUC19, around 2.7 kbp) through several biophysical techniques, aiming at understanding the impact of the conformational dynamics on the physiologic function of the protein.

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A combined microscale thermophoresis and capillary electrophoresis to study protein/small inhibitor interactions directly in cell lysates and in whole cells

T16

Solweig Chartier

This talk did not take place.

Comprehensive characterization of molecular interactions from small molecules to cells using switchSENSE® and RT-IC

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Understanding the dynamics of molecular interactions is pivotal in drug discovery, where precise kinetics dictate therapeutic efficacy. Real-time kinetic measurements with biophysical techniques not only unveil the intricacies of these interactions but also inform rational drug design, enabling the development of potent and selective therapeutics. This talk introduces two advanced technologies, switchSENSE® for the in-depth characterization of molecule-molecule interactions as well as Real-Time Interaction Cytometry (RT-IC) for the analysis of cell-molecule interactions.

switchSENSE® is an automated, fluorescence-based biosensor chip technology that employs electrically actuated DNA nanolevers for the real-time measurement of binding kinetics (k_{on}, k_{off}) and affinities (K_d). Interactions between proteins, DNA/RNA, and small molecules can be detected with femto-molar sensitivity. At the same time, conformational changes and complex binding events can be measured using minimal amounts of sample. We present two case studies using cutting-edge drug modalities. First, we investigate proteolysis-targeting chimeras (PROTACs), small heterobifunctional molecules designed to induce proteasomal degradation of target proteins. Utilizing a Y-shaped DNA nanostructure, we analyse binary and ternary complex formation of different PROTACs in high-throughput. Secondly, we investigate aptamers and demonstrate a screening of single base sequence mutations regarding their influence on small-molecule binding kinetics.

Besides these small molecule- or nucleic acid-based drug designs, biologics become increasingly relevant as therapeutic modality, for example therapeutic antibodies. They often target transmembrane proteins (e.g. PD-1, CD3, etc.) and binding kinetics are influenced by the target‘s density and mobility within the membrane, it‘s transmembrane domain folding or the presence of coreceptors. Molecular interactions of therapeutic antibodies with their targets should therefore be characterized within their native environment to obtain physiologically relevant kinetic data with high *in vivo* predictability. RT-IC enables the measurement of real-time kinetics directly on cells. We explain the technology‘s workflow and demonstrate the importance of measuring real-time kinetic rates on cells for the rational design of immunotherapeutic drugs.

Kinetic mechanism and deteminants of EF-P recruitment to translating ribosomes

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EF-P is a conserved protein that has a shape similar to the one of tRNAs and binds to the E site of the ribosome [1]. EF-P enhances the rate of peptide bond formation during the synthesis of consecutive proline (polyPro) sequences [2]. EF-P is post-translationally modified in most bacteria, the modification varies, for example ß-lysine (ß-Lys), rhamnose or 5-aminopentanol modifications have been described [3].

The interaction of EF-P with ribosomes was studied here at different stages of translation using fluorescence assay and stopped-flow technique to determine which elements of the translating ribosome contribute to the factor binding.

The association rate constants are very similar for all complexes. However, the dissociation rate constants of EF-P from the ribosome differ depending on the identity of the P-site tRNA and on the codon presented in the E site. This concludes a scanning mechanism in which EF-P can bind to any type of the ribosome as long as the E site is empty, interactions with specific P-site tRNA ensure a longer residence time on its substrate complexes, thereby providing the time window for EF-P to enhance peptide bond formation, whereas the factor is rapidly released from non-substrate complexes. The EF-P binding to the ribosomal E site does not inhibit translocation. The ß-Lys modification contributes not only to EF-P function, but is also important for binding. The association rate constant for unmodified EF-P is reduced two-fold, whereas the dissociation rate constant is increased by 5-7-fold.

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Impact of redox alterations on IDO1 inhibitors binding properties

T19

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The indoleamine 2,3-dioxygenase 1 (IDO1) enzyme catalyses the O2-dependent oxidation of L-tryptophan (Trp) to the immunosuppressive metabolite L-kynurenine. This step is the rate-limiting step in the kynurenine pathway, which produces NAD^+ and controls the immune response in host-pathogen interactions. [1] Dendritic cells (DCs) use IDO1 activity to establish an immuno-tolerogenic environment and maintain tolerance to self-antigens. These events occur in tissues and organs that may differ in metabolic parameters like nutrients and growth signals, as well as chemical factors like pH and redox conditions. Early investigations revealed that changing redox conditions, independent of oxygen levels, regulate the expression and catalytic activity of IDO1 in DCs. [2] This could be due to the regulation of the oxidative turnover of the heme cofactor and/or disulfide bridge formation of cysteine residues in IDO1. Several kinds of inhibitors have been designed to modulate IDO1 catalytic activity, with different binding mechanisms that may engage or not the heme group via a coordination bond with the iron atom. [3] In this framework, we have tested the hypothesis that redox conditions may affect the binding properties of inhibitors to the enzyme. At this aim, we have investigated the effect of reducing agents on the interaction between IDO1 and its inhibitors in clinical trials, using MicroScale Thermophoresis (MST) and nano Differential Scanning Fluorimetry (nanoDSF) techniques. Our results show that redox conditions have different effects on the dissociation constant (K_d) of distinct types of inhibitors to the enzyme and suggest how redox imbalances in the tumour microenvironment may contribute to drug resistance mechanisms against specific classes of IDO1 inhibitors, making them less effective for clinical benefits.

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Biochemical and biophysical characterization of *Mycobacterium tuberculosis* SseA, a thiosulfate:cyanide sulfurtransferase and its interaction network

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Tuberculosis remains a global health issue and one of the leading causes of death worldwide. In this frame, the appearance of multi-drug resistance strains has further emphasized the need to identify new targets for diagnostics, drugs and vaccines.

Although its physiological role in *Mycobacterium tubercolosis* (Mtb) has not been clarified yet, the putative thiosulfate-sulfurtransferase SseA appears as a potential drug target candidate since its to involvement in Mtb macrophage infection and oxidative stress resistance pathways has been widely demonstrated.

In particular, this project aims at gathering knowledge on the biochemical and biophysical properties of the rhodanese-like protein SseA. Since bioinformatics allowed the identification, as a neighbouring sseA gene, of a sequence that encodes for a yet uncharacterized SufE-like protein (SufE), and highlighted the co-expression of putative homologs for the genes encoding the two proteins, this project also aims at providing computational and experimental evidence of the interaction between SseA and SufE.

Recombinant SseA and SufE were produced in *E. coli* as well folded monomeric protein, as highlighted by circular dichroism and size-exclusion chromatography measurements. Enzymatic measurements demonstrate the ability of SseA to transfer sulfur from low-MW thiols to cyanide as an acceptor molecular and that its enzymatic activity shows up to a 4-fold increase in the presence of SuFE. ITC and MST measurements demonstrated that this increase results from a direct protein-protein interaction and allowed to measure binding affinities. Ongoing studies are addressing the interaction stoichiometry and mechanism, also by using conveniently designed mutants of the two proteins.

Monolith X: a tool for molecular-scale biophysical measurements

T21

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Free-in-solution measurements confer an advantage for affinity measurements: they enable Kd determination in lysate; they expand the available buffer systems to maintain protein solubility; further, the buffer flexibility and easy assay read-out reduce time required for optimization and data collection. Monolith X uses two biophysical modalities, Spectral Shift Technology and Microscale Thermophoresis, that give unprecedented precision in biophysical measurements with highly sensitive optics to follow target:ligand binding. Here, we will discuss how these methodologies work, and give a few examples of the versatile affinity measurements empowered by Monolith X.

Folding of multi-domain proteins – folding intermediates, hidden kinetic traps and cryptic functional features

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Although more than 75% of the proteome is composed of multi-domain proteins, current knowledge of protein folding is based primarily on studies of isolated domains. In fact, multi-domain proteins tend to be very elusive to an experimental description and commonly display very complex behaviour characterized by multiphasic kinetics and/or irreversible folding. Hence, whilst interesting, these systems have largely escaped a rigorous experimental characterization. In my presentation I will describe our recent work focussed on the kinetics of folding of multi domain complexes, with particular attention on tandem repeats. By taking advantage on the synergy between kinetic experiments and site-directed mutagenesis, I will discuss the effects of the presence of contiguous domains, both in their native and denatured conformations, and will highlight the hidden functional features of misfolded intermediates.

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Computational biophysics meets medicine - from biomolecular simulations to animal models

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Proteins conform the ultimate machinery of Life, executing all processes that sustain living organisms – from complex metabolic pathways to neurotransmission. Far from being static, at physiological temperatures, proteins vibrate and cycle between different states or conformers, sensing external signals: in the same way that primary sequences fold into 3D-sructures, each shape encodes intrinsic functional motions of such relevance for Life that are conserved from bacteria to humans [1]. Nevertheless, although protein conformational mechanisms are key to understand the link between structure and function, they are often elusive for both experiments and simulations. To overcome these limitations and explore them in-depth, our research integrates database mining, AI, coarse-grained and atomistic simulations of “hot” mutations in cancer and mendelian diseases [2-3], with a special focus on path-sampling methods which can provide realistic transition pathways even for very large sub-mesoscopic protein assemblies [4]. Here we present selected examples where these multiscale and integrative approaches have allowed to dissect the essential motions orchestrating function in highly complex systems, yielding key functional insights connecting mendelian mutations and cancer [5] or revealing unexpected intermediate states in receptor signaling validated up to the *in vitro* and *in vivo* level in mice [6].

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Annotation and validation of nucleic acid structures: increasing the quality and interoperability of the data

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The existence of the PDB and the underlying community standards put structural biology into relatively good position, certainly compared to some other fields of life sciences. First it is the accepted culture of depositing newly determined structures into the PDB archives and second and of similar importance, it is now widely accepted implementation of the mmCIF dictionary. Structural biologists can even benefit from the AlphaFold2 able to predict protein structures. These facts and growing power of cryo-EM technique open new possibilities of deciphering accurate 3D structures of large multimeric complexes. The rosy picture is however darkened by some facts about quality of nucleic acid (NA) structures. The quality problems of NA-containing structures can be divided into three groups: (i) inconsistently set and applied target values of the valence geometry, bond distances and angles, for nucleotides; (ii) poorly refined backbone geometries; (iii) incompletely and often incorrectly assigned base pairing topologies. In the talk, I hope to convince you that solution of these problems is possible and indeed even simple. The tools we offer are available from our web application dnatco.datmos.org [1]. The web leads you from easy-to-navigate Annotation TAB, which offers an overview of the analyzed structure accessible even for a non-expert. The Validation TAB enables an in-depth analysis of nucleic acid structures and expert judgement of its quality. This TAB provides a detailed analysis of NA conformation at the level of dinucleotide; this analysis is based on our original system of the NtC classes [2], a new way of validation by rmsd/RSCC scatterplots, and valence geometry validation. Soon, we will provide an integrated tool to analyze base pairs detected in the analyzed structure; now we offer a base pair overview at basepairs.datmos.org. The Browse TAB allows the user to view NA structures from various perspectives. We continue to develop the web services at dnatco.datmos.org to offer tools to modify structures during the process of their refinement.

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Accurate and efficient SAXS/SANS implementation including solvation layer effects suitable for molecular simulations

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The combination of Small-Angle X-ray and Neutron Scattering (SAXS/SANS or SAS) experiments with molecular dynamics (MD) simulations is an effective strategy for the characterisation of biomolecules in solution [1]. On the one hand, the limited resolution of SAS can benefit from the MD contribution, and on the other hand, the inaccuracy of MD can be reduced by integrating experimental data. Although very promising, the latter approach is hampered by its high computational cost. In particular, the multiple scattering intensity calculations performed on-the-fly alongside the MD simulation make this method prohibitively expensive, even on the latest High-Performance Computing systems. One way to overcome this limitation is to calculate the intensity of the system of interest on a coarse-grained model, thus aggregating the scattering behaviour of groups of atoms into larger particles [2]. Previously, we presented a hybrid resolution method that allows atomistic SAXS-restricted MD simulation by using a Martini coarse-grained approach to efficiently back-calculate scattering intensities [3]; in our last work, we enhance this technique by developing a novel hybrid-SAS method that is faster, more accurate, extended to the SANS intensity calculation and that is compatible with both proteins and nucleic acids. Furthermore, an implicit and user-definable solvation layer contribution is included in the calculation to allow the reconstruction of a more realistic scattering behaviour in solution. This layer depends on solvent-solute interactions and, being typically more electron/neutron dense than the bulk solvent, actively contributes to the scattering signal [4]. To ensure a fast and simple use of our method and to broaden its application, we have included it in PLUMED-ISDB, a module part of PLUMED [5], an open-source software designed to enhance and extend various MD engines or to be used as a stand-alone package to perform a wide range of advanced analyses of complex biomolecular systems.

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Probing protein-induced local membrane deformation: a small-angle scattering and computational study

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The complex interplay between integral membrane proteins and lipid bilayers includes the so called hydrophobic matching, which describes the balancing between the hydrophobic thickness of membrane proteins and the acyl-chain region of the surrounding lipids. Deviations in this matching can introduce a free energy penalty, alleviated by membrane deformations or protein oligomerization. So far experimental measurements of lipid thickness near membrane proteins are scarce and have only recently been explored through NMR [1], to the best of our knowledge.

Here we propose an approach combining small-angle X-ray scattering (SAXS) and all-atom molecular dynamics (MD) simulations to probe local membrane deformations induced by the outer membrane protein phospholipase A (OmpLA). OmpLA, an integral enzyme that hydrolyses phospholipids upon dimerization, is reconstituted in large unilamellar vesicles (LUVs), referred to as proteoliposomes [2]. LUVs composed of 1-palmitoyl-2-oleoyl-glycero-3-phosphocholine (POPC) or 1,2-dilauroyl-sn-glycero-3-phosphocholine (DLPC) are selected for their differing hydrophobic thicknesses, exceeding (28.4 Å) or falling short (21.3 Å) of the OmpLA hydrophobic patch (22-24 Å) [3]. A multi-scale scattering model for proteoliposomes is developed, encompassing nanometric transbilayer structure and protein spatial distribution within the spherical frame of LUVs.

According to theoretical expectations, we observed thinning of POPC membranes and thickening in DLPC systems. Specifically, MD simulations reveal lipid deformation within a distance of less than 30 Å from OmpLA for both POPC and DLPC, and support the quantitative SAXS analysis demonstrating average thickness deformations of a few angstroms. Additionally, results highlight that OmpLA maintains its monomeric state in both systems, suggesting that POPC and DLPC model proteoliposomes do not favor the physiological dimerization.

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Identification of ClpC1 inhibitors using an in silico approach

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Mycobacterium tuberculosis ClpC1 is an unfoldase and a member of the class II AAA+ family of proteins, which contains a N-terminal domain (NTD) and two distinct ATP- binding modules, D1 and D2 (1). The potential of ClpC1 as a target against Mtb was proven by the discovery of 4 potent and chemically diverse natural product antibiotics targeting this protein. Indeed, ecumicin, cyclomarin, rufomycin and lassomycin, all targeting ClpC1, are among the most powerful anti-TB molecules to emerge recently.

Though these molecules are promising start points, due to their complex multiring structures, they are challenging for medicinal chemistry and display poor pharmacological properties. Nevertheless, considering the chemical diversity, the sterilizing properties and the variety of mechanisms of action displayed by ClpC1 inhibitors, we believe that small molecules acting on the same binding sites will also be able to block ClpC1-ClpP1P2 activity and represent valid drug candidates.

We have developed an in silico screen to find new molecules that bind to the same pockets as the NPAs targeting ClpC1. Using this approach on a small library of compounds, we were able to identify a ClpC1 binder (Kd 1 μM) and show that this compound binds to the same pocket as ecumicin and cyclomarin (2). In addition, this compound, as well as 18 analogs, were found to be inhibitors of GFPssra degradation by the ClpC1P1P2 complex and ATPase activity of ClpC1. This approach, when applied to larger libraries of compounds, will be an important asset in the development of new drugs targeting ClpC1.

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Orange and Quasar: interactive data exploration for spectroscopy

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Spectroscopy enables insight into materials' physical and chemical properties. Modern spectroscopy techniques collect large amounts of data, which makes data analysis challenging, as we need statistics to make sense of it. Due to a lack of capable, user-friendly, and freely available data analysis software, we adapted Orange, an open-source data mining software [1], for spectroscopy.

In Orange, visual programming is used to create flexible workflows where components process, visualize, or model data. Changes in workflows are propagated immediately, which allows for quick adaptations and helps users gain confidence [2]. Components in Orange were designed so that any result could be analyzed further; therefore, most visualizations are highly interactive.

Quasar builds on Orange and adds data readers, visualizations, and processing needed for spectroscopy, such as spectral preprocessing, integration, peak fitting, and hyperspectral data display [3]. Because Quasar embraces Orange's core data structures, these additions can be immediately used with general data processing and machine learning methods from Orange. Multiple institutions have contributed to Quasar, among them the University of Ljubljana, Slovenia, Soleil Synchrotron, France, Elettra, Italy, Canadian Light Source, Canada, LNLS, Brazil, and NBMU, Norway. Current development focuses on supporting SNOM data.

With its visual interface, spectroscopy-tailored components, and machine learning, Quasar currently represents a unique combination of capabilities while also being freely available. In May 2024, papers on Quasar [3,4] already had 175 citations.

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Molecular Biophysics Database of raw data - MBDB

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Biomolecular research profits from a plethora of biophysical methods that enable characterization of molecular properties, stability assessment and optimization, interaction analysis, etc. However, a standardized solution for deposition and public access to raw measurement data from such methods is missing.

Under the MOSBRI project we have taken the endeavor to define a standard format of metadata for selected techniques of molecular biophysics and create a public database to store raw data files together with metadata descriptions and enable them to be **F**indable, **A**ccessible, **I**nteroperable and **R**eusable. The metadata for individual data sets will consist of a general part and a method-specific part. The general part defines descriptors for key parameters common for different experimental techniques, e.g. source organism, identity of individual molecules, including chemicals, with reference to external databases and unique identifiers for the most relevant types. The method-specific part is devoted to the metadata special for a particular technique (such as MST, BLI, etc.). This approach will eventually enable searching across different techniques and allow direct comparisons of results e.g. interaction parameters for the same molecular system measured by different techniques.

The Molecular Biophysics Database (MBDB) is being built using the Invenio repository platform technology (https://inveniosoftware.org/) and JSON as the key representation format of metadata, in collaboration with the CESNET data storage team and their hardware resources. Based on the previous survey on the use of biophysical methods and the need for databases [1] the first set of covered techniques includes microscale thermophoresis, bio-layer interferometry and surface plasmon resonance.

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Computational tools to get the most out of biophysical data

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Lack of consistency and reproducibility is one of the more important barriers in the application of experimental biophysical data in drug discovery programs and biotechnology developments [1]. Two specific analytical tools implemented into the software AFFINImeter to increase the reliability of biophysical data will be presented: (i) KinITC which aims to coherently combine the thermodynamic and kinetic characterization of molecular interactions by Isothermal Titration Calorimetry[2]. (ii) Multi-technique global fitting, which not only enhances the consistency of orthogonal experimental studies but also sheds light on elucidating complex mechanisms of interaction [3].

The ongoing effort at the MOSBRI consortium in the development of a standardized biophysical database under the FAIR principles (Findability, Accessibility, Interoperability, and Reusability) and how this could set the basis for predictive tools based on machine learning algorithms will also be discussed.

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Instruct-ERIC- pan-European research infrastructure for structural biology. Examples for integrated structural biology studies

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Instruct is a pan-European Research Infrastructure with 16 member states and one member organization. Provision of access is a key priority for Instruct, enabling cutting-edge science, often involving multiple technologies.

In this contribution, examples will be provided for the impact of integrated structural biology studies in the area of Life Science.

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Intrinsically disordered proteins studied by NMR spectroscopy: insights from ^{13}C direct detection

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Highly flexible intrinsically disordered proteins (IDPs) and regions of complex multi-domain proteins (IDRs) introduce an additional dimension in protein function, still exploiting simple modules (highly flexible regions as well as globular domains). This modular protein architecture is shared by many proteins involved in recognition, signaling and regulation, all processes in which structural and dynamic heterogeneity plays a fundamental role. Protein malfunction, linked to the onset of incurable diseases, is often related to highly flexible regions.

NMR represents a unique tool for the investigation of IDPs/IDRs at the atomic level. Challenges however emerge as a consequence of their high disorder and flexibility and the lack of a defined 3D structure. Moreover when globular and disordered domains are simultaneously present in a protein, the NMR spectra can become quite complex. ^{13}C detection offers an elegant approach to study them not only in isolation but also when part of complex multi-domain proteins.[1-5].

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Progress in the integrative analysis of the pyruvate dehydrogenase complex metabolon

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The pyruvate dehydrogenase complex (PDHc) is one of the central enzymes in energy metabolism connecting glycolysis with the citric acid cycle, playing vital roles in aerobic metabolism [1], autoimmune response [2], neurodegeneration [3], and inflammation [4]. Localized at the inner mitochondrial membrane, PDHc utilizes a multidomain-driven reaction to catalyze the conversion of pyruvate to acetyl-CoA – the "*link reaction*". The full endogenous complex structure that includes multiple copies of the catalytic E1-, E2-, E3- and structural E3BP- subdomains is unknown. We applied, together with collaborators, an integrative approach utilizing biochemical assays, mass spectrometry (MS), cryo-electron microscopy, high-speed atomic force microscopy (HS-AFM) and computational methods for the characterization of the full assembly of this 10 MDa metabolon. Especially HS-AFM, performed in the Roos laboratory via MOSBRI enabled the understanding of PDHc catalytic dynamics. Ultimately, we uncovered (1) stoichiometries; (2) the first accurate model for the spatial domain organization; (3) the highest resolved structure of the endogenous catalytic core to date; and (4) conformational dynamics directly related to the multi-domain reaction cycle. Based on these results, we constructed an atomic model for the full endogenous eukaryotic complex that is in line with experimental data. Its refinement against almost ninety years of biochemical data [5] is the is the final step in PDHc analysis, serving as the framework for the next chapter in visualizing the link reaction in respiration at unprecedented detail.

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Spectroscopic insights into the mechanism of hydrazine synthase

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Anaerobic ammonium oxidizing (anammox) bacteria are chemolithoautotrophic microorganisms that make a living by converting nitrite and ammonium to dinitrogen gas, with the rocket fuel hydrazine as a unique free intermediate [1]. The *c*-heme containing protein complex hydrazine synthase catalyzes the formation of hydrazine in anammox metabolism and has been purified directly from native biomass as an αβγ dimer.

Analysis of the crystal structure [2] allowed for the hypothesis of a twostep reaction mechanism where nitric oxide is first reduced to hydroxylamine at active site heme γI. Hydroxylamine then diffuses through an intra-protein tunnel to a second active site heme in the α subunit, where it is proposed to condense with ammonium to form hydrazine. Anammox bacteria thus seem to utilize the oxidative power of hydroxylamine to anaerobically activate inert ammonium, resulting in the formation of hydrazine.

To assess this proposition and to assign individual redox properties to each heme at both active sites, the redox properties of the hemes were determined by spectroelectrochemistry and EPR spectroscopy and this data was combined with structural information and redox-induced FTIR difference spectra. Spectroscopic analysis in the presence of substrates and reaction intermediates was performed, giving first insights into the reaction mechanism of hydrazine synthase.

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Orthogonal spectroscopy-systems for the analysis of protein stability and structural changes

T35

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Spectroscopic techniques to study protein stability and structure are well established. Circular Dichroism detects the difference in absorbance of left and right circularly polarised light in the presence of a chiral molecule. Chromophores from proteins that can be detected include peptide carbonyl bonds, aromatic residues, and disulphide bonds, allowing for comparison of protein secondary structure and individual amino acids. The Chirascan CD spectrometers from Applied Photophysics 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.

Differential Scanning Fluorimetry (DSF) is a valuable and widely used technique that monitors protein unfolding by detecting changes in fluorescence as temperature increases. DSF can be used to optimise protein buffer composition rapidly and effectively before downstream structural analysis or crystallography screening. A primary buffer screen of large global parameters (buffer system, pH, salt, concentration) can be used to optimise protein homogeneity, increase solubility and stability, prevent protein unfolding and aggregation, and minimise consumable costs. The stability of a protein in its initial buffer condition has been directly linked with crystal formation success in subsequent crystallography screens. The SUPR-DSFs 384 well plate format allows simple screening of 96 condition buffer screens, including all replicates, in one thermal ramp experiment, with no proprietary consumables required. The conventional workflow for DSF uses extrinsic dyes that may influence the protein's 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 full spectrum intrinsic fluorescence of proteins in a small volume while avoiding using additional dyes. SUPR-DSF reduces operator time and minimises the risk of errors in multi-step sample preparation while also bringing down the cost of consumables and sample consumption without compromising data quality.

Deciphering the intricate hierarchy: subtleties in clathrin heavy chain binding boxes provide selectivity among adaptor proteins of budding yeast

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Clathrin-mediated endocytosis (CME) is an essential cellular mechanism facilitating the internalization of membrane receptors and solute molecules from the extracellular medium into the cell. This process is crucial for various cellular functions, including signal transduction, nutrient uptake, and membrane recycling. Clathrin, the eponymous protein, is a triskelion comprising three Clathrin heavy chains (CHC) that form the structural backbone of the lattice, with stability and regulation provided by three Clathrin light chains (CLC). Clathin does not directly interact with the membrane but interacts with adaptor proteins (APs) that connect it to the membrane. In particular, the N-terminal domain (NTD), a WD40 beta-propeller structure, is crucial for AP interactions via Clathrin Binding Motifs (CBMs). In S. cerevisiae, several adaptor proteins are involved in endocytosis; many of them, like Ent1 and Ent2, are considered redundant.

Our current study delves into this redundancy question. Are actually Ent1 and Ent2 redundant? Using a combination of X-ray Crystallography, Biophysics, live-cell imaging, and molecular dynamics simulations, we sought to answer that question. Through crystallographic analysis, quantitative binding studies using nano-differential Scanning Fluorimetry (NanoDSF), and Native Mass Spectrometry, we reveal distinct binding affinities and selectivity mechanisms. Our findings show a complex interplay of competition and positive cooperativity between Ent1 and Ent2, highlighting the evolutionary adaptability of CME components. This study provides deep insights into the cellular machinery's ability to handle diverse biological functions through precise molecular coordination.

A closer look at interactions between actinoporins and lipids: insights through Cryo-EM and molecular dynamics simulations

T37

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Pore-forming proteins (PFPs) disrupt normal membrane function by forming pores thus compromising membrane integrity. PFPs are usually produced as soluble monomers that bind to various membrane components. After binding, they oligomerize and undergo conformational changes to form transmembrane pores. These pores are lined by either α-helical clusters or β-barrels. Actinoporins are a well-studied α-PFP family originally discovered in sea anemones. They bind specifically to membranes containing sphingomyelin. The only known structure of the actinoporin pore is that of fragaceatoxin C (FraC) [1] from the strawberry anemone (*Actinia fragacea*). The FraC pore is a protein-lipid complex to which three lipids are stably bound. We explored the structure of the pore of actinoporin homologue Fav from the mountainous star coral (*Orbicella faveolata*) using cryo-electron microscopy (cryo-EM). High-resolution cryo-EM maps reveal numerous lipids associated with the Fav pore, representing lipids in the upper leaflet of the membrane surrounding the pore. Among lipids are also densely packed cholesterol molecules under the pore cap. We also observed phospholipids, which we categorized into three groups based on their position and role. Receptor lipids are located in the membrane binding region. Structural lipids are located between two protomers and interact with both. Bridging lipids, located at the complex's outer edge, have only limited interactions with the protein, but stabilize the pore by forming an interaction bridge between two protomers. Atomistic simulations based on this protein-lipid complex allowed us to observe and quantify the effects of the pore on the lipids in both leaflets of the membrane. This research contributes to our understanding of the interaction between membrane proteins and their lipid environment.

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Integrative structural biology to decipher the mechanism of host cell intoxication by the CyaA toxin

T38

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Bordetella pertussis, the causative agent of whooping cough, secretes an adenylate cyclase toxin (CyaA, an RTX protein of 1706 residues) that plays an essential role in the early stages of respiratory tract colonization. The cell intoxication process of CyaA is still poorly understood. After its secretion through a type 1 secretion system, CyaA intoxicates human cells via a direct translocation of its catalytic domain (ACD) across the plasma membrane. Once in the cytosol, ACD catalyses high amounts of cAMP, leading to cell death. Based on integrative structural biology approaches, our results illustrate how the structural flexibility of CyaA is essential for its secretion, its folding, its translocation across plasma membrane and cell intoxication. All of these steps involve disorder-to-order structural transitions that are finely tuned to the environmental conditions that CyaA successively experiences along its journey from the bacterium to the eukaryotic cell cytoplasm. Our data open new avenues for both basic sciences, as well as biotechnological applications of recombinant CyaA as an antigen delivery vehicle, and as a potential protective antigen in the next generation of pertussis vaccines.

Integrative structural biology on cytokine-receptor assemblies for fundamental and translational science

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We employ integrative structural biology to study the structure, mechanism, and antagonism of proteins and protein complexes pivotal to immunity, inflammation, and cancer. Our recent work on Anaplastic Lymphoma Kinase receptors and their activating cytokines has revealed novel protein folds and unprecedented receptor-cytokine assemblies [1]. Furthermore, our work on complete extracellular complexes of leptin receptor [2], IL-12 and IL-23 [3] have highlighted the need to pursue complete cytokine-receptor assemblies to harness the full potential of the mechanistic and structure-function relationships of such signaling complexes. My contribution will focus on leveraging a diversity of structural biology methods and structure-function data to propose biologically relevant cytokine-receptor assemblies that could be used for further functional interrogation and therapeutic targeting. Furthermore, I will illustrate how the latest developments in protein structure prediction and design [4] can be used synergistically with experiment-driven research to help resolve critical debates on the structure and mechanism of cytokine-receptor assemblies.

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Pushing the boundaries of cryo-EM throughput and resolution at 200kV

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Cryo-electron microscopy (Cryo-EM) has established itself as an essential and versatile tool for structural biology. Among Cryo-EM methods, single particle analysis (SPA) is the workflow of choice for high resolution structural determination of proteins and macromolecules. Over recent years, constant advances aimed at improving the resolution of molecular structures and increasing throughput have contributed to the widespread use of the technique both in academia and in industry.

In this presentation we will review how improvements in Cryo-EM hardware and software are advancing the quality and quantity of structural information that can be uncovered across a wide range of macromolecules. We will discuss the latest results achieved using the next-generation low energy spread electron guns (E-CFEG) in conjunction with the Glacios 2 200kV Cryo-Transmission Electron Microscope (Cryo-TEM). We will also demonstrate the latest implementation of AI-assisted software Smart EPU, helping novice and expert users alike in data acquisition.

Cryo-STEM tomography: a bridge between molecular and cellular morphologies

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The versatility of electron imaging techniques has had a big impact on the field of structural and cellular biology in recent years with rapid technological developments making it now possible to model in three dimensions across length scales from tissue to macromolecular structure.

I will describe the current state of research in cryo-electron tomography, where macromolecular structure can be described in-situ, to large field-of-view volume electron microscopy methods. I will focus on our contributions to developing scanning-transmission electron microscopy methods for elucidating both morphology and chemical content of vitrified intact cells, and will discuss what is on the horizon for future developments with segmented and pixelated STEM detectors, allowing for phase-contrast STEM imaging.

Small variations in protein structure induce selectivity in the aggregation process of insulin

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The interaction between misfolded proteins/peptides brings in the formation of insoluble fibrillar aggregates, generally called amyloid aggregates. The deposition of amyloid fibrils in tissues and organs is the hallmark of a series of pathologies, generally called amyloidosis.

In our previous works [1, 2] we demonstrated that, in some cases, the presence of a fluorescent tag covalently bound to the polypeptide chain can alter the aggregation properties of a protein. To investigate this phenomenon, we used an integrated approach, coupling a super-resolution optical microscope, in particular, a stimulated emission depletion (STED) microscope, with a label-free technique, i.e., an atomic force microscope (AFM). We showed that fibrils obtained in-vitro from the aggregation of a partially labeled monomer solution are not homogeneously labeled; on the contrary, some of the fibrils are totally unlabeled. In a scenario dominated by polymorphism, the asymmetric distribution of the fluorophores indicates that labelled monomers follow just some particular aggregation pathways. More recently, we found that even very small modification of the monomers, induced by the presence of monomeric proteins labelled with two fluorophores with a slightly different structure, gives rise to selectivity in the aggregation pathways of insulin.

This observation brings us to the formulation of some hypotheses on general properties inherently related to the process of protein aggregation.

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Alterations in unfolding of FRET-labelled fibronectin in the extracellular matrix of NIH/3T3 fibroblasts and MDA-MD-231 breast cancer cells

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The conformation of fibronectin (FN) within the extracellular matrix is crucial for regulating cellular interactions in physiological and pathological environments. In pathologies like cancer, FN confirmation differs from that seen in physiological conditions which can induce altered cell behavior by presenting different bioactive sites; RGD domains are an example of this by regulating integrin-mediated cell responses that facilitate cancer-like behavior.

Förster resonance energy transfer (FRET) is a technique that measures energy transfer between two light-sensitive molecules (donor and acceptor fluorophores) that can be used to determine if two molecules are within a certain distance of each other. FRET-labelled FN has previously been developed to determine FN conformational states from relative FRET intensity (1, 2).

At the Laboratory for Advanced Microscopy Bioimaging Spectroscopy (LAMBS) facility at University of Genova, I used a Bruker Nanowizard IV AFM on a STED Leica Stellaris 8 microscope to assess FRET-labelled fibronectin in different conformational states. Specifically, FN was labelled with Alexa 488 (donor) and 546 (acceptor) fluorophores before adding to NIH/3T3 fibroblasts and MDA-MB-231 breast cancer cells for 24 hours before fixation. FRET, fluorescence-lifetime imaging microscopy (FLIM), and fluorescence recovery after photobleaching (FRAP) were performed to assess alterations in FN confirmation. I observed highly fibrillar, aligned FN with low FRET intensity/efficiency and longer scales associated with donor-acceptor distance and quenched donor fluorophore lifetime during FLIM/FRAP; these collectively indicate more unfolded, stretched fibronectin.

By contrast, MDA-MB-231 breast cancer cells showed highly globular, intracellular organisation of FRET-labelled FN. Compared with the fibroblasts, they showed a higher FRET intensity/efficiency and longer scales associated with donor-acceptor distance and quenched donor fluorophore lifetime by FLIM/FRAP.

These results validate the effectiveness of FRET-labelled FN as a tool to measure altered biophysics associated with FN conformation in different cell types and could be useful for phenotyping cells in diseases like cancer.

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Microscopes and antarctic fish: how developing high-resolution microscopy for 0°C imaging sheds a new life on cold-adapted systems.

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The Southern Ocean is one of the only places on earth where all biological processes routinely happen below 0°C.[1] At these temperatures, our models for life fall apart. Diffusion, protein folding, energy and metabolism are all processes that are poorly understood in the cold, where dynamics are slow and energy is scarce. Cells from Antarctic fish seem to also have issues related to proteostasis [2], as hinted by the very high RNA:protein ratio [3] and high constant expression of Heat Shock Proteins [4]. Since a lot of the cells energy seem to be focused on 'holding on to life', one could expect all the other processes to be slowed down as a result. Our team has recently developed a method to perform high resolution imaging of live samples (primary cell lines of Antarctic fish) at 0°C, therefore shedding a new light to observe these systems in physiological conditions. Observing molecular processes at low temperatures is challenging when using traditional optical equipment. The low working distances required by high and super-resolution microscopy meet issues of condensation and very fast heat transfer occurring between the microscope and the sample. The development of this new method offers perspectives for studying the rate of molecular, cellular and sub-cellular processes. Observing cells from Antarctic animals in vivo reveals some surprising dynamics, in particular when comparing the speed of organelle movement in the cold. This technology opens avenues to investigate how the energy budget of cold-adapted cells is spent, and what survival strategies have evolved from such a constrained environment.

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Measuring biophysical properties of cells and nanoscale bioparticles in health and disease

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Remodelling of our cells as response to environmental changes is essential for their survival and function. Ability of immune cells to pass through tight epithelial cell layers from circulating blood during infection, ability of tumour cells to travel through the body during metastasis, migration potential of the cells after epithelial-to-mesenchymal transition could be examples where cells undergo extensive remodelling. Although numerous studies aimed at finding protein markers during such cellular processes, there is a major gap in our understanding of how collective biophysical properties of the cells (such as stiffness, membrane fluidity, viscosity etc) alter during these crucial biological processes. Similarly, our understanding of how biophysical properties of cells change in diseases is also limited. To gain a thorough mechanistic perception of cellular processes and diseases, it is essential to fill this gap and have a clear and quantitative picture of biophysical remodelling of the cells.

We and others have made extensive effort to unravel the biophysical aspects of cells in a quantitative manner. To achieve this, we developed advanced imaging approaches that could reveal the molecular details with very high spatiotemporal resolution. These technologies allowed us to see how biophysical properties of cells play crucial roles for signalling from molecular to cellular level. Although these technologies were extremely useful to study biophysical aspects of cellular life at the molecular level, their low sampling (one cell at a time) has been a major obstacle to apply them to medical problems that require measuring thousands of cells. This can be overcome with high throughput methodologies that can robustly report on the ensemble biophysical properties of cells which require reliable reporters and instruments. Thus, while developing advanced instrumentation, we also develop reliable probes to quantify different biophysical properties of cells. Here, I will discuss our approach from probe development to high throughput biophysical analysis.

Spatial requirements for T-cell receptor triggering probed via a DNA origami-based biointerface

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T-cells detect via their T-cell antigen receptors (TCRs) the presence of single stimulatory antigenic peptide-MHC complexes (pMHCs) which are vastly outnumbered on the surface of antigen-presenting cells (APCs) by structurally similar yet non-stimulatory endogenous pMHCs. While TCR:pMHC binding kinetics, molecular clustering, mechanical forces have been implicated as critical parameters, the precise mechanisms underlying highly sensitized and selective T-cell antigen recognition are not understood. We have devised a DNA origami-based biointerface which allows the experimenter to adjust protein distances with nanometer precision as a means to enhance or disturb signaling while being responsive to large scale reorganization processes during cell activation. Applying this biointerface to study the spatial requirements of T-cell activation we found that single, well-isolated transiently engaging pMHC molecules efficiently stimulate T cells [1]. pMHCs with highly stable TCR-interactions gradually lose this capacity. This points at the dynamics of pMHC:TCR binding as a critical parameter for sensitized antigen detection which we hypothesize to result from serial short-lived engagements of several TCRs in close proximity by single antigenic pMHCs.

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Current trends and developments of biophysics in Africa and the actions organized by Society of Africa Biophysical Societies (SABS)

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Biophysics which is multidisciplinary is offered worldwide as a distinct programme, BSc. Biophysics except in Africa where subfields of Biology are offered yet it provides the essential vehicle for realisation of high-tech economy and 2030 agenda of SDGs [1, 2]. Biophysics is yet to get traction in Africa due to lack of basic equipment, expertise, funding and inadequate infrastructure for remote access to equipment hosted in other countries resulting to macro-scale research or computational approaches [3]. Lack of a distinct Biophysics course at undergraduate level, attributed to lack of expertise, denies Africa of holistic Biophysicists, weakening biophysical foundation for postgraduate scholars and researchers. Evidently, the best 23 universities in biophysics research consited of 10, 9, 3 and 1 universities from Egypt, South Africa, Tunisia and Nigeria respectively out of 54 countries in Africa, creating a glaring gap [2]. A paradigm shift about biophysics research and education is required for successful implementation in Africa. The Society of Africa Biophysical Societies (SABS) provides national and intercontinental networks for Scientists from different disciplines [4]. A data bank of Equipment and Biophysicists is being established for button click access. Website, email and social media accounts have been created. SABS collaborates with IUPAB, and BPS for mutual benefits. SABS achieves its objectives through postgraduate mentorship, monthly online Training, conferences, outreach programmes, workshops, biophysics week, networking events, collaborative teaching and research, thematic meetings are conducted. SABS is working on BSc. Pure and Applied curriculum at Masinde Muliro University of Science and Technology (MMUST) which is a proposed Biophysics Centre of Excellency in Africa. MOSBRI provides new frontier in research, education, equipment, exchange programme and support at molecular level. Success of SABS is pegged on rollout of BSc. Pure and Applied Biophysics, provision of scholarships, funding of SABS activities, research grants, travel grants, donation of equipment, and exchange programmes.

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MINFLUX tracking for real-time observation of biological processes at molecular resolution

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Intracellular processes occur on the molecular level and at various speeds. For a long time, microscopy methods for visualizing dynamic processes were limited to either a high spatial resolution or high acquisition speed, while often exposing the sample to high light intensities.

MINFLUX nanoscopy has paved the way towards investigating the dynamics of (macro) molecular complexes and machines on a nanoscopic level. In MINFLUX, the localization of single fluorophores is accomplished with a minimal number of photons. By tailoring the probing scheme to the dimensions and time scales of the individual experiment, it is possible to collect thousands of data points from a single fluorophore, with a temporal resolution of up to 100 µs and track lengths of several seconds. Thus, MINFLUX allows to track single fluorophores at so far unmatched spatiotemporal resolution.

While alternative approaches often lack molecular specificity or live-cell compatibility, MINFLUX combines single-digit nanometer localization precision with a standard light microscopy setup, allowing to easily implement this technique into common biological workflows in a wide range of applications. We demonstrate MINFLUX as a versatile tool to track single biological molecules in 2D or 3D, in vitro and in living cells at molecular resolution. In addition, we envision and test the capability and potential of parallel two-color tracking with MINFLUX.

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The Triceratops SPR #64 – tap into a new dimension with next generation multiplexing SPR

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Surface Plasmon Resonance (SPR) is a label-free, biophysical technique to elucidate the binding kinetics of two interaction partners. The Triceratops SPR #64 is a multiplexing system bringing together the demands for a flexible and efficient system. With 64 addressable sensor spots on a rotatable 8x8 manifold 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), either by running different running buffers or by a short-term buffer exchange feature. Competition assays such as epitope binning or tertiary complex formation like for PROTACs are supported.

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 and minimal sample consumption.

The Triceratops SPR #64 is a highly flexible system offering broad application possibilities and throughput.

Alto & OpenSPR: Nicoya's label-free solutions for biologics research

T50

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Surface plasmon resonance (SPR) is becoming an indispensable technique for biologics research and the development of safe and effective treatments against diseases. The data derived from SPR can help better understand molecular mechanisms and provide key insights into critical signaling pathways in advancing drug development.

As the world's only SPR system powered by digital microfluidics, *Nicoya's Alto™* revolutionizes real-time interaction analysis by eliminating the need to compromise on quality and time. Users can go from sample to answer within hours while streamlining even the toughest of biologics applications with Nicoya® Alto's intuitive and automated assay methods. The Alto is designed to take the complexity out of SPR and empowers scientists with the data they need to take their discoveries to the next level.

The *OpenSPR™* stands out in the field, offering researchers the high-quality, publication-level SPR data they need. As the world's first benchtop SPR instrument, OpenSPR was designed to democratize SPR analysis, providing flexibility and ease of use to research groups. Its innovative nanoplasmonic sensor technology, robust hardware, and intuitive software interface enable real-time, label-free analysis of binding kinetics data for a wide range of biomolecular applications. With its user-friendly interface and compact benchtop footprint, OpenSPR is the perfect tool for affordable SPR analysis.

By choosing Nicoya's SPR solutions, you're joining a community of hundreds of researchers revolutionizing our understanding of fundamental biological processes and disease progression. OpenSPR™ and Alto™ are not just tools, they are catalysts for advancing biotherapeutic discovery and drug development.

Visit nicoyalife.com to learn more about what *OpenSPR™* and *Alto™* can do for you!

Biacore™ 1 series – Next generation one needle SPR system from Cytiva

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Biacore Instruments have been used for interaction analysis since 1990, recently a new generation of systems, 1K-Series, was launched to the community of biophysics scientists replacing Biacore T200 and S200, which served the scientific needs since 2005.

The new 1K generation of systems fulfill current trends and requirements, e.g. more flow cells, single software solution and modular set-up. High sensitivity, flexibility and new injection commands for certain applications complement the system. Biacore 1 shows robust and reproducible interaction analysis, measurements are simpler and faster without compromising data quality. Industrial users will find latest GxP requirements fulfilled and are supported by new method queueing and increased capacity [1].

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Recent advances in biomolecular characterization by mass photometry

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Mass photometry is a bioanalytical single-particle technology that measures the masses of biomolecules in their native states, in solution. Since its introduction in 2018 [1], it has been rapidly adopted as a new approach for biomolecular characterization. Mass photometry is being used in a variety of applications and on many biomolecules, ranging from assessing sample purity and homogeneity for protein, nucleic acid or gene delivery vector samples all the way to the characterization of complex biomolecular interactions.

Here, I will showcase some exciting advances in mass photometry for the characterization of membrane proteins, early aggregation intermediates of proteins involved in neurodegenerative pathologies as well as the quantification of complex protein interactions.

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Lek, a Sandoz company, is the first and oldest pharmaceutical company in Slovenia. It leads in biosimilars and generics, with over 3,700 associates shaping global research and development. At the beginning of our new journey as a stand-alone company Sandoz started significant investments in Slovenia. Recently, we celebrated the start of construction of the state-of-the-art Biosimilars Development Center in Ljubljana. Around 200 experts will utilize advanced technologies to innovate approaches for cell line development, biotechnological platforms, and drug product formulation. Together with the recently announced investment in production in Lendava, Lek, a Sandoz company is establishing its own capabilities for biosimilar development and production in Slovenia.With ten biosimilars available in nearly 100 countries and another 24 molecules in development, we have more biosimilar products reaching more patients than any other company. And we're confident about our future. And getting there will ultimately come down to one thing: our people. We see our people as our greatest strength. They are the driving force that will shape our future and impact millions of lives. That is why Lek actively engages students and professionals from various fields, offering them the opportunity to work with international experts within the Sandoz global network. These opportunities enable them to acquire new knowledge and skills, unleash their talents and creativity, and advance their careers through scholarships, internships, and opportunities to complete academic theses. Lek's efforts in developing human capital have been recognized with the Top Employer 2024 certificate and the TOP Investor in Education certificate. Furthermore, Lek also organizes special projects and events aimed at inspiring and developing young talent within the pharmaceutical industry. One such project is ScienceBEAT, a recipient of the Golden Practice 2023 award. With inspired associates, a rich heritage, and our agility, we stand proudly in pole position in meeting the patients' needs and are recognized as the most reputable employer in Slovenia.

Single-molecule nanopore proteomics: from enzyme dynamics to protein sequencing

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Nanopore currents are emerging as powerful tools for looking at single molecule. Among the advantages of nanopore technology is that labeling is not requirde, allowing the sampling of individual native molecules with high resolution for up to hours. Arguably, the most notable application of nanopore currents is in the sequencing of DNA. Now a variety of application in proteomics is emerging, from identifying proteins with single amino acid resolution to monitoring tiny conformational changes in real-time of proteins.

Here I will provide an overview on the different approaches our laboratory uses in nanopore proteomics.Special attention will be given to advances towards single-molecule protein sequencing. I will then show examples in which nanopores can be used to monitor conformational dynamics in proteins, suggesting a view of enzyme catalysis in which the soft structure of enzymes guides catalysis through well-defined paths along the reaction coordinates.

Preparation of non-covalent self-assembled nanoparticles for nanomedicine

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An important research area in our group is the design of supramolecular assembled nanostructure based on charged polymers and small molecules. Two different nanosystems based on non-covalent interactions will be presented: Cyanidin 3-O-glucoside (CND) complexed with negative charged polysaccharides and supramolecular citrate poly allylamine hydrochloride nanoparticles (CIT-PAH).

Cyanidin 3-O-glucoside (CND), one of the most widely abundant anthocyanins, is an unstable polyphenol that rapidly degrades when exposed to basic pH, high temperature, and oxygen. Here, we used negative charged polysaccharides for CND encapsulation. Considering the pKa of both CND and polysaccharides, complexes were prepared in a range of pHs from 2.5 to 5 and characterized by UV, circular dichroism (CD), NMR, Dynamic Light Scattering (DLS), Small Angle X Ray Spectroscopy (SAXS), Transmission Electron Microscopy (TEM) and ITC [1]. Our results show how the tunning of pH can affect the organization of CND/polysaccharides complexes having an impact on CND loading and optical properties.

Renal calculi are mainly made by calcium oxalate (CaOx) and can cause different complications including mal function of the kidney. The most important urinary stone inhibitors are citrate molecules. Unfortunately, the amount of citrate reaching the kidney after oral ingestion is quite low. We have prepared nanoparticles by complexation of polyallylamine hydrochloride and citrate (CIT-PAH) [2]. We have shown that CIT-PAH nanoparticles dissolve CaOx nanocrystals as shown by NMR, DLS, TEM and Wide Angle X ray scattering (WAXS) in water and by Raman spectroscopy in synthetic artificial human urine. WAXS and Raman show that the crystal structure of CaOx disappears in the presence of CIT-PAH. NMR proves that citrate ions are released from the CIT PAH NPs during CaOx dissolution, MD simulations showed that oxalates exhibit a stronger interaction for PAH than citrate, explaining the removal of oxalate ions and replacement of the citrate in the polymer nanoparticles.

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MAGNA™: a novel single molecule approach for the real-time analysis of biomolecular interactions using magnetic force spectroscopy.

T56

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Despite the enormous technological advances made in genomics over recent years, the latest tools still fall short in their ability to fully capture the dynamic molecular interactions within cells that are essential for understanding the underlying biology of health and disease.

Based on established force spectroscopy technology, MAGNA is a novel platform for analyzing the complex interactions between different biomolecules, with potential application across a broad range of areas within life science research.

MAGNA™ is a novel single molecule genomic analysis platform based upon magnetic tweezer technology. Large numbers of individual molecules can be captured in their native form, immobilised within a flow cell, and are then available for repeated, non-destructive interrogations that can reveal a broad range of features including base modifications, molecular structure, and interactions with other molecules.

We have already generated rich data sets revealing how nucleic acids, DNA- & RNA binding proteins, antibodies, and small-molecule compounds bind to their targets. As an example of this, we demonstrate how MAGNA can be used to explore the binding events between nucleic acids (in this case RNA), and ligands such as proteins and small molecule compounds, to inform the development of RNA- targeted therapeutics. We also highlight the capabilities of the technology for study of protein-protein interactions.

Forces in chaperone-mediated protein unfolding and cell adhesion

T57

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Many processes in our cells are mechanical. Single molecule methods like optical tweezers allow studying the forces involved in those processes. In my talk, I will discuss 2 examples. In the first part, I will show how the concerted action of the hsp70 chaperone and its co-chaperones completely unfold the glucocorticoid receptor. In the second part of my talk, I will discuss how the cytoskeletal proteins talin and kindlin co-operate to strengthen their mechanical bond to the cell adhesion protein integrin.

Infrared nanospectroscopy a new tool in Biophysics

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The recent coupling of infrared spectroscopy with atomic force microscopy (AFM-IR) overcomes the weak spatial resolution of the usual infrared microscopy and achieve a resolution around ten nanometers [1]. The AFM-IR allows recording spectrum and absorption mapping of many sample (proteins, cells or even tissue). This new technique will be described and results on amyloids will be presented.

In Alzheimer's disease, we observe amyloid plaques in the brain, that are made of aggregated forms of amyloid b peptide. We study the aggregation of the peptide alone and follow changes in the structure of isolated amyloid fibrils [2,3]. Some AFM-IR results were also obtained on neurons and brain sections. The AFM-IR provides molecular information at nanoscale resolution and can be applied on various samples. It will help to better understand diseases related to protein structural changes, especially in the field of protein aggregation.

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Site-directed spin labelling and electron paramagnetic resonance for the study of the human flavoprotein Cytochrome P450 reductase

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Cytochrome P450 reductase (CPR), a crucial membrane protein situated on the cytoplasmic side of the endoplasmic reticulum, plays a pivotal role in drug metabolism. Comprising FAD- and FMN-binding domains connected by a flexible region [1], CPR orchestrates the transfer of electrons from NADPH to cytochrome P450s (CYPs) through its flavin moieties [2]. Crystallographic studies on Rattus norvegicus CPR revealed distinct conformations for the FMN binding domain, labeled as “closed” (WT CPR) and “open” (ΔTGEE CPR) states [3]. The proximity of the two flavin domains in the “closed” conformation facilitates inter-flavin Electronic Transfer (ET), whereas inter-protein ET (FMN to CYPs) necessitates a more “open” conformation.

Understanding the domain movements associated with ET in CPR is paramount for comprehending its role in drug metabolism. To this end, we employed Site-Directed Spin Labeling combined with Electron Paramagnetic Resonance (SDSL-EPR) to study human soluble CPR [4]. By grafting nitroxide labels onto specific sites, we conducted distance measurements using DEER, taking advantage of the endogenous FMN site isolated in its semi-quinone state, $FMNH^{\bullet}$, and inserting a non-canonical amino acid [5].

Our recent findings on the study of human soluble Δ66-CPR [6] unveiled the existence of two major states, “unlocked/open” and “locked/closed,” for the specific redox state FAD/$FMNH^{\bullet}$ of CPR in solution. Under high salt concentrations, the two flavin domains exhibited significant separation, indicating an extensively “open” state. These results are fundamental for future investigations into human membrane CPR. This innovative approach, applicable to flavoproteins with non-modifiable cysteine residues and when the stabilization of semi-quinone states is achievable, holds promise for diverse applications in the realm of drug metabolism and beyond.

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First step toward the development of an optical biosensor based on vibrational spectroscopy for the detection of airborne pathogens

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Vibrational spectroscopy has emerged as a promising tool for pathogens detection [1-5]. Infrared (IR) and the more innovative Terahertz (THz) spectroscopy are widely used for studying biomacromolecules [1,2,6], and for characterization of viruses [2,5]. Coupling the unique structural information provided by vibrational spectra with a sensor platform specifically optimized for airborne pathogens adsorption, would enable the development of a label-free, real-time ultrasensitive optical biosensor. Silicon and/or metal oxides substrates are suitable to be nanostructured and engineered, and their surfaces can be modified with a dedicated bioconjugation in order to increase the selectivity. Other possible approaches, based on silane chemistry or MOFs biocomposites, are considered and illustrated to obtain an optimized sensor capable of concentrating viral capsids, which can then be inspected with IR spectroscopy. Vibrational spectroscopy offers several advantages over gold standards (such as ELISA, RT-PCR, bDNA). It does not require chemical pre-treatment of the sample, measurements are rapid, low-cost and unique for all viral samples. Employing the functionalized sensor platform coupled to spectroscopy it would be possible to perform large scale measurements in an open environment, not simply focusing on the extracted sample as common biochemical assays do.

Here, we explore various strategies and report our recent results about the realization of optical biosensors based on vibrational spectroscopy. We spectroscopically characterize the analytes. The object of our propose is Spike protein (subunit S1) of three variants (α, γ and $\omicron$) of SARS-CoV-2 virus. We prove the potential to discriminate the three proteins from their IR amide I band (1600-1710 cm^{-1}) and to exploit them as a biomarker. A structural investigation is also deepened, leading the spectral differences back to changes in variants S1 proteins conformational structure.

Following, various approaches involving nanostructured platforms and chemical treatments have been developed. Preliminary results are illustrated. These constitute the first steps for the realization of new optical device in the field of airborne biosensing.

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Ultimate DSC, a new tool for protein characterization

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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 Lysosyme or RNase in PSB buffer at low concentration and protein quantities.

Nanoscale dynamics of biomolecular interactions: from viruses to antibiotics

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Bulk methods are often used, however they average out small varieties in underlying sample parameters. Furthermore, activity at the molecular level can often not be recorded in bulk. In order to fill this gap, single molecule/particle methods have appeared over recent years. Here I show how these approaches can be used to characterize stability and dynamics of supramolecular assemblies. In particular, using High Speed-Atomic Force Microscopy (HS-AFM) and fluorescent Optical Tweezers we are now able to scrutinize the dynamics of these processes at the nanoscale, in real time, in liquid. I will show how we are using these techniques to unveil the fascinating world of sub-cellular mechanics and biomimetic assembly processes. This will be illustrated by discussing the stability of single viral and virus-like particles and scrutinizing the relation between mechanics and infectivity. Next assembly and disassembly of protein complexes and its real-time visualization will be discussed. Furthermore, dual-trap optical tweezers studies of the self-assembly of virus-like-particles (VLPs) are shown that reveal real time binding of capsid proteins to dsDNA and the appearance of stable VLP structures around the genome. The formation dynamics of 2D capsid protein assemblies is analysed, particularly showing how complex the kinetics of viral self-assembly can be, with multiple assembly pathways and continuously occurring assembly and disassembly events. Finally the mode of action of antibiotics is discussed and examples are given of how we can scrutinize the dynamic attachment of antibiotic compounds to membranes and the subsequent remodelling of the membrane.

From integrative structural biology to cell biology

T63

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Integrative modeling is an increasingly important tool in structural biology, providing structures by combining data from varied experimental methods and prior information [1-3]. As a result, molecular architectures of large, heterogeneous, and dynamic systems, such as the ~52 MDa Nuclear Pore Complex, can be mapped with useful accuracy, precision, and completeness. Key challenges in improving integrative modeling include expanding model representations, increasing the variety of input data and prior information, quantifying a match between input information and a model in a Bayesian fashion, inventing more efficient structural sampling, as well as developing better model assessment, analysis, and visualization. In addition, two community-level challenges in integrative modeling are being addressed under the auspices of the Worldwide Protein Data Bank (wwPDB). First, the impact of integrative structures is maximized by PDB-Dev, a prototype wwPDB repository for archiving, validating, visualizing, and disseminating integrative structures. Second, the scope of structural biology is expanded by linking the wwPDB resource for integrative structures with archives of data that have not been generally used for structure determination but are increasingly important for computing integrative structures, such as data from various types of mass spectrometry, spectroscopy, optical microscopy, proteomics, and genetics. To address the largest of modeling problems, a type of integrative modeling called metamodeling is being developed [4]; metamodeling combines different types of input models as opposed to different types of data to compute an output model. Collectively, these developments will facilitate the structural biology mindset in cell biology and underpin spatiotemporal mapping of the entire cell.

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Poster abstracts

In the following pages are the abstracts for the posters presented at the conference.

The MOlecular-Scale Biophysics Research Infrastructure (MOSBRI): a transnational initiative to structure and consolidate the European molecular biophysics community

Patrick England[1] and the MOSBRI Steering Committee

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The MOSBRI project (www.mosbri.eu) aims at structuring and consolidating the European molecular biophysics community and is funded until June 2025 by the European Commission through its Horizon 2020 INFRAIA scheme. Its integrated consortium of 13 academic centres of excellence and two industrial partners from 11 European countries carries out three types of activities: 1) Joint Research Activities enabling the emergence of novel methodologies and technologies (in partnership with instrument and software developers), the design, production and dissemination of standard reference proteins, the coining of proficiency accreditation schemes for biophysical techniques, and the establishment of data and metadata archival standards and a universal database for molecular-scale biophysics data; 2) Networking Activities such as the organization of international conferences and courses, and the creation with synergies with like-minded networks and associations, and 3) free of charge Trans-national access (TNA) provision to cutting-edge instrumentation and expertise. By summer 2024, MOSBRI has notably already held three conferences (Paris, Zaragoza, Ljubljana), organized 11 training schools, accepted more than 150 TNA projects and prototyped the first database for molecular biophysics data.

TNA: Apply for access to laboratories of excellence in MOSBRI

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

The MOSBRI programme offers TNA to 13 European biophysical laboratories, widely distributed over Europe and selected to ensure a comprehensive portfolio of technologies and expertise. Access to the MOSBRI TNA sites is based on a proposal submitted via the MOSBRI web-site [1].

MOSBRI has a range of access modalities:

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

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

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

MOSBRI products: Pasteur-PFBMI provides, in remote access mode, protein standards for the calibration and benchmarking of instruments. This will help ensuring that instrument specifications and usage are optimal all over Europe. The first standard is an anti-lysozyme nanobody, which is now available for distribution through the MOSBRI TNA scheme.

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

References:

[1] Apply for TNA through MOSBRI via https://www.mosbri.eu/apply-for-tna/

BIFI-LACRIMA: biophysical instrumentation for protein stability and interactions

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[4]Biomedical Research Networking Centre for Liver and Digestive Diseases (CIBERehd)

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

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

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

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

We are happy to discuss specific needs with potential users!

All equipment is available to external users (remote and in-person access, https://www.bifi.es/access/).

MoB-IBT: Molecular biophysics and more

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The MoB-IBT is part of the Centre of Molecular Structure, which offers wide range of methods of molecular scale biophysics and structural biology. It operates in BIOCEV as part of Institute of Biotechnology, CAS. CMS consists of facilities devoted to crystallization of macromolecules, X-ray diffraction and scattering, biophysical characterization, advanced mass spectrometry, and infrared and fluorescence spectroscopy. The open access services are provided via the Czech Infrastructure for Integrative Structural Biology (CIISB) and Instruct-ERIC. The essential core equipment consists of 15T-Solarix XR FT-ICR (Bruker Daltonics) for mass spectrometry, D8 Venture (Bruker) diffractometer with MetalJet X-ray source (Excillum), crystallization hotel RI-1000 equipped with SONICC (Formulatrix), Prometheus and two Monoliths (Nanotemper) for protein characterization and affinity measurements, and Chirascan for circular dichroism measurements, MP Two (Refeyn) for single molecule mass estimates. Recently, this instrument portfolio was extended by SAXS Point 2.0 (Anton Paar) with MetalJet X-ray source (Excillum) for small angle X-ray scattering studies of biomolecules in solution, MALDI TOF mass spectrometer, excimer laser for induced protein modification, and room for spectroscopy with Fourier-transformed Infrared (FTIR) spectrometer and a FLS1000 spectrofluorometer. CMS was also extended with Protein Production facility.

The Centre of Molecular Structure is supported by: MEYS CR (LM2023042 CIISB), CIISB4HEALTH (CZ.02.1.01/0.0/0.0/16_013/0001776); project „UP CIISB“ (No. CZ.02.1.01/0.0/0.0/18_046/0015974) from the ERDF; project Structural dynamics of biomolecular systems (CZ.02.1.01/0.0/0.0/15_003/0000447) from the ERDF.

Synchrotron radiation circular dichroism at MOSBRI partner AU-SRCD

M5

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MOSBRI partner AU-SRCD is based at the Department of Physics & Astronomy at Aarhus University in Denmark [1]. AU-SRCD utilizes synchrotron radiation (SR) produced by the ASTRID2 storage ring, a facility where SR in the UV to the soft X-ray region is produced [2]. Access to the AU-SRCD partner through MOSBRI is offered to advanced Circular Dichroism (CD) beam lines on ASTRID2. Synchrotron Radiation Circular Dichroism (SRCD) spectroscopy offers significant improvements to the well-established method of conventional CD (cCD) spectroscopy. The high photon flux, over a wide range of wavelengths, results in higher signal-to-noise ratios and enables the collection of data at lower wavelengths than possible with cCD spectrometers.

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

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Biophysical approaches for molecular interaction analysis at the National Institute of Chemistry, Ljubljana, Slovenia

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The biophysical properties of different nanoscale systems play a crucial role in their interactions with the biological environment and their potential impact. Biophysical approaches can provide insights into molecular interactions, which is important for optimizing the design of nanoscale systems for various applications. In our Department of Molecular Biology and Nanobiotechnology at the National Institute of Chemistry, we use different approaches to study molecular interactions: Surface Plasmon Resonance (SPR), which allows real-time investigation of biomolecular interactions with high sensitivity, Isothermal Titration Calorimetry (ITC), which provides a comprehensive understanding of binding thermodynamics, microscale thermophoresis, and soon mass photometry, which allows accurate determination of molecular weight. The combination of these approaches can improve our understanding of molecular interaction dynamics and functionality, driving innovation in areas such as protein research, structural biology and applications. We provide access to these techniques and our expertise through MOSBRI Trans-National Access.

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

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

The EPR facility hosted in the laboratory of Bioenergetics and Protein Engineering in Marseille (France) (https://bip.cnrs.fr/epr-facility/) is one of the four french EPR centers included in the national infrastructure Infranalytics and comprises 4 high-end EPR spectrometers for both continuous-wave or pulse EPR experiments operating at various frequencies and temperatures.

Since the beginning of MOSBRI in July 2021, 20 TNA projects have been accepted, covering a variety of proteins/enzymes addressing different biological questions: from catalysis to protein structural dynamics [1-5]. Both physical and remote accesses are offered by the facility. We welcomed about twenty researchers from nine different European countries.

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DSB-UROM TNA: Exploring protein and cell function in real-time.

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Located in the Department of Biochemical Sciences of Sapienza University of Rome, DSB-UROM offers both remote and in-presence access to cutting-edge installations for studying kinetic processes and real-time energy metabolism in live cells. The results from TNA accesses allowed publications of 4 joint papers with TNA users, and further publications are programmed.

In detail two installations are available: the ***ARP (Analysis of Rapid Processes) facility*** is dedicated to the measurement of rapid kinetic processes (micro- to milliseconds) on purified macromolecules in vitro by different spectroscopies (absorbance, fluorescence). The facility offers stopped-flow and T-Jump instruments, continuous flow system based on capillary mixing methodology and instruments for sample analysis. In a stopped-flow apparatus, two solutions are rapidly mixed and subsequently stopped, allowing for the recording of spectroscopic signal changes over time, that include variations of absorbance, fluorescence, and/or circular dichroism. This technique is suitable for monitoring both fast and slow reaction kinetics, meanwhile a T-jump apparatus addresses ultrafast measurements. Applications include analysis of protein folding and denaturation, pre-steady state enzyme reactions, substrate channeling and rapid binding interactions between macromolecules or between a macromolecule and a small ligand.

The ***Hyp-ACB (Hypoxic Analysis of Cell Behavior) facility*** provides real-time sensing of the metabolic activity of both prokaryotic and eukaryotic cells. By offering access to the Seahorse technology by Agilent, it is possible to monitor metabolic parameters in living cells seeded on a 96-well plate. The Seahorse measures oxygen consumption rate (OCR) and extracellular acidification rate (ECAR), reflecting cellular respiration and glycolytic activity, respectively. This enables detailed insights into cellular metabolism under physiological conditions or after specific treatments. Tailored drug addition sequences can be applied to investigate specific metabolic pathways. Measurements under low oxygen conditions (down to 3% oxygen) are available, offering versatility in experimental design. Cell biology equipment is available alongside a Real-Time PCR and Image reader plate.

Infrared spectroscopy, an extended TNA offer

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Our laboratory offers a complete infrared analysis solution to our TNA visitors. We developed infrared expertise in the analysis of biomolecules over decades now, both in the recording and the interpretation of spectra. We have developed our own analysis program. The interest of infrared spectroscopy in the study of biomolecules and especially proteins is numerous. Fourier transform infrared (FTIR) spectroscopy is fast (a few minutes), required minute amounts of samples (10-100 ng) and study proteins without any external labeling or chemical modifications. Our laboratory is using mainly the attenuated total reflection (ATR) sampling method which allow an easy study of membrane proteins in their native lipidic environment but also poorly or insoluble proteins like amyloids. Information about post-translational modifications like glycosylation or phosphorylation can be acquired at the same time. For amyloids, we can distinguish oligomers from fibrils and follow aggregation kinetics.

We recently acquired a state-of-the-art AFM-IR equipment. This technology gives the best of both methods: AFM and infrared spectroscopy. Most importantly it provides infrared spectra with the AFM spatial resolution (~10 nm) giving to infrared spectroscopy an almost single molecule resolution. This new methodology is now available and included in the TNA offer provided by the laboratory.

The ISMB protein crystallography and biophysics centre (BiophysX)

M10

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BiophysX is a core facility of the UCL/Birkbeck Institute of Structural and Molecular Biology (ISMB) which provides access to a wide range of instrumentation and expertise covering the characterization of protein stability, crystallization and biomolecular interactions. We offer on-site an remote access to state-of-the-art liquid handling equipment and automated imaging systems for monitoring aggregation or crystal growth. On-site access is available for characterisation of structure via temperature-controlled CD for secondary structure and multi-angle light scattering attached to size exclusion chromatography for accurate sizing of molecular species. Molecular interactions and reactions can be monitored by UV and fluorescence spectrometers, biolayer interferometry and isothermal or differential scanning calorimetry.

BiophysX is experienced in working with a wide range of users. In addition to long experience of helping structural biologists, we are working on establishing reference protein interactions systems, engineering proteins for solubility and stability, ligand discovery, antibody therapeutic development and the assembly of protein and nucleic acid membrane spanning complexes. As a MOSBRI partner, BiophysX is providing free Trans-National Access to European academic and industrial researchers to individual instruments or to muti-instrument pipelines for biomolecular characterization.

The SPC core facility for biophysics at EMBL Hamburg

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The Sample Preparation and Characterization Core Facility (SPC) at EMBL Hamburg provides a diverse array of biophysical techniques for characterizing biomolecules and their interactions with binding partners. Our facility welcomes both academic and industrial users, offering comprehensive services such as consulting, training, equipment access, sample drop-in, and data analysis. We are affiliated with various organizations facilitating transnational access for academic researchers, such as MOSBRI and Instruct ERIC. Our data analysis web server, **spc.embl-hamburg.de**, hosts tools for biophysical data analysis [1], continually expanding to include the latest modules, such as dedicated analysis for dynamic light scattering (DLS) data [2]. Furthermore, we actively engage in method development within the biophysics field, including techniques for determining binding affinities from thermal shift data [3], and developing a pipeline for characterizing and optimizing membrane proteins before cryo-EM experiments [4].

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ProLInC – 'Protein folding and Ligand Interaction Core' facility. A transnational access site for MOSBRI focussing on the "difficult" targets!

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The LiU core facility ProLinC (liu.se/en/research/prolinc) is the Swedish node in MOSBRI. ProLinC offers a dedicated biophysics facility with staff scientist support, making it possible for SciLifeLab users to fully explore their protein/complex properties and refine conditions for downstream applications, as well as explore the nature and dynamics of its interactions. roLinC actively sustains transnational access (TNA) as well as research activities in protein/peptide molecular studies and its applications within cancer, regenerative medicine and neurodegenerative and infectious diseases. When needed, our research environment allows for sample preparation and analysis according to Biosafety level 3 (BSL-3).

ProLinC provides facile one-stop access to complementary and advanced biophysical techniques jointly with experts in their use, which creates unmatched sample and experiment synergies. In the same lab, ProLinC holds a near-complete set of instrumentation for the biophysical analysis of proteins and their interactions, which enables parallel investigation of essential properties such as oligomerisation, interactions, stability and conformational change, with complementary verification by at least two independent methods. Several of our instruments are designed to cope with small amounts of material, offering the possibility to screen many sample conditions and/or libraries with little sample consumption.

Our user pipelines showcase lines of investigation tailored to facilitate/enable the investigation of novel "difficult" targets in systems of high complexity and/or difficult-to target samples. Where structural data is already available, these pipelines will be able to add functional data related to interactions, dynamics and stability and thereby increase scientific value and applicability.

Protein quality control and buffer conditioning: monodispersity, folding, stability

- DLS and nanoDSF, SEC-MALS, CD, DSC

Protein-ligand/peptide interaction: affinities, stochiometry

- MST and/or SPR (tailor-made surfaces available) ITC, fluorescence, CD

Unstable protein characterization : IDPs, aggregating and/or unstable proteins/complexes

- fibrillation kinetics (fluorescence spectro/microscopy (hyperspectral, confocal, FLIM))
- multi-technique small-volume assays (nanoDSF, MST, DLS, SPR)

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

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

RUG-BP: Molecular Biophysics research in Groningen

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

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Available techniques at LAMBS facility

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The Laboratory for Advanced Microscopy Bioimaging Spectroscopy (LAMBS) is placed in Genoa (Italy). LAMBS facility is made of two different sites one based at the University of Genoa (UNIGE) and the second at the Istituto Italiano di Tecnologia (IIT).

DIFILAB is an integrated research laboratory at the Department of Physics of UNIGE, designed and built as part of the ministerial project "Departments of Excellence, 2018-22" to achieve the goal of enhancing research on biomedical science and nanotechnology. Nanoscopy Lab is based at IIT and its core activity is related to the development of novel instrumentations and approaches for advanced diagnostics. The two laboratories are deeply interconnected and are carrying out common projects in the field of microscopy and spectroscopy.

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

Non specificity of I-motif ligands and antibody demonstrated by bio-layer interferometry (BLI)

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i-Motifs of DNA (hereafter, *i*-DNA), known *in vitro* for nearly three decades, are unusual, four-stranded structures, in which cytosines are intercalated *via* a stack of hemi-protonated C–C base pairs (CH^+:C) (Fig. 1A, B). Some of these structures have been well characterized *in vitro* but their biological relevance is being investigated.

Relatively few molecules were reported to interact with i-DNA. The main issues in this regard are the strong pH-dependency, flexibility, polymorphism and complex folding behavior of i-DNA that introduce potential bias into screening methods. In particular, low-pH conditions used to induce the formation of i-DNA can increase the non-specific interactions and afford false-positive compounds. The interactions of the molecules reported as i-DNA ligands were demonstrated by FRET, pH transition or thermal melting methods but few affinity constants were measured.

In this context, we have developed a method to synthetize a constrained structure of i-DNA of the human telomeric sequence (hTeloC) which was found more stable in particular at neutral pH. Using Bio-Layer Interferometry (BLI), we were able to screen and study the interactions between i-DNA and already reported ligands (TMPyP4, mitoxantrone, IMC-48, berberine, *etc*) at physiologically relevant pH. We demonstrated that none of the reported ligands were shown to discriminate between folded and unfolded *i*-motif structures [1,2]. In fact, at acidic pH, the interaction between the ligand and i-DNA were mostly governed by electrostatic interactions due to the protonation of many ligands, which can strongly increase their non-specific nucleic acid binding.

In a similar context, we also showed by using BLI that commercial *i*-motif antibody (*i-Mab*) is not able to recognize selectively the *i*-motif structure. These results were confirmed by other bi-physic technics: Circular dichroism, Pull down test [3].

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Native agarose gels and contact blotting as means to optimize the protocols for the formation of antigen-ligand complexes

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Background. Protein complexes provide valuable biological information but can be difficult to handle [1]. Therefore, technical advancements designed to improve their manipulation are always useful.

Methods. We investigated the opportunity to exploit native agarose gels and the contact bot method for the transfer of native proteins to membranes as means for optimizing the conditions for obtaining stable complexes [2]. As a simple model of protein-protein interactions, an antigen-ligand complex was used in which both proteins were fused to reporters.

Results. At each step, it was possible to visualize both the antigen, fused to a fluorescent protein, and the ligand, fused to a monomeric ascorbate peroxidase (APEX) and, as such, a way to tune the protocol. The conditions for the complex formation were adapted by modifying the buffer conditions, the concentration of the proteins and of the cross-linkers.

Conclusions. The procedure is rapid, inexpensive, and the several detection opportunities allows for both the monitoring of complex stability and the preservation of the functionality of its components, which is critical for understanding their biomedical implications and supporting drug discovery. The overall protocol represents a handy alternative to gel filtration, uses very standard and ubiquitous equipment, and can be implemented rapidly and without specific training.

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Toehold mediated strand displacement in coiled coil proteins

P3

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The ability to accurately regulate the speed of protein complex dissociation has significant practical applications in synthetic biology, ranging from activating split enzymes to altering protein conformations. Toehold-mediated strand displacement, is a non-enzymatic reaction that allows for the controlled displacement of one nucleic acid strand by another. This invading strand first establishes interactions through a previously unpaired toehold region. Such a mechanism can significantly reduce the half-life of a DNA complex, enabling us to achieve kinetic control over the reaction [1]. Given that the interactions between two coiled-coil proteins resemble those between two strands of DNA, we suggest that toehold-mediated strand displacement could be applied to protein complexes with overhangs based on the coiled-coil (CC) motif. This would allow for precise kinetic control over their dissociation.

Previous attempts to demonstrate toehold-mediated displacement in short, charged coiled-coil were not successful [2]. We propose that lengthening CCs in the complex can result in extended dissociation times, and the introduction of buried asparagines at the interaction surface can enhance orthogonality and prevent the formation of off-target states [3]. We postulate, that these modifications enable design of more stable CC complexes with very long dissociation times.

We monitor kinetic parameters of protein complex dissociation through techniques such as split luciferase activity, Fluorescence Resonance Energy Transfer (FRET), or Surface Plasmon Resonance (SPR). We compared the dissociation constants of protein complexes with and without a toehold to assess the viability of integrating this mechanism into proteins.

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Biophysical and biochemical characterization of rhizobial L-asparaginases as zinc metalloproteins

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L-Asparaginases, which catalyze the hydrolysis of L-asparagine, are divided into 3 structural classes. Representatives of Class 1 are important as effective drugs against acute lymphoblastic leukemia. The mysterious Class 3, represented by ReAIV and ReAV from *Rhizobium etli*, have no sequence similarity to other asparaginases. Both isoforms have the same fold, active site, and quaternary structure, despite low sequence identity. This work examines the consequences of their sequence difference. ReAIV is almost twice as efficient as ReAV in asparagine hydrolysis, with the kinetic K_M, k_{cat} parameters of 1.5 mM, 770 s^{-1} and 2.1 mM, 603 s^{-1}, respectively. The temperature optimum of ReAIV is ~50°C but 37°C for ReAV. The activity of both isoforms is boosted by low and optimal concentration of zinc, which is bound three times more strongly by ReAIV than by ReAV, as reflected by the K_D values of 1.2 and 3.3 μM, respectively. Perturbation of zinc binding by a Lys→Ala substitution in the coordination sphere drastically decreases the enzyme activity. Different divalent cations inhibit both isoforms in the following order, from the strongest to weakest inhibitors: Hg^{2+} > Cu^{2+} > Cd^{2+} > Ni^{2+}. ReAIV is more sensitive to Cu^{2+} and Cd^{2+}, while ReAV is more sensitive to Hg^{2+} and Ni^{2+}. Cd^{2+} (as Zn^{2+}) improves substrate specificity of both isoforms, suggesting its role in substrate recognition. The activity of ReAV is eight times less sensitive to Cl^- anions. This work utilized a number of modern biophysical techniques, such as microcalorimetry, mass photometry, or nDSF. The discovered complementary properties of the two isoforms help us better understand their environmental role and the inducible character of ReAV, provide guidance for the preparation, activity testing, and storage of these proteins, and will promote future research towards application of these enzymes for ALL therapy.

Nucleotide-based compounds to selectively target a nanoRNase involved in mitochondrial homeostasis

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Mitochondria, in addition to their role in energy production, are involved in supporting the development and progression of cancer, being involved in viable (energy) and lethal (cell death) cell activities. In mitochondria, nucleases activity is important in balancing the synthesis and degradation of mitochondrial RNA, which is crucial for governing mitochondrial gene expression. REXO2, an exonuclease specialized in the elimination of nanoRNA residues (2-5 nucleotides), allows effective recycling of nucleotides within the mitochondria, thus preventing the accumulation of nanoRNAs and antisense RNAs [1]. Given its pivotal role in cell proliferation and its observed overexpression in multiple tumor type [2], Rexo2 emerges as an attractive target for potential anticancer therapies.

To pursue this approach and starting with the evidence that REXO2 binds with higher affinity 2-nucleotides substrates, we screened in vitro a library of dinucleotide-based compounds, already successfully employed as anti c-di-GMP in bacteria [3], for potential inhibitory effects on the recombinant Rexo2 protein. This set of compounds is characterized by the replacement of the charged and hydrolyzable phosphodiester group of a dinucleotide with the neutral and stable 1,2,3-triazole ring. We performed an initial screening of the compounds using a spectroscopic assay in a 96-well plate with pNP-derived compund as a hydrolyzable analog substrate and following product accumulation at 420 nm [4]. Subsequently, the most promising compounds were validated and their IC_{50} values were determined by RP-HPLC assay [5]. With this method we tested the residual activity of Rexo2 by following at 252 nm the separation of the dinucleotide substrate pGpG and its hydrolysis product, GMP. Two promising hits have been identified so far and their effect on cell will be tested.

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Improving stability of bryoporin nanopores using genetic code expansion

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Nanopore sensing enables the analysis of individual molecules of various analytes. Interactions between the molecules of the analyte and the nanopore lead to changes in the measured ionic current flowing through the nanopore, from which properties of the analyte can be derived [1]. Protein nanopores are typically used for this purpose [2]. However, some sensing applications can only be performed under conditions where this type of pores are not stable. For this reason, we employ genetic code expansion (GCE) to incorporate the non-canonical amino acids (ncAAs) *m*-Cl-tyrosine and *p*-pentafluorosulfanyl phenylalanine, which can increase protein stability [3, 4], into the pore-forming protein bryoporin [5]. In this way, we aim to engineer bryoporin pores that can be used for nanopore sensing under harsh conditions.

Several bryoporin variants containing ncAAs were prepared and screened for efficiency of ncAA incorporation and solubility by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), as well as for proper folding and activity by circular dichroism (CD) spectrometry and hemolysis, respectively. The most active and soluble variant was used for pore formation on large unilamellar vesicles. The pores were then solubilized with detergents and purified by size exclusion chromatography. The purified pore samples were characterized by native-PAGE, cryo-electron microscopy (cryo-EM) and planar membrane experiments. Native-PAGE showed that the pore samples of the ncAA-containing variant were more homogeneous than those of the wild-type pores prepared by the same method. Opportunely, the prepared pores readily inserted into planar membranes.

Given the promising performance of the prepared ncAA-stabilized bryoporin nanopores in the experiments on planar membranes, our goal is to examine their stability in the presence of a strong denaturant, guanidinium chloride, to develop the optimal conditions for translocation and consequently identification of full-length protein analytes.

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Novel pore-gorming proteins from Molluscs with potential for nanopore sensing

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Pore-forming proteins (PFPs) are a large class of proteins found in all kingdoms of life. They are produced as water-soluble monomers which, upon binding to the target membrane, undergo conformational rearrangement and oligomerisation, leading to membrane insertion and pore formation. Depending on the secondary structure of the membrane-spanning region, PFPs can be classified into two groups – α-PFPs and β-PFPs – where the region consists of α-helices and β-barrels, respectively [1].

Due to the variety of pore sizes and specificity, PFPs represent a valuable tool in the field of sensing, which is based on the detection of analytes using electrochemical methods (resistive pulse sensing, current-voltage measurements). To date, protein nanopores have been used for the detection of various molecules, including metal ions, small molecules, nucleotides, and proteins [2]. As the demand for the detection of different molecules increases, new protein nanopores with unique characteristics are needed to offer sensing with high sensitivity and specificity.

In this work, we performed a thorough bioinformatic analysis of PFPs from molluscs, which are a rich source of PFPs [3, 4]. Using the BLAST suite, we searched the genomic and transcriptomic databases for sequences similar to known PFPs. By aligning the acquired sequences with the sequences of known PFPs, we focused on sequences with unusual properties (e.g. insertions, gaps or extensions at either termini). Finally, we employed AlphaFold2 to model the obtained sequences and compared how the predicted structures differ from those of the known PFPs. Using this approach, we obtained several interesting PFP sequences. The majority showed similarity to the actinoporin, aerolysin and haemolysin families of PFPs.

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Biophysical characterization of biomolecules at Centre of molecular structure

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Biophysical characterization at the Centre of molecular structure employs several techniques to give insight into biomolecules high-order structures, functions and activities.

The techniques provided for the determination of size, molecular mass, structure and stability of biomolecules, study of conformational changes and thermodynamics of temperature transitions including mass photometry, circular dichroism spectroscopy, spectrophotometry, Fourier-transform infrared spectrometry, fluorescence spectrometry, differential scanning fluorescence, multiangle dynamic light scattering, and differential scanning calorimetry.

Isothermal titration calorimetry, microscale thermophoresis, surface plasmon resonance and bio-layer interferometry techniques are available for the characterization of biomolecular interactions.

Centre of molecular structure of Institute of Biotechnology is a member of Instruct-ERIC, Czech Infrastructure for Integrative Structural Biology (CIISB) and Molecular-Scale Biophysics Research Infrastructure (MOSBRI).

All relevant information is on the web pages: https://www.ibt.cas.cz/cs/servisni-pracoviste/centrum-molekularni-struktury/, https://www.ciisb.org/open-access/core-facilities.

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A new standard protein to ensure better research practice and data reproducibility

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A reliable and rigorous research process relies on robust data collection, which can be achieved by regular assessment of the instrument performance and the skills of researchers. To this avail, the development of standard samples is one of the necessary tools. In the frame of the European MOSBRI project, the Pasteur-PFBMI site, 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 date, one recombinant VHH camelid antibody fragment (nanobody) has passed all the quality controls and performed well in biophysical assays, i.e. hydrodynamic characterizations, structural assays in solution and interaction assays, etc. In this poster, we will highlight the versality for biophysical characterization of this anti-lysozyme nanobody, which is now available for distribution through the MOSBRI TNA Product access mode. This standard can notably be used for benchmarking activities, user training and routine assessment of biophysical instrument operation. We hope it will help ensuring better research practice and data reproducibility throughout Europe.

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

P10

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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 [1]. The intracellular signaling molecule cyclic diguanylate (c-di-GMP) has been identified as a critical regulator of biofilm formation and dispersion in Pseudomonas aeruginosa [1]. 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 [2]. Our current work focuses on one-component signal transducers whose domains architecture involves a periplasmic nutrient-sensing domain [3, 4], a transmembrane portion, and a cytoplasmic moiety including the GGDEF and/or EAL domain; a redox switch tuning the enzymatic activity has been predicted as a strategy to sense the reducing power [4]. Membrane proteins are particularly challenging to isolate and characterize. Here we show novel results on the extraction, purification, and activity of such transducers, relevant to biofilm formation and maintenance.

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Diverse Folding Properties of $d(G_4C_2)_n$ repeats related to ALS and FTD

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The DNA molecule is best known for its double helix structure, but research in the field of structural genomics reveals that its structure is much more complex. Guanine-rich DNA sequences can self-assemble into highly stable four-fold structures called DNA G-quadruplexes (G4s). DNA G4 structures play an important role in the regulation of gene expression and chromosome dynamics. We studied the guanine-rich DNA sequence $d(G_4C_2)_n$ with n = 1, 2, 4 found in the non-coding region of the *C9orf72* gene. A large number of $d(G_4C_2)$ repeats are associated with fatal neurological disorders such as amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). The formation of G4s by $d(G_4C_2)$-repeats limits the normal functioning of the C9orf72 gene and may trigger the pathological pathways leading to ALS and FTD. In our previous research, we used dynamic light scattering (DLS) and atomic force microscopy (AFM) to confirm that all three sequences form DNA G4s [1]. We determined the translational diffusion coefficients and the length of the larger aggregates formed in the solution. Oligonucleotide $d(G_4C_2)$ forms extremely long aggregates with lengths above 80 nm, oligonucleotide $d(G_4C_2)_2$ is characterized by a short stacked dimeric G4, while $d(G_4C_2)_4$ forms aggregates of multimers corresponding to seven stacked intramolecular G4. Here, we used nuclear magnetic resonance (NMR) and complementary methods to gain insight into the structural features of $d(G_4C_2)_n$ oligonucleotides in solution. Our data are consistent with $d(G_4C_2)_n$ repeat forming a mixture of different G4 structures stabilized by K^+ ions. Diffusion coefficients determined by NMR for the three sequences revealed the presence of G-quadruplexes composed of a single G4 unit and higher-order structures based on stacking of G4 units, in good agreement with our previous light scattering studies. Different folding properties of the three sequences may rationalize the formation of aggregates of different sizes as observed previously by DLS.

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Curvature-dependent adsorption of surfactants in water nanodroplets and nanobubbles

P12

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Adsorption of surfactants at curved air-water interfaces plays a major role in phenomena involving nanodroplets and nanobubbles, such as emulsification, wetting, heterogeneous catalysis, self-assembly and cloud formation. For small enough droplets or bubbles, the large curvature of the interface can influence adsorption. We investigate this phenomenon for short-chain surfactants, using molecular dynamics simulations, in nanoscale droplets and bubbles. We show that the curvature of the interface enhances adsorption for droplets and reduces it for bubbles, affecting also other interfacial properties, such as the preferred surfactant orientation. We relate this behavior to the dependence on curvature of surface tension, described by the Tolman length correction, and show that the influence of curvature on adsorption depends also on the size of the hydrophobic tail of the surfactant. Finally, we use our theoretical model to predict the behavior of larger surfactants and of larger droplets.

In vitro Characterization of LSR interactions with ApoE isoforms

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The LSR (Lipolysis Stimulated lipoprotein Receptor) is a membrane receptor involved in peripheral and central lipid homeostasis. LSR deficiency leads to disturbances in lipid homeostasis associated with several diseases, such as Alzheimer's disease (AD) [1], making it a potential new therapeutic target, but the mechanisms involved remain unclear. The LSR recognizes lipoproteins through specific interaction with its surface protein called apoprotein E (ApoE). The *ApoE* gene has three major alleles called ε2, ε3 and ε4, which respectively code for the ApoE2, ApoE3 and ApoE4 isoforms. Particularly the e4 allele, is genetically linked to the incidence of AD [2]. We hypothesise that the specificity of LSR for the different ApoE isoforms may explain dyslipidemia as a risk factor for AD.

In this context, my project involves *in vitro* characterizing, at a molecular level, the interactions between the LSR and ApoE isoforms by interdisciplinary approaches combining protein engineering and protein/ligand interaction studies using Surface Plasmon Resonance technology. The extracellular N-terminal domain of LSR, presenting an immunoglobulin-like structure, is proposed to contain the ligand-binding domain (LBD). The N-terminal domain was immobilized on a CM5 sensor chip and peptides of the binding regions of ApoE together with free recombinant ApoE isoforms have been tested as ligands.

The results will be presented and discussed according to what was described in the literature.

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Thermodynamic and kinetic effects on the activity and selectivity of antimicrobial peptides

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Biophysical studies of the interaction of antimicrobial peptides (AMPs) with artificial membranes are pivotal in developing models of the mechanisms of pore formation. However, quantitative data on the behaviour of AMPs in real cells are scant. We developed assays allowing the thermodynamic and kinetic characterization of peptide interaction with live bacterial cells [1, 2, 3]. Our results clarified some key aspects of AMP function but also led to several new questions. For instance, a concentration range wherein some bacteria are killed, while others survive, is usually present. Within this interval (often called “mutant selection window”, or MSW) resistance can develop. Phenotypic or genetic differences between individual cells could be the origin of the heterogeneous bacterial response to AMPs. However, this explanation cannot apply to the similar behaviour observed on liposomes, which are extremely homogeneous in terms of size and composition. Our result indicate that the thermodynamic equilibrium of water/membrane partition equilibrium plays a key role in the heterogeneous response observed in liposomes. When the membrane-bound peptide concentrations are considered, rather than total concentrations, the MSW is essentially abolished. However, in situations where two cell populations (bacteria and host cells) or two different liposome types are present, thermodynamics alone does not explain the observed selectivity, suggesting that kinetic effects could be at play. For this reason, we are currently studying the kinetics of peptide/cell interactions. Our findings indicate that peptides bind to bacterial membranes in less than a second, perturb them within a few minutes, causing bacterial death, and ultimately, localize inside the cells. Overall, a combined thermodynamic and kinetic characterization provides a unique perspective on the activity and selectivity of AMPs.

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Interaction studies of molecules involved in DNA-protein crosslink repair

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DNA-protein crosslinks (DPCs) are DNA lesions that occur when a protein is irreversibly covalently bound to DNA. DPCs have adverse effects on the organism, including cancer, premature aging and neurodegenerative diseases. Due to their bulky nature, DPCs affect all DNA transactions such as replication, transcription and repair, making DPC repair an essential cellular pathway [1].

DPC repair involves several mechanisms that are still largely unexplored. We are studying two proteins of human origin involved in the DPC repair process: a DNA dependent metalloprotease called SPRTN and a hexameric AAA+ unfoldase p97. The N-terminal protease domain of SPRTN is well structured, while the C-terminal part of the protein is intrinsically disordered. There are many interaction motifs within this unstructured region. One of these is the SHP motif, which is thought to be the main interaction motif between the SPRTN and p97, but it is also utilized by many other proteins that bind to p97 [2, 3].

We have studied the molecular interactions between p97 and SPRTN, and between SPRTN and DNA using various biochemical and biophysical approaches such as pull-down assays, size exclusion chromatography (SEC), microscale thermophoresis (MST), surface plasmon resonance (SPR) and isothermal titration calorimetry (ITC). Due to the low sample consumption, rapid assay set-up and optimization, the initial interaction experiments were performed using MST. The results of the MST experiments were then confirmed with ITC experiments. The interaction studies were performed using both the full-length SPRTN and a truncated version, which has a shorter unstructured part, but still contains the SHP interaction motif. We have shown that SPRTN binds to p97 with an affinity in the low micromolar range, comparable to other p97 adapters.

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Structural fluctuations of human calmodulin at low calcium saturations

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Calmodulin is a calcium-dependent molecular switch, activated by the binding of four calcium ions to EF-hand motifs, resulting in the accessibility of previously hidden apolar surfaces for the binding of numerous protein targets with diverse roles in signal transduction. It is well known that the calcium binding is cooperative and can be modeled by the allosteric equations developed for hemoglobin, nevertheless, the molecular details of this allostery is still unknown. Calmodulin can bind multiple partners, so there must be a mechanism for target-selection, depending on the primary receptor activations responsible for the intracellular calcium rise. By analyzing experimental calmodulin-target structures, canonical binding modes were identified and hence the hypothesis of induced-fit emerged. In a living cell, however, several targets are available at the same time for the few micromolar concentration of calmodulin to bind upon calcium concentration increase. Intriguing observations are i) the presence of small amplitude calcium „puffs" causing only a slight increase in the cytoplasmic calcium concentration, and ii) the large temporal profile variation of the calcium signals originating from the pattern of activated receptors. We show by red-edge enhanced fluorescence lifetime spectroscopy and low-wavenumber FTIR spectroscopy, that calmodulin displays a markedly increased conformational heterogeneity, both with and without partner peptide, exactly in the low calcium-load range originated from the "puffs" and receptor-dependent transient calcium signals. All-atom molecular dynamics simulation shows elevated extremely low (< 5GHz) frequency fluctuations in the Van der Waals energy term in the C-terminal only-saturated calmodulin. These results suggest that the input information of which source caused the calcium transient may be encoded in the low frequency oscillations of calmodulin, and by resonance this mechanism enables and enhances the subsequent selective binding to the downstream signaling partners. Thus, calmodulin's function can be described by a conformational resonance-selection mechanism.

C-terminus acting as the third conformational switch of small GTPase Ran

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Ran (Ras-related nuclear) is the main regulator of the nucleocytoplasmic transport through the nuclear core complex. As other small GTPases it functions as a molecular switch cycling between the GDP-bound inactive and GTP-bound active state.

Ran consists of a globular domain and a C-terminal region, which is bound to the G-domain in the GDP-bound states. The crystal structures of the GTP-bound form complexed with Ran binding proteins (RanBP) show that the C-terminus undergoes a large conformational change, embracing Ran binding domains (RanBD), whereas in the crystal structures of macromolecular complexes not containing RanBDs the structure of the C-terminal segment remains unresolved, indicating its large conformational flexibility. This movement could not be followed either by experimental or simulation methods.

Here, by using molecular dynamics (MD) and Molecular Dynamics with excited Normal Modes (MDeNM) simulation methods, we present the nucleotide specific dynamical behaviour of the C-terminus and interpret its functional effect.

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Nep1-like proteins – how many sugars they prefer?

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Necrosis- and ethylene-inducing peptide 1 (Nep1)-like proteins (NLPs) are protein effectors from plant pathogens [1]. Upon plasma membrane recognition, they form small pores of transient nature to damage the cell [2]. Their receptors are plant glycosphingolipids glycosyl inositol phosphoryl ceramides (GIPCs) [3]. Although the core structure of GIPCs is well-defined, they represent a family of various molecules [4]. Their polar headgroups, known to interact with NLPs, differ not only in the number and identity of sugar moieties but also in the way of their connection. GIPC-rich plant tissue extracts have been used to study the NLP-GIPCs interaction. Such extracts contain GIPCs with different sugar headgroups as well as impurities. Therefore, the NLP preference for GIPC sugar headgroup composition remains poorly understood.

To obtain a more detailed knowledge of NLP binding specificity, we developed a method for purification of GIPCs from GIPC-rich plant extracts. We separated tobacco and leek extracts by high-performance thin-layer chromatography (HPTLC) to yield GIPC fractions with (more) homogenous sugar headgroups. Fractions were analyzed by tandem mass spectroscopy (MS/MS) and isolated. We used the isolates for various experiments to test the binding of NLPs: sedimentation assays, TLC blotting followed by immunostaining, and surface plasmon resonance (SPR) analyses. GIPCs with three sugar moieties in their headgroup were consistently shown to be the preferred target of NLPs, resulting in the most stable interaction. The importance of binding stability for the following steps of pore formation needs further investigation.

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The influence of pH on the G-quadruplex ↔ i-Motif ↔ DNA duplex transition

P19

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It is known that in specific regions of double-stranded genomic DNA (such as telomeres, promoter regions of oncogenes, centromeres, etc.) there exist G-rich and C-rich short regular sequences capable of forming four-stranded G-quadruplex and i-Motif structures. Cells utilize the formation of such structures as molecular switches to regulate cellular processes under physiological conditions. The discovery of this fact has become the basis for the formation of a new direction in anti-tumor therapy, based on the targeted generation or disruption of G-quadruplex and i-Motif in selected DNA regions.

In this work, using circular dichroism and differential scanning calorimetry methods, we investigated structural transitions in a 22-mer segment of human telomeric DNA: the G-rich d[5'-A(GGGTTA)3GGG-3'] and the complementary C-rich d[3'-T(CCCAAT)3CCC-5'], as well as their 1:1 mixture depending on the pH of the environment. Studies were conducted at three physiological pH values (5.5, 6.5, and 7.0). The formation of G-quadruplex and i-Motif was monitored by circular dichroism spectra. Calorimetric melting curves of G-quadruplex, i-Motif and their 1:1 mixtures at various pH values were then recorded. The research results showed that upon mixing equimolar amounts of G-quadruplex and i-Motif, the formation of a duplex is enthalpically preferable at all pH values. However, in an acidic environment (pH 5.5) the i-Motif is enthalpically favorable and can coexist with the duplex. Increasing the pH to neutral reduces the enthalpy of melting of the i-Motif structure to zero, thereby shifting the equilibrium towards the duplex.

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The binding of new structural classes of C-terminal inhibitors to human Hsp90 studied by ligand-based NMR methods

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Heat shock protein 90 (Hsp90) undergoes large conformational changes during its chaperone cycle which is essential for its client proteins to be correctly folded, activated and stabilized. Hsp90 is a potential target for cancer treatment since many of its client proteins are oncogenic. Apart from N-terminal inhibitors (NTI) which target its active site, there were also C-terminal allosteric inhibitors (CTI) developed. However, compared to NTI, there is no co-crystal structure of allosteric CTI bound to Hsp90.

Using ligand-based NMR methods Saturation Transfer Difference (STD) NMR and transferred NOESY, we have investigated the interactions between Hsp90 and a new class of triazole-based CTI and TVS21 analogs [1,2]. For this purpose, group epitope mapping (GEM) analysis was performed using data obtained by 1D ^{1}H STD NMR experiments of CTI in the presence of human Hsp90. Using TR-FRET technique, it was confirmed that TVS21 analogs bind to the C-terminal domain and not to the N-terminal domain of Hsp90 [2]. Furthermore, GEM analysis of the selective TVS21 analog differed in the absence or presence of a non-hydrolysable ATP analog resulting in an open and a closed conformation of Hsp90α, which would not be expected for surface binding. The most promising CTI inhibited cell proliferation and induced apoptosis *in vitro* and limited tumor growth *in vivo* [1,2]. In addition, the most promising TVS21 analogs induced the degradation of key oncogenic proteins associated with TNBC progression, without inducing a heat shock response [2]. In the future, we plan to investigate the binding of CTI using protein-based NMR methods and human Hsp90 with selectively isotopically labelled methyl groups and fluorinated amino acids.

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Exploring the impact of MurD conformational dynamics on ligand binding with NMR and MD simulations

P21

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MurD (UDP-*N*-acetylmuramoyl-L-alanyl-D-glutamate ligase) is a 47.7 kDa multidomain protein, crucial for intracellular peptidoglycan synthesis, making it potential target for novel antibacterial agents. In its interactions with substrates, it undergoes significant structural changes, transitioning between its open, semi-closed, and closed states.

Efforts to develop potent MurD inhibitors have seen limited success, with the most effective reaching only single-digit µM IC_{50} values. This is mainly due to the complex interplay between the inhibitors' flexibility and the protein's intrinsic dynamics, where subtle yet persistent domain movements can change ligand conformation, potentially causing stretching or detachment from the binding site. Despite modifications to improve ligand binding, the dynamic nature of protein-ligand complexes continues to pose significant challenges and static structural data prove insufficient for structure-based design.

In this study, we explored the dynamics of ^{2}H and ^{15}N isotopically labeled MurD enzyme in its apo and ligand-bound states using NMR methods. Utilizing Reduced Spectral Density Mapping combined with Principal Component Analysis, we characterized the spectral density functions $J(\omega)$ across the protein's backbone, identifying distinct dynamics of residues and regions across the three states on the ps-ms timescale. Our findings reveal three categories of dynamic behavior: residues with enhanced ps-µs dynamics, residues showing increased rigidity, and residues undergoing conformational exchange on the µs-ms timescale. Additionally, conformational clusters of MurD were identified in 1 µs molecular dynamics simulations, suggesting new insights into the protein's approach to binding and the potential existence of rarely populated states. Further ^{13}C relaxation measurements of methyl-bearing side chains in MurD could enable the identification of these rarely populated states and slow exchange dynamics.

This new understanding of MurD's conformational variability offers potential avenues for rethinking drug design strategies targeting this promising antibiotic candidate.

Investigation of the interactions between the potyviral coat protein and the helper component proteinase

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Potyviruses are plant RNA viruses that represent a significant threat to crops worldwide. Despite their economic importance, the molecular mechanisms underlying the infection process of potyviruses remain poorly understood. Our research focuses on two key potyviral proteins, the coat protein (CP) and the helper component proteinase (HCPro). The CP primarily serves as a structural component that forms flexible, filamentous virions, yet it exhibits multifunctionality. In particular, CP is involved in aphid-mediated transmission of potyviruses, where interactions with HCPro, another multifunctional protein, are crucial. In this study, we used cryo-electron microscopy (cryo-EM) to determine the three-dimensional structure of potato virus A (PVA) and PVA virus-like particles (VLPs) generated through bacterial expression of ^{PVA}CP. These structures showed a high similarity between the external surface architecture of virions and VLPs. On this basis, we used VLPs to study the interactions with HCPro. We have established an *in vitro* system for investigation of potyviral CP-HCPro interactions, employing VLPs alongside HCPro in fusion with the globular affinity tag (AT) at the N-terminus (AT-HCPro). Using affinity chromatography, we isolated AT-HCPro-VLP complexes and visualized them with cryo-EM. In addition, the VLP-HCPro binding affinities were assessed with microscale-thermophoresis. Our findings demonstrate the binding of AT-HCPro onto the surface of VLPs. Moreover, the same procedure was repeated with VLPs and HCPro based on potato virus Y (PVY), showing that both intraspecies (PVA-PVA, PVY-PVY) and interspecies (PVA-PVY) CP-HCPro interactions occur with comparable affinity. This suggests conservation of binding modes across potyviral species.

Core facility for biomolecular and cellular anaylsis (BmCA) at BOKU university

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The BOKU Core Facility Biomolecular & Cellular Analysis (BmCA) is a state-of-the-art analytical laboratory offering a wide range of equipment and methods. The facility is dedicated to advancing research in three main areas: (I) the characterization of biomolecules and their interactions, (II) cellular analysis, and (III) X-ray crystallography.

Central methods for the characterization of biomolecules include differential scanning calorimetry (DSC), size exclusion chromatography - light scattering (OMNISEC), and dynamic light scattering. For investigating biomolecular interaction, we offer surface plasmon resonance (SPR), bio-layer interferometry (BLI), and isothermal titration calorimetry (ITC). Flow cytometry enables comprehensive cell analysis, allowing researchers to study cell dynamics and phenotype, while advanced sorting systems facilitate the isolation of specific cell populations. The third research focus of the core facility is X-ray crystallography for the elucidation of three-dimensional protein structures with atomic resolution. We streamlined the preparation of crystallization plates through automated formulation of crystallization screens and a pipetting robot. Additionally, our crystal imaging hotel provides a convenient online platform for observing these plates remotely.

At BmCA, we are committed to fostering scientific excellence through professional training and consultation services. We offer training programs for specific techniques, as well as expert guidance on experimental setup and design. Additionally, we provide a full-service option, which includes the measurement of your samples by trained staff and the generation of experimental reports.

Metabolism and DNA repair: clues from the role of human Serine Hydroxymethyltransferase

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Serine Hydroxymethyltransferase (SHMT) is a metabolic enzyme involved in the reversible conversion of serine and tetrahydrofolate, into glycine and N5,N10- Methylenetetrahydrofolate. This tetrameric protein is PLP dependent enzyme, that works as a dimer of obligate dimers.SHMTs is a part of a complex network of metabolic pathways, the one carbon metabolism (OCM) that fuels cancer cells proliferation. Therefore, SHMTs are overexpressed in several types of tumors, being an interesting target for cancer therapy. In humans, there are two genes encoding SHMT, a cytosolic (SHMT1) and a mitochondrial (SHMT2) isoform [1].

In addition to having catalytic activity, it has shown SHMT1 also has nucleic acids binding affinity. In cytosol SHMT1 binds SHMT2 transcript 5'UTR, through which negatively regulates SHMT2 expression and in turn serine to glycine reaction, catalyzed by SHMT1, is negatively ribo-regulated by the same mRNA [2]. Furthermore, SHMT1 translocates into the nucleus during S and G2/M phase or in response to DNA damage, to form a protein complex with DHFR and TYMS for de novo thymidylate biosynthesis in situ [3] [4] [5].

To understand how SHMT is involved in DNA repair, we characterized the molecular basis of SHMT1-DNA interaction in vitro and in cellulo. In vitro SHMT1 preferentially binds ssDNA, which affects the enzyme catalytic activity. Cell studies show how SHMT1 knock out leads to an increased interferon-β expression and a higher phosphorylation of H2AX histone.

Moreover, preliminary studies show it leads also to a major accumulation of uracil in the genome and to an elevated content of fragmented DNA.

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Aptamer-based platform for accelerated biologics development

P25

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With the strategic realignment of Novartis into a company fully focused on innovative medicines, novel biologics formats are on the rise. Consequently, traditional purification methods based on protein A chromatography require re-evaluation. Recognizing this paradigm shift, we are exploring alternative purification approaches. Custom-made aptamers, renowned in academia as potent reversible protein binders, offer a promising - yet rarely tapped - opportunity for support within the pharmaceutical industry. Our aim is to investigate their potential application in the purification of molecules for analytical purposes at an early stage of the development pipeline.

Following established protocols [1], we synthesized aptamers tailored to specific molecules and screened their binding efficiency using surface plasmon resonance. Subsequently, we investigated setups for the purification of the target molecules from the harvest material and identified the optimal aptamer-based resin for integration into our process analytics framework.

Building on this foundation, we aim to establish a robust platform for the routine generation of aptamers tailored to new biological entities [2]. This platform can be seamlessly integrated into high-throughput purification processes, titer determinations and bioassay development. Given the imperative to accelerate the development of biologics, this approach promises significant time savings and allows critical milestones in early process development to be achieved faster than with traditional separation approaches. By embracing the aptamer technology, we anticipate not only accelerated processes but also substantial cost savings, reflecting our commitment to advancing biologics development in a sustainable and innovative manner.

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Killing cells – MLKL and membrane disruption

P26

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Mixed lineage kinase domain-like (MLKL) protein plays a central role in necroptosis [1,2], a regulated form of cell death characterized by membrane disruption. Understanding the molecular mechanisms underlying MLKL effector function is essential for elucidating the intricate processes of necroptosis.

Oligomerization of MLKL is critical for the execution of necroptosis. To gain insight into the oligomeric state of MLKL, we performed cross-linking experiments, size-exclusion chromatography and RALS/LALS analysis. Our data revealed the presence of MLKL dimers, trimers, tetramers and higher-order oligomers, supporting the hypothesis that oligomerization is a crucial step in MLKL-mediated membrane disruption.

To further investigate the functional implications of MLKL in membrane disruption, we generated stable inducible FlpIn HEK293 cell lines expressing different MLKL constructs. This allowed us to investigate the properties of MLKL variants and their effect on cell death. Using confocal fluorescence imaging of fixed cells and functional assays, we observed that MLKL expression resulted in membrane localization and disruption, consistent with the protein's role in necroptosis.

To better understand the interaction between protein and membrane, we prepared different liposome mixtures and tested liposome leakage. The experiments showed a concentration-dependent liposome leakage and a positive effect of inositol phosphates on membrane disruption.

Further investigations into the structural properties and dynamics of MLKL oligomers will contribute to a comprehensive understanding of MLKL effector function and may offer potential therapeutic targets for diseases involving dysregulated cell death pathways.

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Dissecting annexin A11 in its domains: structural and functional characterization

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Annexins are a family of Ca^{2+}-dependent phospholipid-binding proteins with several functions. Among these, there is Annexin A11, whose mutations have been found in patients with ALS, a neurodegenerative disease. Annexin A11 has the longest N-terminus domain within Annexins, formed by 197 amino acids of which most are glycine, proline, and tyrosine, and a very conserved C-terminus that hosts four calcium-binding motifs that has been suggested to be involved in the binding to phospholipids[1]. One of the functions recently attributed to this protein has been the ability to passively transport RNA granules, via its C-terminus that tethers to lysosomes, enabling protein translation *in* situ in the intrasynaptic cleft.[2]. The aim of the present work has been the dissection of the Annexin A11 in its structural and functional domains, with a particular focus on measuring the affinity of the C-terminus for the calcium and the effect of the latter on the binding between the C-terminus and membrane phospholipids. After obtaining the two domains soluble, circular dichroism has been performed showing that the C-terminus has mostly alpha-helices whereas the N-terminus is unstructured. The binding affinity of calcium to the C-terminus domain has been measured by fluorescence spectroscopy, leading to a Kd of 0,6 µM. Following, pull down assays have been conducted *in vitro* with lysosomes-like liposomes, confirming that the purified C-terminus domain is able to interact with membrane phospholipids in a calcium-dependent manner, as suggested *in vivo*[2]. Lastly, MST has been performed to obtain a Kd for the interaction between C-t and phospholipids, both with and without calcium. The results obtained made it possible to confirm the interaction but not to quantify it yet.

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Biochemical and structural characterization of a newly identified lipase in hazelnut (*Corylus avellana*)

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Rancidity is common in oilseeds. Although the molecular basis of this process has not been clarified, lipases play an essential role in the release of free fatty acids (FFA) that undergo rancidity much faster than triacylglycerols (TAG) [1]. From the industrial standpoint the identification and characterization of such enzymes as potential predictive markers of raw material shelf-life is essential to a more sustainable and efficient food processing and storage [2].

To this purpose, we established a new protein purification protocol, combined with a lipolytic enzymatic assay, to isolate lipase(s) in hazelnut. This led to the identification of a purified protein of 9 kDa, active towards synthetic and natural substrates. Surprisingly, this new enzyme shares little if no homology with canonical lipases.

Different approaches were applied for its biochemical, biophysical, and enzymatic characterization. As a first step, a measurement of its apparent molecular mass by size exclusion chromatography (SEC) was carried out confirming the monomeric state of the protein. The structural characterization via circular dichroism reveals a predominantly α-helical structure, characterized by two minima at 208 and 222 nm. The stability of this novel lipase was assessed through a thermal ramp, indicating a gradual unfolding with almost 60% loss of secondary structure. This exceptional stability is attributed to the presence of 8 cysteines involved in 4 disulfide bridges. The study on thermal activity revealed that the optimal conditions for conducting experiments are at 37°C and pH 8.00.

In summary, our preliminary data highlight that the newly discovered enzyme is structurally stable as a lipase, but its activity is easily compromised.

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Novel genetically encoded probes for functional imaging of cell signaling

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Genetically encoded fluorescent probes convert specific biomolecular events into optically detectable signals. Typically, such probes work by modulating the absorption or emission spectrum of the fluorophore, through a suitable fluorescence quenching process. Here we present a very different, widely applicable design of genetically encoded fluorescent probes that takes advantage of an unrelated detection principle: directionality of optical properties of fluorescent proteins. The probes offer an extremely simple design, high sensitivity, multiplexing capability, ratiometric output, resilience to bleaching artifacts, without requiring any modifications to the proteins of interest. The probes are applicable to imaging cellular activity of G protein coupled receptors, G proteins, arrestins, receptor tyrosine kinases and other signaling proteins.

Characterization of a lectin from *Clitocybe nebularis*

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Lectins, a ubiquitously occurring and structurally diverse group of carbohydrate-binding proteins, have a variety of functions. Their biological functions include the binding and clustering of glycoproteins, such as cell surface receptors and components of the extracellular matrix, and the subsequent initiation of signal transduction pathways. Due to their versatility, lectins find applications in biotechnology, ranging from cancer diagnostics to the development of bioinsecticides, wherever specific carbohydrate targets are present [2]. Notably, the fungal species clouded agaric (*Clitocybe nebularis*) harbors interesting lectins such as CNL, which exhibits potent anti-nutritional activity against Colorado potato beetle larvae, and CnSL, which shows particularly pronounced insecticidal activity against *Drosophila melanogaster* [1].

The isolation of CnSL from the extract of the *Clitocybe nebularis* fruiting bodies by affinity chromatography enabled further steps such as determination of the N-terminus, cDNA sequencing and cloning into expression vectors for further characterization. The expression of a recombinant CnSL variant in *Escherichia coli* using an isotope-labeling medium enabled further structural determination using nuclear magnetic resonance spectroscopy (NMR). Characterization efforts continue, with a focus on studying the interaction partners of CnSL using carbohydrate microarrays and elucidating the interactions between the lectin and its ligands using NMR and complementary techniques. In addition, the functional properties of the recombinant CnSL will be compared with those of the natural isolate.

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The antioxidant activity of the *Calluna vulgaris* (L.) Hull. extracts obtained by supercritical CO_2 extraction

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Calluna vulgaris (L.) Hull. is an evergreen perennial plant of the *Ericaceae* family possesses noteworthy pharmacological properties and has long been used in traditional medicine for addressing many medical conditions [1]. A plethora of bioactive compounds have been discovered in C. vulgaris, with phenolics being the primary source of its diverse health benefits, including antioxidant, anti-inflammatory, and antimicrobial properties, among others [2, 3]. However, a detailed study of its antiradical activity has not been conducted before. Herein, the supercritical CO_2 extraction of *C. vulgaris* branches wild pomegranate peel has been employed to obtain an extract rich in polyphenolic compounds. Supercritical CO_2 extraction, a green technology method, replaces classical solvent-based extraction, enabling the isolation of plant compounds in their purest form without degradation of the sample [4]. Owing to its high sensitivity and specificity, Electron paramagnetic resonance (EPR) spectroscopy has been utilized to access the extract's scavenging activity against free radical species (DPPH and hydroxyl radicals). The spin-trapping technique was employed to detect activity against short-lived hydroxyl radicals.

The results demonstrate that the *C. vulgaris* brunch extract is a significant free-radical scavenger. It exhibited remarkable selectivity in eliminating hydroxyl radicals (68.5% compared to 21.7% for DPPH), which is particularly noteworthy as hydroxyl radicals are considered the most reactive oxygen radical species, biologically relevant, and responsible for a number of pathologies. These findings underscore the therapeutic potential of *C. vulgaris* brunch extract in combating oxidative stress-related medical conditions but also suggest possibilities for further investigation into its mechanism of action and formulation into pharmaceutical or nutraceutical products.

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Differential interactions between ATP and NGF/proNGF revealed ATP as modulator of Neurotrophins biological activity

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The prototype of the neurotrophin family, Nerve Growth Factor (NGF), is essential for the development and maintenance of neurons and is crucial in immune and endocrine systems and in the pain pathway. NGF precursor, proNGF, whose pro-peptide is an intrinsically unstructured domain (IUD), is endowed with different biological properties. The binding to TrkA, p75NTR and sortilin receptors activates the NGF/proNGF signaling pathways. Much is known about NGF in neuronal physiology. However, few reports described essential endogenous ligands as modulators of NGF biology.

Recently, the binding of ATP to NGF was identified. To determine the molecular elements of this binding, we used integrative structural biology to unveil for the first time the binding cartography of ATP to NGF [1]. ^{1}H-^{15}N HSQC NMR, coupled to the determination of the 3D solution NMR structure of NGF and MD simulations, helped identifying the likely binding mode of ATP on NGF. ATP/NGF binding to the receptors was investigated through Surface Plasmon Resonance (SPR). We also undertook a complementary biophysical study on the binding of ATP to proNGF. Our results reveal a different binding profile for mature and precursor proteins. A combination of Small Angle X-ray Scattering (SAXS), Hydrogen-Deuterium Exchange Mass Spectrometry (HDX-MS) and limited proteolysis showed that ATP binding induces a change in the conformation and/or dynamics of proNGF, predominantly in the IUD pro-peptide [2].

Combined, these results suggest a functional role for ATP in modulating the biological role of proNGF/NGF in health and disease states.

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Structure, affinity, and anti-SARS-CoV-2 efficacy of M^{pro} and cathepsin inhibitors

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As the COVID-19 pandemics emerged in 2020 the urgent need for specific antiviral drugs targeting various stages of viral cell entry and replication led to a massive X-ray crystallographic screen of two repurposing drug libraries of more than 10.000 compounds (1). With this approach, we identified Calpeptin as a covalent active site inhibitor of SARS-CoV-2 main protease (M^{pro}) and as such a promising drug candidate. However, due to a moderate activity in M^{pro} inhibition assays, we conducted a follow up study (2) investigating potential of dual-targeting effect of Calpeptin and its derivatives (S-Calpeptin, GC-376) towards cysteine cathepsins. Inhibition of cathepsins L, V and K in picomolar range indicated that in the cellular context several cysteine cathepsins are inhibited more effectively than viral M^{pro}. Treatment of SARS-CoV-2 infected hamsters with the S-Calpeptin resulted in a considerable decrease in viral load in trachea, emphasizing its therapeutic potential. Because of its widespread tissue distribution, cathepsin L appears a prospective drug target, so our subsequent study focused on the role of cathepsin L in SARS-CoV-2 infection (3). We characterized the anti-SARS-CoV-2 activity of a set of carbonyl- and succinyl epoxide-based inhibitors, which were previously identified as inhibitors of cathepsins or related cysteine proteases. Calpain inhibitor XII, MG-101, and CatL inhibitor IV inhibited cathepsin L in the picomolar Ki range. They also showed antiviral activity in the very low nanomolar EC50 range in Vero E6 cells. We showed a relevant off-target effect of cathepsin L inhibition by the coronavirus main protease α-ketoamide inhibitor 13b. Crystal structures of cathepsin L in complex with 14 compounds at high resolutions provide a basis for structure-guided understanding and optimization of cathepsin L inhibitors aiming at drug development.

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Analyzing enzymatic kinetics in model extracellular matrix: a biophysical approach

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The extracellular matrix provides not only a physical scaffold in which the cells are embedded, but also regulates many cellular processes including growth, migration, differentiation, survival, homeostasis and morphogenesis [1]. It consists of a variety of different molecules like collagen, elastin, fibronectin, laminins and glycan conjugates forming a dynamic complex system that constantly undergoes changes. Deregulation of kinetic pathways will alter the composition of the extracellular matrix (ECM), which may favor various pathological conditions such as tumor formation. Before addressing the enzymatic kinetics in such complex natural media, we have resorted to the studies in ECM mimics that are suitable enough for investigating (i) the properties of such media (viscosity, diffusion, excluded volume) and (ii) interactions between components (i.e. enzyme – substrate, enzyme -crowder). We mainly focus on the activity of three enzymes operating in the ECM: hyaluronidase, elastase and collagenase. The reactions kinetics of hyaluronidase was previously investigated in the presence of polyethylene glycol (PEG) [2] and the quantification of products with capillary electrophoresis allowed us to derive a kinetic model valid for crowded conditions as well [3]. Here we present our recent work related to the activity of elastase and collagenase. The ECM is modelled with dextran macromolecules of three different sizes (10, 40 and 470 kDa). The enzymatic activity is followed with spectrophotometry by measuring fluorescence recovery after substrate cleavage (we use short peptides with appropriate labelling). The obtained data are carefully analyzed to extract kinetic parameters, and to get a better insight into the reaction mechanism under crowding conditions.

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Mapping the interaction site of the antibiofilm protein cocaprin 1 with *Listeria* cell wall components

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A fungal protein from *Coprinopsis cinerea* named cocaprin 1 (CCP1) has recently been described as a protease inhibitor that exhibits a completely new arrangement of inhibitory functions than other currently known fungal beta-trefoil peptidase inhibitors [1]. It shows strong antibiofilm properties against *Listeria* by interfering with bacterial adhesion, but its activity is not the result of protease inhibition. Due to sequence and structural similarity with the fungal lectins MpL and CNL, *fast photochemical oxidation of proteins* (*FPOP*) was used to test the possibility of binding of bacterial cell wall components, peptidoglycan and wall teichoic acids, by CCP1. Mutanolysin digested *Listeria* cell wall sacculi were used to detect significant changes in protein oxidation upon binding of cell wall components to CCP1. Peptides corresponding to protein residues 39-48, 49-58 and 82-104 were significantly protected from oxidation after binding ($p \leq 0.05$), while the other peptides showed no significant oxidation changes. This result confirms that the protein binds to the cell wall components, either carbohydrate or peptide moieties, and suggests that these protected regions are either directly involved in binding or are affected by allosteric changes after binding. Studies are currently being carried out to identify the protein residues and cell wall components involved in the interaction.

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Molecular analysis of peptidase-inhibitor interactions

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Peptidases play vital roles in the body's metabolism and its regulation by diverse signaling pathways. For elucidating the physiological role of the peptidases, small molecule inhibitors can be developed that selectively target the enzyme of interest and can serve as leads for future drug or probe development. Using Isothermal Titration Calorimetry (ITC) and Grating-coupled Interferometry (GCI, Creoptix WAVEdelta), we aim at analyzing molecular interactions of proline-specific peptidases, all members of the S28 and S9 families of serine proteases with such small molecule inhibitors. Since the detection methods rely on basic aspects of the interactions (ITC: heat exchange, GCI: mass accumulation upon binding), no detection labels are needed on the molecules under study. By combining ITC and GCI, we verify and determine the affinity (K_d) and kinetic aspects (k_{on} and k_{off}), as well as the thermodynamic characteristics stoichiometry (N), entropy ($-T * \Delta S$) and enthalpy (ΔH) of interactions of the peptidases with the inhibitors. In this way, we aim at characterizing the binding of the inhibitors for modulating the peptidase activity and uncovering the physiological and molecular role of the peptidases under study.

Biophysics instrumentation from Malvern Panalytical

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Malvern Panalytical develops a number of analytical methods valuable to the biophysicist and biochemist. We provide an overview of some of these techniques, and opportunities for early career researchers and collaboration potential. Isothermal titration calorimetry (ITC, the PEAQ-ITC product) characterises binding affinity and thermodynamics, in solution and label-free, by measuring heat released upon molecular interactions. Grating coupled interferometry (GCI, the WAVE product) characterises binding affinity and kinetics on a 2D biosensor chip, based on changes in refractive index in the vicinity of the biosensor surface upon molecular interactions. Dynamic light scattering (DLS, the Zetasizer product) characterises molecular size and surface charge, the former quantity by the auto-correlation of (stochastic) scattered light, the latter by rate of (coherent) movement through an electric field. Finally, nano-tracking analysis (NTA, the Nanosight product) measures particle size at the single-particle level by taking videos of the diffraction patterns of diffusing particles.

Cooperative dynamics of PARP1 zinc-finger domains in the detection of DNA single-strand breaks

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The process of repairing single-strand breaks (SSBs) in DNA is a complex mechanism that begins when the PARP1 (poly(ADP)ribose polymerase-1) protein detects an SSB [1,2]. This protein recognises the break using its zinc finger domains, Zn1 and Zn2. Although the role of these domains in identifying SSBs has been extensively studied, the complex dynamics of the repair process remain elusive. To gain more insight, we conducted all-atom Molecular Dynamics (MD) simulations focusing on the interaction between the SSB and the Zn1-2 domains of PARP1. These simulations were performed on various SSB configurations, each with different terminations at the 3' and 5' ends of DNA. Additionally, we studied the action of PARP1 as a dimer, which revealed a cooperative dynamic between the Zn2 and Zn1 domains. Our results align with experimental observations that show a rapid interaction of Zn2 with the DNA, followed by Zn1's engagement with the break site. The simulations suggest that DNA strands can naturally open up due to thermal fluctuations, making the break accessible for Zn2 to bind with the exposed nucleotides. Such spontaneous openings are quite rare in long, free DNA strands. Furthermore, we observed DNA bending in specific configurations when the phosphate backbone grip of Zn1-2 clamps onto the DNA. This facilitates the formation of hydrogen bonds between Zn1-2 & the major/minor grooves of the DNA. Although Zn1's involvement is secondary to Zn2's, its position relative to the terminal nucleotides and Zn2 is critical for the entire process. In summary, our study sheds light on the intricate dynamics of SSB repair facilitated by PARP1, particularly emphasising the collaborative roles of the Zn1 and Zn2 domains in this essential cellular function.

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CpH-MetaD: coupling wt-metadynamics and CpHMD in the study of RNA oligomers

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RNA molecules have a wide range of biological functions due to their highly flexible structures. Their flexibility stems from complex H-bonding networks defined by both canonical and non-canonical base pairs. With some non-canonical base pair interactions requiring (de)protonation events to either stabilize or perturb H-bond networks.

Constant pH molecular dynamics (CpHMD) methods provide a reliable tool to describe the conformational space of dynamic structures and to obtain robust calculations of pH-dependent properties (i.e. pK_a). However, pH-sensitive methods have rarely been explored in the field of nucleic acids, despite growing biological evidence concerning pH regulation of certain motifs' H-bond networks.

In this work, we present an extension of the stochastic CpHMD method [1] to RNA from the standard XOL3 AMBER force field [2] and demonstrate the accuracy of our method to reproduce pK_a's of RNA oligomers.

Poly-U trimers and pentamers with a single central titrable site were characterized for method validation [3]. To tackle their high degrees of freedom, we have integrated a well-tempered (wt) metadynamics approach [4] into the CpHMD methodology (CpH-MetaD). The CpH-MetaD technique significantly expanded the sampled conformational space, allowing for more robust and accurate estimates of the oligomers' pK_a shifts with respect to the single nucleoside pK_a's: 0 and 0.4 (A3mer and A5mer); 0~0.1 and 0.7 (C3mer and C5mer).

The predicted pK_a values - A3mer: 3.55 (0.05); A5mer 4.0 (0.2); C3mer: 4.7 (0.2); C5mer: 5.0 (0.1) - and relative shifts are in good agreement with experimental data [3]. Nucleobase stacking and electrostatic interactions with phosphate groups clarify the intramolecular phenomena which dictate the experimentally observed pK_a shifts. This work highlights the robustness and accuracy of CpHMD/CpH-MetaD applied to RNA oligomers, and the proton binding affinity sensitivity to phosphate group content in the RNA backbone.

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Cavitation in water: significant impact of nonpolar impurities

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The stability of water is a long-standing problem in physics, studied since the 17th century [1] and continuing to be a subject of investigation today. There is a notable discrepancy between experimental findings and theoretical predictions, as well as inconsistency in measurements across different experiments. While theory predicts that water should be remarkably stable against cavitation, experiments show quite the opposite. [1,2]

In this talk, I will present our work on the conditions that lead to catastrophic cavitation events in decane and water. Additionally, I will discuss how the tensile strength of water is influenced by hydrocarbon impurities, such as oil droplets. We use a framework that combines classical nucleation theory with molecular dynamics simulations.

We find that while pure bulk water is exceptionally stable against cavitation, the presence of even tiniest amounts of decane is enough to destabilize water and reduce its tensile strength to experimentally measured lower values. Using our numerical analysis, we find that a decane droplet of a radius of around 1 nm in a macroscopic volume of water is enough to destabilize the system. This is the reason why even in ultra-pure water, the measured tensile strength is significantly lower compared to theoretical predictions. We also find that the curvature correction of surface tension is important to take into account when studying cavitation, nanodroplets, or nanobubbles.

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Rationalizing the Berg limit of 65° in surface phenomena

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I will discuss the puzzling universality of the fundamental water contact angle of 65°, known as the "Berg limit," [1] which is essential for phenomena such as long-range hydrophobic attraction, macromolecular adhesion, and biofouling in aqueous environments. By conceptualizing these interactions as three-phase problems, involving a surface, water, and a representative oil-like material indicative of nonpolar entities, we have recently offered a new understanding of this threshold [2, 3]. Our analysis, supported by molecular simulations [3], indicates that attraction and adhesion correlate with surfaces displaying underwater oleophilic properties, a condition termed "hydrophobicity under oil." This condition typically arises when the contact angle exceeds 65°. The insights gained from this approach not only clarify the mechanistic underpinnings of the Berg limit across various scenarios but also suggest broader implications for technological applications and material design. I will also discuss the complexities introduced by real-world variations such as surface roughness, heteroatoms in the hydrophobic components, and the electrostatic properties of surfaces, which contribute to the observed range of behaviors and the practical applicability of the Berg limit in diverse environmental and biological contexts.

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Alphafold model of the flexible protein CP12 from a diatom challenged by a combination of SDSL-EPR, SAXS and Molecular Dynamics

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The chloroplastic protein CP12 is a conditionally disordered protein described in several photosynthetic organisms (land plants, green microalgae, cyanobacteria): its disordered state depends on the day/night cycle and the redox conditions of the chloroplast. CP12 regulates the Calvin cycle by interacting with two key enzymes: glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and phosphoribulose kinase (PRK) [1].

A new CP12 from the marine diatom *Thalassiosira pseudonana* has been recently discovered. The regulation of the Calvin cycle in diatoms significantly differs from other photosynthetic organisms and remains enigmatic. From a structural point of view, this newly discovered CP12 is atypical, being *dimeric* unlike all other CP12 that are monomeric, with a predicted coiled coil domain [2].

We have used Alphafold to generate a 3D structure of this CP12. However, this model does not account for the SAXS data obtained on the protein. We have therefore undertaken a strategy based on the use Site-Directed Spin Labeling combined with Electron Paramagnetic Resonance (SDSL-EPR) spectroscopy to improve this model and explore the structure and dynamics of this CP12 in solution. With various labeled variants of CP12 we explored the local structural dynamics of different regions of the protein and demonstrated a high flexibility of the C-terminal region. Additionally, using Double Electron-Electron resonance (DEER) experiments we measured inter-label distance distributions and showed the antiparallel orientation of the coiled coil. Finally, the Alphafold model was refined by using harmonic restrained all-atom molecular dynamics simulations to generate distributions of conformations that meet both DEER and SAXS data.

This unique combination of biophysical approaches allowed us to decipher the structural and dynamic behavior of this atypical protein. These results question about the function of this chloroplastic protein in the regulation of the Calvin cycle in a diatom as its structural properties are completely different from its homologous counterparts from Planta and cyanobacteria.

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Enhancing computational design for nanoparticle-based delivery vectors in the CRISPR/Cas9 system

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CRISPR/Cas9 therapy offers effective targeted gene editing with numerous advantages over traditional gene therapies. A critical aspect of implementing this technique is the design of gene delivery vectors that can precisely target mutations within the genome. Additionally, the use of oligonucleotide-functionalized gold nanoparticles has been successful in delivering CRISPR/Cas9 for the treatment of inherited diseases like muscular dystrophy. It is important to develop delivery vectors with reduced risk of insertional mutagenesis by leveraging a molecular understanding of the systems involved, especially as current CRISPR/Cas9-Gold therapy trials are still in early stages of development. To address these challenges, we optimized such a design through computational methods in two stages. Initially, we fine-tuned the DNA loading on various sizes of gold nanoparticles in nanoparticle-oligonucleotide conjugates. Subsequently, we conducted molecular dynamics simulations of the Cas9-sgRNA complex. This study is crucial for enhancing the design of CRISPR/Cas9-Gold-based delivery vehicles.

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De novo design of cyclic homooligomers to be used as fiber anchors

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Protein nanomachines have a potential to be used for various applications, from therapeutics to sensors and nanomaterials. *De novo* designed proteins are especially well-suited for such applications due to their high thermal stability and variability in shapes and functions. Our goal is to design proteins capable of moving along the tracks made of self-assembling protein fibers. To better functionalize the fibers we would like to specifically attach structures to their ends. Therefore, we need to design protein "anchors" that consist of a cyclic oligomer connected to half of the protein interface (a protein cap).

Here we present the design of novel C10 oligomers. We used a combination of deep learning methods: RosettaFold [1] was used to generate protein backbone, ProteinMPNN [2] was used for side chain generation and then the structures were predicted with AlphaFold2 [3]. To broaden the search space, we designed the oligomers with monomers that were 60, 90 and 120 amino acid residues long. We ranked our designs based on established Rosetta and AlphaFold2's metrics, such as charge, free energy of binding (ddG), predicted lddt; we also considered RMSD between the AlphaFold2 model and initial backbone predicted by RfDiffusion.

Overall, based on the combination of metrics we chose 14 designs for further work.

We ordered the designs as synthetic genes, expressed them in E. coli and purified them via Nikel affinity chromatography. We present the basic experimental characterization of the designs, including expression and SEC-MALS (Size exclusion chromatography with multi-angle static light scattering) that can measure oligomeric state.

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Stop the biting: toward modulation of fast inactivation of voltage-gated sodium channel

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Diseases spread by mosquitoes lead to the death of 700,000 people each year. The main way to reduce transmission is vector control by biting prevention with chemicals. However, the most commonly used insecticides lose efficacy due to the growing resistance. Therefore, a thorough understanding of molecular mechanisms of insecticide action and resistance is urgently needed to develop new ways of insect control.

Voltage-gated sodium channels (VGSCs), membrane proteins responsible for the depolarizing phase of an action potential, are targeted by a wide range of neuroactive compounds, such as animal toxins, local analgesics, and insecticides. Here, we use steered molecular dynamics (SMD) to investigate the impact of ligands on the fast inactivation process of VGSCs, which is crucial in sodium ions' conductance and termination of the action potential. By measuring the forces required to inactivate the channel, we can assess the ligand efficacy to enhance or block the sodium ions' conductance. We also design and test the inhibitory potential of sulfonamide insecticides that target less conserved channel areas than ion-conducting pore – voltage sensor domains – by lead optimization of sulfonamide inhibitors investigated in clinical trials as potential pain modulators.

Our molecular modeling study is the first step in the development of a new class of selective insecticides. It can also give an insight into the allosteric pathway of conformational changes in the VGSC in response to neuroactive ligands binding.

Exploring uracil base opening dynamics in dsDNA through enhanced molecular dynamics simulations

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Uracil can exist in dsDNA by spontaneous cytosine deamination or by misincorporation of dUMP instead of dTMP during the replication process[1]. A highly specific Uracil-DNA glycosylase (UDG) enzyme recognizes and excise the flipped uracil base from the dsDNA helix; this mechanism is part of the base-excision repair (BER) pathway[2]. There remains a lack of complete understanding in the debate over the DNA base flipping mechanism—whether base flip happens due to the DNA helix's distortion or if it is induced by the UDG enzyme during BER. However, the dynamic mechanism of uracil base flipping at an atomic level is still not understood. To gain an insightful understanding, we perform metadynamics (MtD) simulations to generate free energy profiles for uracil base flipping out of its helical stack in the dsDNA in this work. The uracil base causes a slight perturbation (intrahelical) towards the major groove side initially, and the opening angle is ~50º with an energy barrier of ~6 kcal/mol. The energy barrier difference from the intrahelical to extrahelical state is about ~2kcal/mol. We docked intrahelical and extrahelical poses of uracil base in dsDNA with the UDG enzyme using molecular docking tools to understand the UDG enzyme activity in the base flipping mechanism. The obtained docking confirmation indicated that the extrahelical state of uracil in dsDNA fits well into the catalytic pocket of the UDG enzyme. Our simulations suggest that uracil base flipping may be the key step that permits UDG to recognize and excise uracil from the dsDNA.

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eSPC, an online data analysis platform for molecular biophysics

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To aid researchers gain knowledge from molecular biophysical data and democratize access to advanced analysis tools we have developed an online user-friendly platform called eSPC (spc.embl-hamburg.de). It contains, to date, six different tools that handle data from various techniques, such as microscale thermophoresis (MST), differential scanning fluorimetry (DSF), mass photometry (MP), dynamic light scattering (DLS) data and circular dichroism (CD) [1-4]. These interactive tools are useful for the assessment of biomolecular interactions, complex formation, and sample quality, at least. During 2023, counting only interactions of more than 30 seconds, the eSPC had 3643 visits, underscoring its utility for the biophysics community.

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Assembly and dynamics of the outer membrane exopolysaccharide transporter PelBC of *Pseudomonas aeruginosa*

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Biofilms, the most abundant life form on Earth, ensure survival of the embedded bacterial communities in hostile environments, facilitate the capture of nutrients, and protect cells from antibiotics and desiccation. The human pathogen *Pseudomonas aeruginosa* secretes several exopolysaccharides, including Pel, to build up the biofilm matrix [1]. The dedicated synthesis/secretion system spans the cell envelope, and the PelBC complex mediates the translocation of the Pel chain across the outer membrane. As understanding the determinants of the PelBC assembly and its conformational dynamics may help combating *P. aeruginosa* biofilm formation, we set out to unravel structural and functional details of the membrane complex.

Cryo-EM resolved the 2.4 Å structure of the *in vivo* assembled and nanodisc-reconstituted complex of ~250 kDa built of a β-barrel PelB and a dodecameric ring of PelC lipoproteins at the periplasmic interface, as well as multiple lipid and LPS. The α-helix of PelB preceding the barrel domain forms extensive electrostatic interactions with the neighboring PelC subunits, and it appeared essential for the complex assembly. As the PelB barrel is sealed by multiple loops, we are studying the conformational dynamics of the transporter and its variants via single-channel conductivity measurements combined with all-atom molecular dynamics simulations. The on-going experiments have visualized transitions between “closed” and “open” states of PelB in lipid bilayers, where deletions of individual loops have affected the protein dynamics.

Though the functional role of the PelC ring is not understood yet, the protein is essential for the exopolysaccharide secretion. Using *in vitro* tools, we show that the N-terminal acylation is required for PelC:lipid binding, and the interaction is sensitive to the ionic strength. Both *in vitro* and *in vivo*, PelC is targeted to the membrane even in absence of PelB, but it remains to be shown whether the β-barrel is required for the ring assembly.

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Riboregulation as a new player in the control of cellular metabolism: clues from the cryo-EM structure of serine hydroxymethyltransferase-RNA complex

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Recent evidence suggests that RNA can directly control protein activity, either by affecting its function or by mediating the assembly of multiprotein complexes, thus modulating many cellular processes, including metabolism [1]. This novel mechanism is known as riboregulation.

Interstingly, in recent years many metabolic enzymes have been found to display non canonical RNA-binding properties in living cells, but only a few examples of RNA-mediated regulation of their activity (riboregulation) are available [2-3], and none of them has been structurally characterized.

Here we present the cryo-EM structure of the complex between cytosolic serine hydroxymethyltransferase (SHMT1) and its cognate RNA modulator, shown to selectively inhibit serine cleavage activity but not its reverse reaction, serine synthesis. The RNA binds with a 1:4 stoichiometry and its position in the structure suggests that the tetrameric assembly, characteristics of eukaryotic SHMTs, is necessary for RNA binding. Upon RNA binding, two out of four protein subunits adopt a conformation characterized by a high degree of disorder of the active site. Our results suggest that RNA works as conformational switch, allosterically regulating the enzyme and locking it in a conformation that does not allowed the conformational re-arrangement necessary for the serine cleavage reaction, offering a mechanistic explanation on how RNA affects enzyme function in a substrate-specific manner. This observation not only provides important insights on how RNA can allosterically control the activity of metabolic enzymes, but also offers an approach for improved targeting of these enzymes in RNA-mediated therapies.

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Dissecting the structural properties of the nickel binding sites in the urease chaperones HypA and UreE

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Urease is a nickel-dependent enzyme exploited by many virulent bacteria and fungi to infect the host and exert their virulence. Its maturation pathway requires the protein UreE as a metallochaperone to supply Ni(II) ions to the enzyme. In *Helicobacter pylori* urease maturation also requires HypA, an accessory protein that is commonly associated with nickel-dependent hydrogenase activation. HypA and UreE dimer ($UreE_2$) form a complex that is functional for metal ion delivery. This complex forms in the absence of Ni(II) and brings Ni(II) binding residues of $UreE_2$ and HypA in close proximity.

In the present work, the nature of this metal binding site and the residues that are involved in Ni(II) binding will be investigated[1]. The Ni-binding properties of HypA and $UreE_2$, as well as of their complex HypA•$UreE_2$, were investigated by isothermal titration calorimetry (ITC) using a global fitting strategy that included all the relevant equilibria. This analysis showed that the protein complex contains a single Ni(II)-binding site with a sub-nanomolar K_D, not present in the isolated proteins. The structural features of this novel Ni(II) site were elucidated using proteins produced with specifically deuterated amino acids, protein point mutations, and the analyses of hyperfine shifted NMR features, as well as molecular modeling. The results show that the complex contains a six-coordinate, high-spin Ni(II) site with ligands provided by both component proteins.

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Half way to hypusine. Molecular basis of (deoxy)hypusination

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(Lys50 in human eIF5A). Hypusination is essential to resolve ribosomal stalling during the formation of proline-rich polypeptides. Recent findings show that the hypusination of eIF5A plays a role in many important cellular processes, including autophagy, senescence, polyamine homeostasis, and the determination of helper T cell lineages. Malfunctions of the hypusination pathway, including those caused by mutations within the pathway encoding genes, are associated with such conditions as cancer or neurodegeneration. Therefore, hypusination seems as an attractive molecular target for therapeutic interventions.

Hypusination involves two distinct enzymatic steps. First, deoxyhypusine synthase (DHS) catalyzes the transfer of 4-aminobutyl moiety of spermidine to a specific lysine of eIF5A precursor in an NAD-dependent manner. Subsequently deoxyhypusine is further hydroxylated to the mature form hypusine by second enzyme: deoxyhypusine hydroxylase (DOHH).

Here, we present the cryoEM structure of the human eIF5A-DHS complex at 2.8Å resolution and a crystal structure of DHS trapped in the key reaction transition state. Furthermore, using combined structural biology and biochemical analysis, we show that DHS variants that cause neurodegeneration influence complex formation and hypusination efficiency. Hence, our data provide the molecular basis of deoxyhypusine synthesis and reveal how clinically-relevant mutations affect this crucial cellular process.

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Structural characterisation and autoproteolysis of Cwp5 cell wall protein from *Clostridioides difficile*

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C. difficile is a Gram positive spore-forming bacterium and a leading cause of antibiotic-associated diarrhea that can lead to life-threatening complications [1]. Its surface protein layer (S-layer) is an essential virulence factor composed of cell wall proteins (Cwps) that share a cell wall-binding trimer of CWB2 domains, one of the two evolutionary conserved S-layer-anchoring modules in Gram-positive bacteria [2]. The aim of our studies is to gain structural insight into the Cwps to understand the functions of these proteins acting either alone or as a part of the S-layer assembly. **Methods:** Mature Cwp5 (i.e. without the signal sequence) from *C. difficile* 630 was overexpressed in *Escherichia coli* BL21(DE3), purified by Ni-affinity and size exclusion chromatography. After spontaneous degradation of Cwp5 the resulting C-terminal fragment was identified by mass spectrometry and crystallized by sitting-drop vapor-diffusion technique using optimised commercial screens. Platinum derivatives of the crystals were prepared by soaking. **Results:** Single-wavelength anomalous dispersion method revealed a two-faced right-handed β-helix crystal structure of the functional domain of Cwp5. Cleavage site-directed mutagenesis showed that Cwp5 undergoes intramolecular autoproteolysis, most likely similar to the maturation mechanism of CwpV [3]. **Conclusions:** Our results indicate a possible common autoprocessing mechanism of *C. difficile* CWPs and other CWB2 module-containing S-layer proteins of Gram-positive bacteria.

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Activities of the cryo-EM facility at the National Institute of Chemistry, Slovenia

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The field of cryo-electron microscopy (cryo-EM) has seen rapid development and significant technical improvements in the last few decades. Advances in sample preparation, microscope hardware, direct electron detectors and data analysis have ushered in the so-called 'resolution revolution'. Cryo-EM with its three main methodological approaches, single particle analysis (SPA), tomography (cryoET) or microcrystal electron diffraction (MicroED), has become a powerful tool for gaining structural insights into individual biological molecules, their complexes, cellular organelles, whole cells, tissues, viruses and synthetic nanoparticles, as well as peptides and even small organic molecules, at atomic or near-atomic resolution.

The cryo-EM facility at the National Institute of Chemistry was established in 2019 and remains the only one in the region. It includes the 200 kV Glacios™ cryogenic transmission electron microscope, which allows users to perform SPA, cryo-ET and MicroED experiments. Samples are vitrified by plunge-freezing (Vitrobot) and the Falcon 3 EC and Ceta D detectors enable the acquisition of high-quality data. High performance computing (HPC) infrastructure is available for data storage and analysis.

The cryo-EM facility is part of the Centre for Molecular Interactions and Structural Biology within the Department of Molecular Biology and Nanobiotechnology. It is open to internal and external users and we actively collaborate with various academic and industrial partners.

I will present several successful structural projects that have been completed in recent years, from plant viruses to virus-like particles [1,2], pore-forming proteins[3,4], to extracellular vesicles[4,5] and more.

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The National Facility for Structural Biology at Human Technopole: a new infrastructure for the Italian structural biology community

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Human Technopole [1] is a recently established life science institute which has the mission of both performing basic research in human biology and physiology, and provide support to the Italian research community with cutting-edge technology and expertise in the institute's research areas. In this framework, Human Technopole has set up a number of National Facilities, which will provide free of charge access to their services to the members of the Italian research community.

One of these is the National Facility for Structural Biology, which stands as a one-of-a-kind scientific and technological hub for integrative structural biology. The Facility comprises six Infrastructural Units (IUs) working together to *i)* provide and support conventional practices, and *ii)* promote and establish innovative workflows in integrative structural biology.

The Cryo-Electron Microscopy Unit (UI1) aims at identifying, visualising, and characterising biological players of interest, isolated and within their cellular compartments. The Biomass Production Unit (UI2) provides access to different cell lines for protein expression and performs scale-up of bioprocesses for large-scale productions. The Biophysics Unit (UI3) is a technological platform for the biophysical characterisation of macromolecules and their interactions. The Structural Proteomics Unit (UI4) relies on crosslinking mass spectrometry (XL-MS) to provide topological and structural restraints on protein-protein interactions in samples ranging from purified protein complexes to cellular fractions. The Dynamic Single-molecule Unit (UI5) can visualise biological processes in real time with single-molecule sensitivity thanks to cutting-edge instruments that combine optical tweezers with fluorescence and label-free detection modules. The Technology Development Unit (UI6) will be where all other units converge when it comes to pushing technological limitations in integrative structural biology. Taken together, the synergy of expertise and technological offerings unquestionably makes the National Facility for Structural Biology more than the sum of its parts.

References:

[1] www.humantechnopole.it

Biomolecular Interactions and Crystallography Core Facility at CEITEC MU

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The Biomolecular Interactions and Crystallography Core Facility (CF BIC) at CEITEC MU in Brno, Czech Republic belongs to a group of core laboratories offering their services to a broad range of internal and external users. At CF BIC, we grant access to the state-of-art instrumentation for biomolecular sample analysis, characterization of molecular interactions and X-ray crystallography. Among our instrumentation can be found advanced tools for analytical ultracentrifugation, surface plasmon resonance, bio-layer interferometry, isothermal titration calorimetry and micro-scale thermophoresis. Various properties of biomolecules can be studied via differential scanning fluorimetry and calorimetry, dynamic light scattering, circular dichroism, analytical SEC or small-angle X-ray scattering. We also provide a complete crystallography pipeline from initial screening, through optimization, up to in-house data collection and structure solving. CF BIC provides services and enables access to one hundred individual users each year being an important source of knowledge in the region.

In addition to standard instrument access, CF BIC participates in various activities, such as teaching, workshop organization, methodology development [1], instrument benchmarking [2], etc. Our members are actively involved in pan-European activities, e.g. in Core Technologies for Life Sciences (CTLS) or Association of Resources for Biophysical Research in Europe (ARBRE).

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Binding dynamics of IgM Mannitou with Paucimannosidic antigens: a biophysical insight into cancer biomarkers

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Paucimannosylation, marked by the presence of truncated mannose residues, is increasingly recognized as a pivotal biomarker in cancer detection and progression, affecting a multitude of oncogenic processes, including cell signaling and tumor invasion [1]. Recent advancements have underscored the specificity of *Mannitou*, an IgM antibody, in identifying tri-mannosylated glycans on *tumor-associated carbohydrate antigens (TACAs)*[2], which may be instrumental in cancer diagnostics and treatment [3]. This poster presents an in-depth exploration of the IgM Mannitou's binding interactions with diverse TACAs, such as *AHNAK and antigens from Schistosoma mansoni eggs*. We elaborate on the methodologies for producing and purifying the IgM antibody and AHNAK, including their structural validation by Small Angle X-ray Scattering (SAXS), Circular Dichroism (CD), and mass spectrometry (MS). Furthermore, the kinetic and stoichiometric characteristics of IgM Mannitou's interactions with various glycoproteins are scrutinized using Surface Plasmon Resonance (SPR) and Isothermal Titration Calorimetry (ITC). A significant portion of this investigation addresses the disparity between the concepts of *affinity and avidity* in IgM Mannitou, illuminating the essential differences through comparative analyses of binding efficiencies to paucimannose moieties, thereby contributing to the foundational understanding of antibody-antigen dynamics in cancer biology.

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Protein-protein interaction inhibitors (PPI) to target the AuroraA/N-MYC complex in *MYCN*-amplified Neuroblastoma cells

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Neuroblastoma is the most frequent solid cancer in paediatric age accounting for 10% of all infant cancer. Notably, amplification of *MYCN* oncogene is seen in approximately 25% of patients and it is associated with the poorest prognosis. This oncogene belongs to the MYC family of transcription factors, and it is considered as the main responsible for this type of cancer transformation. A direct correlation between N-MYC amplification and tumour growth has been demonstrated [1]. Thus targeting the N-MYC transcription factor would be the specific treatment, but transcriptional factors remain difficult to hit with small molecule inhibitors, for the intrinsic plasticity of the DNA binding site. The discovery of the role played by the AuroraA kinase as a stabilizing factor of N-MYC degradation, opened the possibility to study this interaction to find an Achilles's heel to target N-MYC [2]. Some small molecules were studied for their ability to change the AuroraA conformation and prevent the complex formation [3], to date some of these allosteric modulators enter the preclinical trials but showed high grade of toxicity.

In my PhD project we are developing *in silico* three fusion peptides based on the sequence of Protein kinase A Inhibitor (PKI), since PKA is a structural homologue of AuroraA. The aim is to create protein-protein interaction (PPI) inhibitors capable of disrupting the AuroraA/N-MYC complex and let the proteasome machinery eliminate the excess of N-MYC protein. I started to characterize the structure of these three peptides using NMR, before characterizing the AuroraA/peptide complex structure. Understanding the residues at the core of this interaction will allow to improve the PPI inhibitors and then will help to synthesize some more specific peptidomimetics.

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Integrative approach in drug discovery against SARS-CoV-2: a biochemical and biophysical study on the Papain-like protease PLpro

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Among potential pharmacological targets of the SARS-CoV-2, the cysteine proteases Mpro and PLpro are highly promising, due to their essential role in viral replication and infection development [1]. The PLpro, a domain of the sizeable non-structural protein 3 (nsp3), exhibits deISGylating and deubiquitinating activities in addition to cleaving the viral polyprotein pp1ab. PLpro represents indeed one of the main viral defence mechanisms against the immune response, interfering with the cytokines pathways regulated by the Ubiquitin and ISG15 modification system [2]. Despite many efforts to discover PLpro inhibitors, only a few molecules have been validated as PLpro inhibitors. In this poster we present our efforts to identify inhibitors of this enzyme within a repurposing library, and the characterization of differences between the commonly used construct of the PLpro and the double-domain construct PLpro_NAB, containing the nucleic acid-binding domain of nsp3, both produced in the Elettra Protein Facility. By collaboration of all partners providing orthogonal skills led to the identification of a potent inhibitor. Applying an integrative approach of biochemical (fluorescence-based activity assay, limited proteolysis), computer-aided (docking) and biophysical techniques (differential scanning fluorimetry, microscale thermophoresis), we elucidated the mechanism of action of false-positive compounds which at first looked promising, highlighting instead a real inhibitor, CPI-169, for which we further proved the antiviral activity in a cell-based assay. The binding affinity was suitable to be studied by ligand-based NMR techniques, which allowed the validation of the computational and biochemical readouts. Besides the screening, we investigated also the interaction of PLpro with ISG15. Grating-coupled interferometry of ISG15 and its precursor (proISG15) allowed to detect discrepancies in the binding and catalytic activity of PLpro_NAB $S_{466}R$ and $T_{467}K$, two mutants discovered in the Delta-variant of SARS-CoV-2.

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Studies on pore-forming Aegerolysins and their MACPF partners from fungi

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Aegerolysins from fungi are soluble proteins that have been shown to interact with various membrane lipid receptors in target organisms. Their biological function is poorly understood, but more is known about their potential application. The formation of pores by aegerolysins in combination with their protein partners, such as proteins containing a membrane attack complex/perforin (MACPF)-domain, is one of the possible adaptations of organisms to a presumed competitive exclusion in the ecological niche [1]. Such bicomponent pairs can already be observed at the gene level and can be biotechnologically exploited as environmentally friendly bioinsecticides.

Similar methods used to study some other pore-forming proteins have also been used to study the interactions between the two components aegerolysins and MACPF-domain-containing proteins from fungi with receptor lipids in membranes and target organisms (mainly insects). These methods include bioinformatics, production of recombinant proteins, preparation of artificial lipid vesicles, sedimentation assays, surface plasmon resonance measurements, calcein release assays, haemolytic assays, and cytotoxic activity against Sf9 insect cells or other cells containing target lipids [2]. Cryo-electron microscopy can explain in detail the interactions between aegerolysins and MACPFs multimers and the target lipids.

To learn more about their biological function, we investigated whether or not aegerolysins and their partner proteins contribute to the survival ecology of extremophilic generalists and specialists. It is known that fungi have fewer competitors under extreme conditions; consequently fungal specialists produce less diverse and complicated profiles of specialised molecules. We have used bioinformatics methods to show how unevenly aegerolysins are distributed across fungal taxa and how these extremotolerant and extremophilic fungi have evolved in numerous branches of the fungal tree of life. Bicomponent pore-forming proteins - aegerolysins and partners - are less abundant in extremotolerant fungi and absent in extremophilic fungi, supporting our hypothesis that these pore-forming proteins play a role in competitive exclusion.

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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, it binds nickel [2] and lipids, and is functionally regulated by phosphorylation. 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. The interaction of NDRG1*C with lipids was followed by FT-IR spectroscopy, circular dichroism, isothermal titration calorimetry, co-sedimentation assay, electron paramagnetic spectroscopy. These techniques suggest that there is an interaction between the NDRG1*C and the negatively charged lipid DMPG (1,2-Dimyristoyl-sn-glycero-3-phosphoglycerol). Lipid binding induced a change of the secondary structure of the unmodified NDRG1*C, while phosphorylation prevents protein-lipid interaction.

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Insights into amyloid aggregate formation and fluorescent ligand labeling: a correlative microscopy study

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Amyloid aggregate formation represents a coordinated cellular process, often triggered by changes in the cellular environment and genetic mutations. The accumulation of these aggregates within the body is commonly associated with degenerative diseases. Despite variations in the composition of polypeptide chains, amyloid fibrils share common structural and biophysical properties, characterized by a fibrillar morphology and a cross-β structure. In recent years, the development of small fluorescent ligands has enabled the monitoring and visualization of protein aggregates by specifically binding to the β-sheet structure. In this study, we evaluated the effectiveness of two thiophene-based fluorescent ligands, the pentameric formyl thiophene acetic acid pFTAA [1] and the thiophene derivative HS169 [2], in identifying Alzheimer's disease-related Abeta fibrils. Using confocal laser scanning microscopy (CLSM) and stimulated emission depletion (STED) microscopy in conjunction with atomic force microscopy (AFM) for surface mapping, we assessed the labeling efficiency of these ligands. We followed standard protocols to lebel fibres. Our analysis revealed that only a fraction of the fibrillar aggregates exhibited fluorescence under these conditions, with a labeling percentage of 0.46 ± 0.04 for pFTAA-labeled fibers and 0.53 ± 0.03 for HS169-labeled fibers. We repeated the experiment by increasing the fluorophore concentration to verify whether the number of labelled fibrils would increase. Upon analysis, we noted that the fibers were nearly entirely labeled, although with a persistent strong fluorescence inhomogeneity. Our findings highlight correlative microscopy's potential as a powerful technique for studying molecular events at the nanoscale level and provide insights into the intricate nature of these labelling processes.

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Generating CRISPR/Cas9 mediated DPP3 gene knock-out in human embryonic cells

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An efficient and accessible CRISPR/Cas9 genome-editing tool has been used to delete DPP3 gene in 293FT human embryonic cells. Dipeptidyl Peptidase 3 (DPP3) protein encoded by the DPP3 gene, located on the human chromosome 11, belongs to the M49 metallopeptidase family and is enrolled in the terminal stages of protein turnover, but it may also have a role in the regulation of blood pressure and pain through the degradation of bioactive peptides. Another role of DPP3 is the regulation of NRF2-KEAP1 signaling pathway, where it acts as a competitive interactor of KEAP1 that activates NRF2-mediated oxidative stress response. Activation of NRF2-KEAP1 signaling pathway by DPP3 is quite important in cancer, since the increased levels of DPP3 mRNA were found in squamous cell lung carcinoma, correlating with the increased activity of NRF2[1]. In order to understand the biological role of DPP3, we have generated DPP3 gRNAs targeting first and second coding exons and cloned them into lentiCRISPR v2 vector. Constructed plasmids were transformed into chemically competent E. coli NEBStable recombination-deficient bacteria and isolated. 293FT cells were transfected with lentiCRISPR v2-gRNAs using Lipofectamine 2000, and with the additional vectors pMD2.G and psPAX2 for lentivirus production. Collected viral supernatants were harvested and subjected to 293FT cells with the addition of cationic polymer polibren. Cells were further seeded in T25 and in T75 flasks with puromicin, and passaged. Single cell clones were selected from the selected pool in complete growth media with puromicin. Genomic DNA was harvested for screening analysis. Proteins were isolated for Western blot in order to confirm DPP3 knock-out, while high-quality total RNA was isolated from WT and DPP3-KO cells and sequenced by RNAseq (Novogene Corporation Inc., UK) to identify differentially expressed genes.

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Content-aware nanospectroscopy improves acquisition efficiency for advanced modalities

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Advanced fluorescence microscopy and spectroscopy methods, such as super-resolution stimulation emission depletion (STED) nanoscopy or fluorescence correlation spectroscopy (FCS), can provide key additional insights into the structure and dynamics of cellular components down to the molecular level. However, their high spatial and/or temporal resolution often require long acquisition times and a skilled operator to select a handful of "representative" regions of interest (ROIs) where the final data are acquired. The slow, tedious and bias-prone acquisition process limits the applicability of otherwise powerful methods for quantitative high-throughput screening.

To alleviate these concerns, we automated the acquisition of data with such microscopy modalities at ROIs autonomously selected by the acquisition software during the coarse screening of the sample based on predefined criteria. These can involve deterministic analysis algorithms, such as filtering and thresholding to identify the locations of the structures of interest, or deep-learning based methods, e.g. for event/object recognition or cell segmentation. For instance, on-the-fly segmentation allows for acquisition of balanced datasets mimicking a human operator that would take a certain number of datapoints per cell. In addition, precise determination of coordinates for acquisition minimises the acquisition time as well as optimises the signal quality.

As the first demonstration, automated STED imaging enabled us effortless acquisition of two-colour 3D STED images of hundreds of nuclear condensates in cells, allowing precise quantification of the distribution of condensates' dimensions and dyes' co-localisation. With FCS, we next investigated the viscosity of the plasma membrane in live cells exposed to TiO2 nanoparticles, which are known to interact with the membrane and modulate cells' lipid synthesis. We show that the autonomously acquired data are of equal or higher quality (evaluated by the analysed transit time and molecular brightness) as from manual acquisition by a skilled operator. Efficiently amassing higher volumes of reliable data with the desired content and quality now allows us to quantitatively explore biophysical and cellular mechanisms in healthy cells and disease models.

The biophysics of electric field-mediated rolling of cancer cells inside blood-vessel-on-chip devices: applications in metastasis research

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Adhesive rolling dynamics of cells plays a critical role in determining different biophysical processes in health and disease [1,2]. For instance, the adhesive motion of cancer cells on endothelium bears strong influence on the overall metastasis process [3]. Although, numerous studies have been reported regarding modelling of the stochastic behavior of rolling cells on functionalized substrates, the effect of an external stimulus, in the form of electric field, on the rolling adhesion of cancer cells has by far remained elusive. In this study, we demonstrate experimental results related to the interplay of fluid shear and axial electric field on the rolling dynamics of cervical cancer in *blood-vessel-on-chip* devices.

Using microfabrication procedures, we have developed biomimetic blood-vessel like microchannels with integrated electrodes and utilized high-speed imaging techniques to capture the cellular motion through the functionalized channels. The results clearly portray that an external electric field, even within the physiologically relevant regime, can significantly influence the adhesive behavior of cancer cells, quantified in terms of the altered average rolling velocity and frequency of adhesion. The fundamental biophysics behind such phenomenon could be explained by analyzing the effect of electric field on the kinetic rate of bond breakage between receptor-ligand pairs using statistical analysis of the experimental data. Moreover, similar experiments with non-cancerous cells such as red blood cells and fibroblasts have also been carried out in order to rule out the possibility of any detrimental effect of electric field on normal cellular motion.

This study has a strong potential to open up a new paradigm of controlling cellular adhesion using localized electric fields in physiological vasculature, giving rise to a new school of treatment protocol of '*electro-therapy'* as an alternative to procedures involving toxic drugs or radiation hazards.

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Molecular mechanisms of enteric virus inactivation under oxidative conditions

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Human noroviruses and hepatitis E viruses, two pathogenic ssRNA(+)-containing enteric viruses, are a major cause of foodborne diseases leading to millions of gastroenteritis and hepatitis cases worldwide and thus to huge health and societal costs. During their replication cycle, viruses are subjected to various oxidants (HOCl, $ONOO^-$, H_2O_2) in the human body, and also in the environment, leading to their inactivation. However, the way by which this inactivation occurs remains unexplained. To address this gap in current knowledge, the rather simple architecture of the enteric F-specific RNA phages combining ssRNA(+) and capsid proteins makes them attractive and easier to handle models because mechanistic studies can be performed at different scales. Such phages are also recognized as surrogates to describe the behavior of noroviruses and hepatitis E viruses under oxidative conditions. Recent data obtained on MS2 and Qβ bacteriophages suggest that oxidative conditions promote the formation of cross-links between the capsid proteins (CPs) and the genomic RNA that could abrogate virus infectivity [1].

Using a multidisciplinary approach combining protein biochemistry, RNA molecular biology and mass spectrometry, we aim at identifying the molecular basis responsible for the formation of such oxidative modifications. To achieve that, simplified *in vitro* models were developed using mutated recombinant Qβ and MS2 CPs assembled into dimers and their cognate RNA operators. Preliminary data confirmed that cross-links can indeed be formed between CP dimers and 5'-fluorescently labelled RNAs upon oxidation. Optimized protocols for mapping RNA-protein cross-links are developed and these studies will be extended to multiscale phage-derived *in vitro* models including Virus-Like particles.

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Secondary structure investigation of SARS-CoV-2 spike protein Domains via attenuated total reflectance infrared spectroscopy

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All coronaviruses are characterized by Spike (S) glycoproteins, the largest structural membrane proteins, which are the first involved in the anchoring of the host receptor through the receptor binding domain (RBD). The S protein is composed by two main subunits: S1, responsible for recognition/anchoring to the host receptor and containing the RBD; S2, responsible for the membrane fusion [1,2]. The knowledge of this protein secondary structure and of its protein domains is the first step for shedding light on various aspects, from functionality to pathogenesis, finally to spectral fingerprint for the design of optical biosensors. The aim of this work is the characterization of the whole monomeric SARS-CoV-2 S protein and its domains (S, S1, S2 and RBD) at a serological pH (7.4) by measuring their amide I infrared absorption bands through Attenuated Total Reflectance Infrared spectroscopy (ATR-IR), one of the main and suitable techniques used in the secondary structure analysis of proteins [3,4]. Experimental data in combination with MultiFOLD predictions, Define Secondary Structure of Proteins (DSSP) web server (DSSP) and Gravy value calculations, provide a comprehensive understanding of proteins' domains in terms of their secondary structure content, conformational order and interaction with the solvent.

Finally, being the S1 involved in the viral process, it is useful to study its secondary structure alterations induced to chemical/physical environmental modifications. We performed a study on the S1 (variant of March 2020) subunit under pH [4] and temperature variations, observing how the S1 protein adapts its secondary structure to different inhospitable surroundings. The high stable configuration is present at endosomal pH 5.5 and at temperature of 30°C, proving that the protein evolution modifications yield the protein more performant [4].

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Engineering protein folding for real time, continuous biosensing

P67

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Continuous, real-time biosensors measure molecular analytes without specialized equipment or personnel, allowing fully personalized medicine [1]. Most biosensing technologies, however, require multistep protocols that prevent their use for continuous, real-time measurements. And the few that do, such as the glucose continuous monitor, are not generalizable to other analytes.

In response, we are developing a new sensing technology that is reagent-less, selective enough to work in biological fluids, and generalizable to many different targets. To achieve this, we use nanobodies, single domain antibodies capable of binding with high affinity and specificity many different analytes [2]. Inspired by the biophysics of conformational signaling, we are engineering nanobodies to couple ligand binding to a folding structural change. To design them, we combine artificial intelligence and computational prediction tools, as well as biophysical and structural characterization.

As proof of concept, we have developed a nanobody-based conformational receptor for chorionic gonadotropin hormone, a biomarker of pregnancy, which we have used to develop a fluorescent biosensor. The convenient, real-time measurement of pregnancy hormones will improve the monitoring of pregnancy, enabling early detection of miscarriage in high-risk pregnancies, or the monitoring of embryo implantation during fertility treatments.

Given the great versatility of nanobodies and conformational signaling, our approach is generalizable to many different target molecules. This will facilitate the development of biosensors capable of continuous, real-time measurements, which will greatly improve health monitoring, but it will also enable responsive biomaterials or smart imaging probes.

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Genetic engineering as an approach to modulate the lipid-binding properties of the Flowering locus T, the member of the Phosphatidylethanolamine-binding protein family

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The Intergovernmental Panel on Climate Change (IPCC) forecasts a temperature rise of about 1.5 degrees Celsius (2.7 degrees Fahrenheit), by 2050 and 2-4 degrees Celsius (3.6-7.2 degrees Fahrenheit), by 2100, which would have a detrimental impact on the plant life cycle, accelerating the flowering and production of fruits and seeds, leading to the decreased yield and suboptimal food supplies. The climate changes have been already impacting the flowering, as several perennial and annual crops have flowered earlier by about 2 days per decade, during the last 50 years.

Although the process of flowering is well-understood at the genetic level, recently it became clear that one more layer is present in the control of this complex process: the interaction between the flowering locus T (FT; florigen), and lipid membranes changes with the temperature increase, with FT being sequestered onto the membranes at 16 degrees Celsius, but released with the temperature increase to 22 degrees Celsius (the range of ambient temperature for the Arabidopsis plants, as well as for several crops), indicating that the increase by 6 degrees Celsius facilitates the deployment of FT from the membranes, enabling it to travel to the plant apex, to interact with the transcription factor FD, leading to induction of the transcription of the flowering genes [1].

The gene engineering approach will be utilized to deliver the mutated protein species with diminished and enhanced lipid-binding properties, respectively, when compared to the wild-type florigen. The mutant versions will be screened using the lipid overlay assay and liposome sedimentation assay [2], whereas the kinetics and affinity of the protein – liposome interaction will be quantified using the surface plasmon resonance (SPR) technique. Our preliminary protein structure predictions, using ColabFold [3], suggest that the florigen's N- and C-terminal domains might be candidates to interact with the negatively charged phospholipid membranes.

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Combining optical tweezers with various imaging methods: the power of manipulation in dynamic single molecule analysis

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Biological processes involving nucleic acids, proteins, membranes, condensates, or cytoskeletal filaments are key to cell metabolism and life. Detailed insights into these processes provide essential information for understanding the molecular basis of life and the pathological conditions that develop when such processes go wrong. Being able to manipulate, measure and observe in real-time at single-molecule level are crucial to validate and complete current biological models.

Some techniques are exceptionally equipped to study structure and composition of molecules (e.g. x-ray crystallography or cryo-EM). On the other hand, dynamic functionality of molecular mechanisms can be captured with live cell microscopy or SPR, which requires many molecules to pass the detection threshold. Dynamic single molecule technologies combine these two crucial aspects: single molecule resolution and real-time dynamics. LUMICKS C-Trap is the world's first dynamic single-molecule microscope combining optical tweezers, imaging, and advanced microfluidics in a truly integrated system. By manipulating, measuring and seeing single molecules and their interactions, complex molecular mechanics can be addressed in great detail.

Here, we present our efforts for further enabling discoveries in the field of biology and biophysics using the combination of optical tweezers with correlative fluorescence microscopy (widefield, TIRF, confocal and STED) and label-free Interference Reflection Microscopy (IRM). We present several examples in which our correlative technologies enhanced the understanding of DNA-proteins interactions, protein folding and dynamics, cellular structure and phase separation.

Furthermore, we show that advances in hybrid single-molecule methods can be turned into an easy-to-use and stable instrument that has the ability to open up new avenues in many research areas.

Characterizing Glucagon aggregation using computational modeling and microfluidic modulation spectroscopy for experimental validation

P70

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Glucagon is a peptide hormone that plays a central role in glucose homeostasis by increasing the body's blood sugar levels and acting as a counterbalance to insulin. Unfortunately, glucagon is prone to aggregation, and the aggregated form is non-functional. Fully understanding the aggregation pathway of glucagon is fundamental to not only hypoglycemia research, but amyloidogenic research as well since the aggregates take on the cross beta-pleated sheet structure similar to other amyloidogenic peptides like amyloid-beta.

In this study, we computationally simulated the aggregation pathway and potential of glucagon alone and also in the presence of lactose or 2-hydroxylpropyl-b-cyclodextrin (2-HPbCD). Interestingly, both the computational modeling and experimental research using Microfluidic Modulation Spectroscopy (MMS) showed that 2-HPbCD is more effective at inhibiting aggregation than lactose. MMS is a novel technique which very precisely and accurately measures the structure of biomolecules, seeing structural changes that are undetectable by traditional techniques. These results can be used to support formulation development of glucagon therapeutics in order to keep the molecule stable, soluble, and active.

Aggregation properties of beta-amyloid (Aβ) and mutants in presence of iron

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Alzheimer's disease (AD) is the leading cause of dementia worldwide [1]. One of the peptides involved in AD is the amyloid-beta (Aβ) peptide, whose pathological aggregation is believed to be a central event in the etiology of AD [2]. However, there are still questions regarding the triggering of Aβ's aggregation and the subsequent development of AD. Some hypotheses have been tested, such as the metal ions dyshomeostasis hypothesis [3]. It is now a well-established fact that the onset of AD disturbs the equilibria of physiological metallic ions, named biometals, in the brain such as iron [3, 4]. It is not yet well-understood how various concentrations of iron can influence the relationship existing between the structure, the morphology and the toxicity of Aβ aggregates.

To further detail and investigate these effects, we propose a biophysical study of Aβ's aggregation, notably using atomic force microscopy coupled to infrared spectroscopy (AFM-IR). AFM-IR combines a nanometric resolution imaging to the analytical power of infrared spectroscopy [5]. It allows for nanoscale recording of infrared spectra, thus breaking the diffraction limit encountered in Fourier-transform infrared spectroscopy (FTIR).

In this work, we study the aggregation properties of Aβ peptide in the presence of iron. We used various biophysical techniques to characterize the aggregated species. We highlight here specific Aβ aggregation properties related to the presence of iron and its concentration. We show specific effects of iron concentration on the type of Aβ aggregates.

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Schistosoma mansoni micro-exon gene (MEG) proteins: hyper-variable motifs as a foe for the host immune system?

P72

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The parasitic worm *Schistosoma mansoni* has a complex genome structure, including 7 autosomes and one pair of sexual chromosomes [1].

Among other genes, it expresses a peculiar superfamily of secreted proteins called micro-exon genes (MEG) products. MEGs are encoded by very short exons (6-81 bp), interspersed by long introns (0.5-1.5 kbp) and flanked by transposon-like sequences. This genetic structure is difficult to detect with automated bioinformatic assignment. However, the proteins abundance in worms and eggs secretions may suggest a role in host-pathogen interactions. Nevertheless, their real function is at present unknown. We have determined by multidimensional NMR, with normal isotopic abundance, the first 3D structure of 3 isoforms of the MEG 2.1 family, demonstrating their IDP nature and their stickiness [2].

In parallel, we have also analyzed all the 87 validated protein sequences of the MEGs and proposed a model for their filiation, duplication and spread over the genome. In the poster we shall also present the strategies we have applied after the failure in heterologous expression in bacteria, yeast, insect cells and cell-free, in order to perform the structural analysis by Circular Dichroism, Dynamic Light Scattering and NMR [3].

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Breaking bias: unmasking affinity determination challenges in molecular binding.

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Researchers are increasingly turning to orthogonal approaches to comprehensively understand the complexities of molecular interactions, recognizing the limitations of single-method approaches in capturing the full complexity of these processes [1]. Integrating diverse affinity measurement techniques such as MST, ITC, SPR, and NMR, a holistic understanding of molecular binding events emerges.

This study highlights the concept of global fitting analysis, emphasizing its pivotal role in overcoming experimental discrepancies and biases across techniques and experiments. These methods emerge as a powerful tool in harmonizing data, facilitating robust comparisons and interpretations, gaining deeper insights into the cooperativity and dynamics within molecular systems.

Furthermore, the evolution of global fitting methods represents a significant advancement in data analysis, enabling researchers to extract more meaningful information from single-technique and multi-technique datasets. This sophisticated analytical approach not only enhances the reliability of affinity determination but also reveals the cooperative behaviors and conformational changes underlying molecular interactions.

Ultimately, multitechnique analyses and global fitting serve as indispensable tools in advancing our understanding of fundamental biological processes [2]. By elucidating the molecular mechanisms governing complex biological phenomena, these approaches pave the way for the development of novel therapeutic strategies and the engineering of biomolecular systems with unprecedented precision and efficacy.

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DetectSchisto: when analytical chemistry meets molecular parasitology

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Schistosomiasis is a chronic parasitic disease caused by blood flukes (trematode worms) of the genus *Schistosoma*. *Schistosoma mansoni* is the most prevalent species in Africa, the Middle East, the Caribbean, Brazil, Venezuela, and Suriname. It has a high infection rate estimated to be 20 million new cases per year and is co-endemic with other pathogens, such as HIV and malaria [1]. This disease is transmitted via vectors, with freshwater snails serving as intermediate hosts. Transmission typically occurs when infected individuals contaminate freshwater reservoirs with fecal matter containing parasite eggs, which subsequently hatch in water. The resultant larvae penetrate the skin, developing into adult schistosomes within the body. These adults primarily congregate around the liver, where females release eggs. If left untreated, schistosomiasis can lead to significant morbidity and potentially mortality, although the precise extent remains a matter of debate. According to the Global Burden of Disease Study 2019, schistosomiasis contributes to an estimated 1.6 million disability-adjusted life years (DALYs) globally [2], a number that could be lowered through early diagnosis. However, due to the absence of early diagnostic tests, our objective is to develop an early antibody-based diagnostic tool. During its life cycle, S. mansoni produces 28 isoforms of venom-allergen-like (VALs) proteins, which are differentially expressed as the parasite develops [3]. Our research focuses on those VALs exhibiting early expression levels, particularly SmVAL13. We have heterologously expressed the latter in E. coli and are currently characterizing it using various biophysical techniques such as NMR, SAXS, CD, and MX. In parallel, we have generated and immobilized on gold beads highly specific antibody (Ab) targeting SmVAL13, and we are investigating its interaction with the protein using techniques like SAXS, SPR, MX, Fluorescence Spectroscopy, and Native-MS.

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Assessment of the reduction partners of Vitamin K oxidoreductase, a membrane enzyme target of anticoagulation drugs, by an original kinetic strategy

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Vitamin K epoxide reductase C1 (VKOR), is an essential membrane enzyme of the endoplasmic reticulum that supports blood coagulation in Vertebrates. VKOR is the target of anti-Vitamin K drugs that inhibit coagulation, in medicine (to prevent blood clots), and in rodent population control. VKOR catalytic mechanism involves one pair of Cys residues in the active site and one pair on a luminal loop (*LL*), Cys 43 and 51, that are oxidized as disulfide bonds. Regeneration of VKOR active form requires reduction of the Cys43-Cys51 bond by a disulfide oxidoreductase, likely from the thioredoxin (Trx) family. A previous study identified potential candidate redox partners from the ER membrane or lumen, that include ERp18, PDI, TMX1 and TMX4 [1].

The aim of the present work is to clarify the question of the physiological partner of VKOR using a strategy based on kinetic evaluation of the reductase candidates efficiency in order to determine which one most likely acts as a reducer. Because VKOR is an integral membrane protein, a soluble VKOR *LL*-scaffold chimera was obtained as a *LL* model using an optimized scaffold protein [2] and the four human reductase candidates were produced heterologously in *E.coli* then purified and characterized.

The reducing activity of candidate partners was measured in the presence of the *LL* model chimera oxidized as a Cys43-Cys51 disulfide, and monitored by intrinsic Trp fluorescence using fast kinetics apparatus. Alternatively, Cys43 or Cys51 were activated as mixed disulfide by a fluorescent probe [3], allowing to monitor the reaction by the released probe fluorescence upon reduction. Initial results obtained using the *E.coli* thioredoxin 1 as a control indicate the suitability of this strategy to assess Trx-like enzymes activity with the oxidized *LL* models. Preliminary results obtained with other reductase candidates will be presented and discussed.

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Single-vesicle study on NLP toxins affecting GIPC-rich membranes: microfluidic method

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Single-cell studies have deepened the understanding of cellular heterogeneity and enabled insights into the intricate mechanisms driving biological processes. Microfluidic technologies offer precision and control in cost-effective studies at the single-cell level. Here, we present a microfluidic system designed for single-cell experiments, employing giant unilamellar vesicles (GUVs) in interaction with various membrane affecting substances [1]. The study focuses on the response of the GUVs with plant sphingolipid glycosyl inositol phosphoceramide (GIPC) containing membranes to the proteins of the necrosis- and ethylene-inducing 1-like protein (NLP) family. As NLPs have been found to permeate GIPC-containing membranes [2], we investigate the leakage of the vesicle's inner solution to the surroundings and correlate it with the binding of the toxin to the membrane. To obtain data on both the leakage and the toxin binding level at the same time, we use different fluorescent markers for inner vesicle solution and for the toxins, continuously monitoring the intensities of the two fluorescence signals throughout the experiments. This approach enables a clear visualization of the protein binding to the membrane, of the membrane permeation and the consequent leakage process. Through the analysis of the data it was found that the vesicle response to the NLP stimuli can be described by a simple model for protein-to-membrane binding kinetics.

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Striking a pose: Capturing the unique interaction of the oomycete NLP cytolysin with the plant plasma membrane

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Necrosis and ethylene-inducing peptide 1-like proteins (NLPs) are produced by a variety of plant pathogens that infect a wide range of important crops and pose an alarming threat to global food security. Many NLPs are cytotoxic, causing cell death and tissue necrosis by disrupting the plant plasma membrane. Glycosylinositol phosphorylceramides (GIPCs), the most abundant class of plant sphingolipids, are receptors for NLP binding to membranes [1]. Recently, we have shown that this lipid recognition is electrostatically driven and leads to shallow membrane binding, protein aggregation at the membrane plane and transient pore formation [2]. However, the precise molecular mechanisms of NLP-mediated membrane damage, which has been shown to be unique and highly adapted to the specific plant membrane environment, remain to be elucidated.

We utilise various model lipid systems, consisting of plant-isolated GIPCs, e.g. cell-sized vesicles (GUVs), which can be conveniently observed with confocal microscopy. By optimising the electroformation protocol, GUVs from such complex lipid mixtures purified from natural sources, were successfully produced. Visual information after NLP– membrane interaction on localised toxin binding, changes in vesicle morphology and differential leakage of probes of different sizes allow predictions on the membrane-damaging mechanism. NLP_{Pya}, a model NLP protein from the oomycete *Pythium aphanidermatum*, forms small openings in membranes of GUVs. Furthermore, toxin binds to discrete locations on the membranes and triggers morphological changes such as membrane evaginations. This phenomenon could result from protein insertion exclusively into the outer lipid monolayer and confirms our previous observations that NLP_{Pya} does not cross the membrane like the typical pore-forming toxins. The current noise observed in our planar lipid measurements in the presence of NLP_{Pya} confirms the formation of small pores, which are transient in nature but gradually open and subsequently end in membrane rupture.

Our study will provide specific molecular insights into the toxic NLP- plant membrane interaction that are crucial for the development of better strategies for crop protection.

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Interaction analysis with nucleic acids as targets

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Structures of nucleic acids are critical to their biological activity. The secondary and tertiary structures can be adopted in response to extrinsic factors and give raise to specific functions [1]. For RNAs structure is known to impact translation, post-translational modifications, susceptibility to degradation and binding to proteins and other ligands.

Studies of the binding interaction between nucleic acids and proteins, other nucleic acids, oligonucleotides and synthetic compound may inform on structure and function as well as enable modulation of these key properties of nucleic acids, and guide discovery and development of drugs.

Microcalorimetry and biosensors are two biophysical approaches broadly employed in characterization of binding interactions [2]. In late years the use of the biosensors in these area is accelerating. Here we give examples of how isothermal titration calorimetry (ITC) and grating coupled interferometry (GCI) can be applied in nucleic acid research and drug development.

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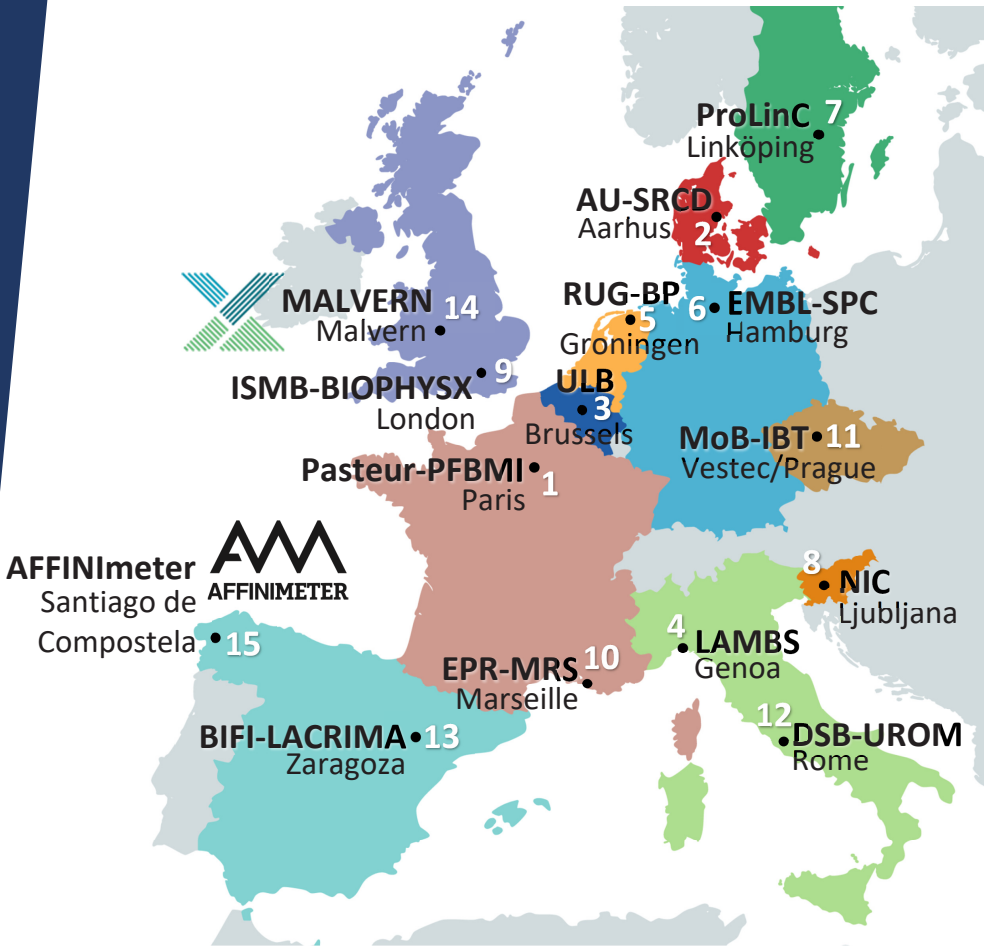

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