FINAL iNEXT-DISCOVERY CONSORTIUM MEETING AND 4TH SYMPOSIUM ON RECENT ADVANCES IN CRYO-EM

10–13|06|2024

BRNO, CZECHIA

BOOK OF ABSTRACTS

ORGANISERS

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The conference will be held under the auspices of the Mayor of the City of Brno, JUDr. Markéta Vaňková.

B R N O

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* 16 April 1951 – † 13 April 2024

IN MEMORIAM

WELCOME SPEECH

Welcome to Brno and to the final meeting of the iNEXT Discovery Consortium.
We are very pleased to welcome the structural biology community here again, following the successful 2nd iNEXT user meeting in 2017. We believe that Brno is the perfect setting for this event, with its rich scientific heritage dating back to Gregor Mendel, the father of modern genetics. It was here, in this city, that Mendel conducted his pioneering experiments on pea plants, laying the foundations for the field of genetics. The legacy of scientific discovery continues in Brno today, with more than 65,000 students spread across nine universities and institutes such as CEITEC, which promote ground-breaking research in the life and material sciences. Over the past 30 years, Brno has developed into a technological centre that currently develops and manufactures more than 30% of the world's production of electron microscopes and is home to international companies in the cybersecurity or aerospace. During the conference you will have the opportunity to take a guided tour of the Brno Castle and enjoy the conference dinner in the Mendel's Abbey premises to experience the essence of Brno.

As you explore the conference programme, we hope that you will find contributions that will stimulate your research or initiate new collaborations. Your participation and contributions are invaluable to the iNEXT-Discovery project.

Jiří Nováček

PROGRAMME

Monday, June 10, 2024

12:00–13:45	Registration
13:45–13:50	Opening
13:50–14:00	Welcome (Anastassis Perrakis; NKI)
	Chair: Jiri Novacek
14:00–14:30	Christiane Berger-Schaffitzel: Discovery of a druggable pocket and a drug in SARS-CoV-2 spike glycoprotein
14:30–15:00	Mikael Akke: Resolving Protein–Ligand Binding Pathways by NMR relaxation: Conformational Selection vs Induced Fit
15:00–15:30	Richard Stefl: Mechanisms of transcription attenuation and condensation of RNA polymerase II by RECQ5 helicase
15:30–16:00	Coffee break
	Chair: Manfred Weiss
16:00–16:30	Elspeth Garman: Radiation damage and metal identification in Structural Biology: why do we care?
16:30–17:00	Petra Wendler: Visualizing electrons in redox proteins by single particle cryo-EM
17:00–17:45	Poster flash talks
18:00–21:00	Poster session and BBQ dinner in the hotel

Tuesday, June 11, 2024

	Chair: Jiri Novacek
8:30–9:00	Gergely Papp: EasyGrid: a versatile platform for automated Cryo-EM sample preparation and control
9:00–9:30	Carlos Oscar Sorzano: Advances in the stream processing of single particles by CryoEM
9:30–10:00	Enrico Luchinat: Detecting protein-drug interactions in human cells by real-time 19F NMR
10:00–10:30	Bernhard Brutscher: Light-NMR: what can we learn about photo-transformable fluorescent proteins?
10:30–11:00	Coffee break

PROGRAMME

	Chair: Eva Pereiro (Partners' JRA)
	Enrico Luchinat: Detecting protein-drug interactions in human cells by real-time 19F NMR
11:00–11:30	Evgenia Zagoriy: Streamlining cryo-corellative multi-scale imaging of multicellular samples
11:30–12:00	Joaquin Oton: Advancing Correlative Microscopy: Automated Alignment of cryo-3D-SIM and Soft X-ray Tomography Data – Strategies and Implementation
12:00–12:30	Genevieve Evans: Building a Fragment Screening Data Pilot Repository: Challenges and Opportunities
12:30–13:00	Sponsor's contribution – Bruker: Magnet Innovations, Liquid State NMR Probes and Software News from Bruker BioSpin
13:00–13:10	Group photoshoot
13:00–14:00	Buffet lunch
	Chair: Rebecca Thompson
14:00–14:30	Andras Perczel: Chemical evolution of the aggregation-prone regions by means of amyloid formation
14:30–15:00	Daniel Rozbesky: Molecular mechanisms of MICAL signaling in cytoskeletal dynamics
15:00–15:30	Gustavo Fuertes: A divide-and-conquer structural biology approach to elucidate the photocycle of light-regulated transcription factor EL222
15:30–16:00	Coffee break
	Chair: Hans Wienk
16:00–16:30	Kevin Gardner: Using structural biology to transform nature's switches into our biotech tools and therapeutics
16:30–17:00	Elda Bauda: Ultrastructural details of the bacterial spore revealed by in situ cryo-electron tomography
17:00–18:00	Round Table: Panel members on iNEXT-Discovery
18:30–20:30	Spilberk Castle visit / Free evening (tips on where to go and where to eat will be provided)

PROGRAMME

Wednesday, June 12, 2024

	Chair: Christoph Müller
8:30–9:00	Slavica Jonic: Hybrid methods for analyzing conformational variability in cryo-EM and cryo-ET data
9:00–9:30	Katja Petzold: What do microRNAs and ribosomes have in common? Dynamics!
9:30–10:00	Francesca Rossi: Impact of osteosarcoma cells differentiation on biomineralization and mitochondrial morphology
10:00–10:30	Johannes Thoma: In-situ characterization of the bacterial outer membrane protein A
10:30–11:00	Coffee break
	Chair: Jiri Novacek
11:00–11:30	Marina Mapelli: Structural and functional characterisation of mechanisms controlling epithelial tissue integrity
11:30–12:00	Lukas Trantirek: Characterizing biomolecular structure and interactions in living human cells synchronized in defined physiological states using NMR spectroscopy
12:00–12:30	Rolf Boelens: Lac Repressor allostery
12:30–13:00	Outlook and Concluding remarks iNEXT AUM (Anastassis Perrakis; NKI, Coordinator)
13:00–13:20	Sponsor's contribution – Thermo Fisher Scientific: Advances and Frontiers in Cryo Electron Microscopy
13:20–14:00	Buffet lunch
14:00–14:50	Carlos Oscar Sorzano: Deep learning for SPA
14:50–15:40	Lukas Palatinus: 3D electron diffraction: a tool for understanding pharmaceutically and biochemically relevant small molecules
15:40–16:00	Coffee break
16:00–16:50	C. Shan Xu: Volume Electron Microscopy: from Room Temperature to Cryo
16:50–17:40	Katerina Naydenova: Structure determination by cryoEM at 100 keV
18:30–22:00	Gala dinner in the Augustinian Abbey, Mendel Square

PROGRAMME

Thursday, June 13, 2024

8:30–9:20	Bruno Klaholz: High-resolution cryo-EM analysis of ribosome and virus complexes
9:20–10:10	Jurgen Plitzko: Serial-lift-out - The path to the molecular anatomy of whole organisms by cryo-electron tomography
10:10–10:40	Coffee break
10:40–11:30	Simone Mattei: Ribosomes hibernate on mitochondria during cellular stress
11:30–12:20	Holger Stark: How to improve resolution and quality in single particle cryoEM
12:20–12:50	Sponsor's contribution – TESCAN GROUP: Accelerating Cryo-TEM Sample Preparation with TESCAN plasma FIB-SEM
13:00–14:00	Buffet lunch / lunch to go

ABSTRACTS OF SPEAKERS

01 Discovery of a druggable pocket and a drug in SARS-CoV-2 spike glycoprotein

Prof Christiane Berger-Schaffitzel
School of Biochemistry, University of Bristol, UK

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), represents an ongoing global threat. Key to SARS-CoV-2 therapeutic development is unravelling the mechanisms that drive high infectivity, broad tissue tropism, and severe pathology. We discovered a druggable pocket in SARS-CoV-2 spike (S) glycoprotein. Our cryo-EM structure revealed that the receptor-binding domains, which mediate viral infection, tightly bind the essential free fatty acid linoleic acid (LA) in three composite binding pockets locking S in a non-infective state. The pocket is highly conserved: a similar pocket is also present in the highly pathogenic SARS-CoV and MERS-CoV which caused the 2003 and 2012 outbreaks, respectively. Importantly, all SARS-CoV-2 Variants of Concern, including Omicron, contain this pocket which is thus evolutionary conserved. We showed that LA binding to the pocket precludes binding of S to the cellular receptor ACE2 outside of cells, and inside of cells suppresses viral replication. These results set the stage for the development of an urgently needed antiviral drug to inhibit infection and transmission of SARS-CoV-2.

02 Resolving Protein–Ligand Binding Pathways by NMR relaxation: Conformational Selection vs Induced Fit

Mikael Akke
Lund University, Sweden

Protein–ligand binding is essential for biological function. A long-standing question is whether proteins bind ligands via conformational selection (CS) or induced fit (IF). We resolved the CS and IF binding pathways by measuring protein conformational exchange associated with ligand binding over a wide range of ligand concentrations using NMR relaxation dispersion. We determined the relative flux through the two pathways using a four-state binding model that includes both CS and IF. In the absence of ligand, the protein galectin-3 exchanges between the ground-state conformation and a high-energy conformation similar to the ligand-bound conformation, demonstrating that CS is a plausible pathway. Stepwise additions of the ligand lactose induce progressive changes in the relaxation dispersions that we fit collectively to the four-state (CS+IF) model, yielding all microscopic rate constants and binding constants. The lactose binding constant is significantly greater for the high-energy, bound-like conformation than for the ground state, as expected for the CS pathway. Nonetheless, the IF pathway contributes greater than 70% of the total flux even at very low lactose concentrations. The higher flux through the IF pathway is explained by considerably faster exchange between the two protein conformations in the lactose-associated state. Thus, the ligand decreases the activation barrier between protein conformations in the same way that enzymes stabilize the transition-state of reactions involving ligand transformations.

03 Mechanisms of transcription attenuation and condensation of RNA polymerase II by RECQ5 helicase

M. Sebesta[1], K. Skubnik[1], W.S. Morton[1], M. Kravec[2], K. Linhartova[1,3], V. Klapstova[1,3], J. Novacek[1], K. Kubicek[1,3,4,5], V. Bryja[2], R. Vacha[1,3,4], R. Stefl[1,3]

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[5] Institute of Molecular Genetics of the Czech Academy of Sciences, v.v.i.; Prague, Czechia

The elongation rates of RNA polymerase II (RNAPII) require precise control to prevent transcriptional stress, which can impede co-transcriptional pre-mRNA processing and contribute to many age- or disease-associated molecular changes (*e.g.*, loss of proteostasis). Additionally, mesoscale organization of transcription is thought to control the transcriptional rates and multiple factors have been reported to form biomolecular condensates and integrate RNAPII through the interaction with the C-terminal domain (CTD) of the largest subunit, RPB1. However, the structural organization of these condensates remains uncharacterized due to their small size and inherently dynamic nature. Here, we investigated the molecular mechanisms by which a general transcription factor – RECQ5 – associates with hyperphosphorylated RNAPII elongation complex (P-RNAPII EC) and controls translocation of RNAPII along genes. We combined biochemical reconstitution, electron cryomicroscopy, cryotomography, and coarse-grained simulations. We report two mechanisms by which RECQ5 modulates RNAPII transcription. At the atomic level, we demonstrate that RECQ5 uses the brake-helix as a doorstop to control RNAPII translocation along DNA, attenuating transcription. At the mesoscale level, RECQ5 forms a condensate scaffold matrix, integrating P-RNAPII EC through a network of site-specific interactions, reinforcing the condensate's structural integrity. Our integrative, multi-scale study provides insights into the structural basis of transcription attenuation and into the molecular architecture and biogenesis of a model RNAPII condensate.

04 Radiation damage and metal identification in Structural Biology: why do we care?

Elspeth F. Garman[1]
[1] Department of Biochemistry, Dorothy Crowfoot Hodgkin Building, South Parks Road, Oxford, OX1 3QU, U.K.

Structural biology relies on X-ray crystallography to provide much of the three dimensional information on macromolecules that informs biological function [1]. My group has helped to establish improved methods for macromolecular crystallography (MX) to enable problems not previously accessible to structure solution to be tackled. A notable example has been the development of protocols to cryocool protein crystals prior to diffraction data collection at 100K, reducing the rate of radiation damage (RD) [2] by around a factor of 70 compared to holding the crystal at room temperature. However, even at 100 K, RD is still a limiting problem as it can prevent structure determination and the changes can mislead the experimenter when interpreting the biology of the structure.
Our contributions have included full dose modelling of the diffraction experiment (RADDOSE-3D, RADDOSE-ED and RADDOSE-XFEL, available at www.raddo.se) to allow data collection optimisation strategies [3,4,5], as well as the recent identification of a single metric, B_{net}, by which the level of damage in a single PDB entry can be assessed [6].
We have also developed a method to unambiguously identify metal atoms in both liquid and crystalline protein samples. Metalloproteins comprise over one-third of proteins, with approximately half of all enzymes requiring metal to function. Identifying the bound metal and its environment is a prerequisite to understanding biological mechanism. However, there are no routine analysis methods with the sensitivity and quantitative accuracy to do this.
We have developed microProton Induced X-ray Emission (PIXE) as a tool for quantifying metals in proteins using the known sulphur content (methionines and cysteines) as an internal standard. We have automated this method to permit high throughput analysis of many samples, validating the approach by using it to analyse four distinct sets of 30 proteins identified as metalloproteins in the Protein Data Bank (PDB) [7]. In all four sets, we found that over half of the metals had been misidentified in the deposited structural models. The PDB is a critical resource for researchers worldwide and in 2021 there were on average 1.86 million downloads per day in the US alone, suggesting that over 350,000 models downloaded per day may not contain the correct metal. This has profound implications for those using the models, whose understanding of them may therefore be flawed.

References, if relevant.
[1] EF Garman (2014) Developments in X-ray Crystallographic Structure Determination of Biological Macromolecules. Science 343: 1102-1108
[2] EF Garman. (1999) Cool Data: Quantity and Quality. Acta Cryst. D55: 1641-1653.
[3] OB Zeldin, M Gerstel & EF Garman (2013) RADDOSE-3D: time- and space-resolved modeling of dose in macromolecular crystallography J.Appl.Cryst. 46: 1225-1230
[4] OB Zeldin et al & EF Garman (2013) Predicting the X-ray lifetime of protein crystals. PNAS 110: 20551-20556
[5] JL Dickerson, PTN McCubbin & EF Garman (2020) RADDOSE-XFEL: Femtosecond time-resolved dose estimates for macromolecular XFEL experiments. J.Appl.Cryst. 53, 549–560
[6] KL Shelley & EF Garman (2022) Quantifying radiation damage in the Protein Data Bank. Nature Communications 13:1314- 1325
[7] GW Grime et al. & EF Garman (2020) High-Throughput PIXE as an Essential Quantitative Assay for Accurate Metalloprotein Structural Analysis: Development and Application. J Am Chem Soc. 142(1):185-197

05 Visualizing electrons in redox proteins by single particle cryo-EM

Petra Wendler[1], Christin Radon1, Jakob Ruickoldt[1], Gerd Mittelstädt[2], Paulina M. Dominiak[3]

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Scattering of electrons by atoms in electron microscopy produces coulomb potential maps and hence EM maps reflect the charge of atoms in protein complexes. At resolution ranges between 5 Å and 10 Å, atomic scattering amplitudes are usually weaker the more negatively charged atoms are [1]. Here, we compare the cryo-EM structures of redox proteins in different oxidation states to visualize negative charges on electron accepting atoms in the electron transfer chain of the reduced map. Two redox systems are examined: *Rhodobacter capsulatus* formate dehydrogenase (FDH) and *Carboxydothermus hydrogenoformans* CO dehydrogenase acetyl-CoA synthase complex (CODH/ACS).
FDH catalyzes the reversible oxidation of formate to carbon dioxide. The electrons gained in this reaction at the active site molybdenum atom are transferred to nicotinamide adenine dinucleotide (NAD^+) via an electron-transfer pathway formed by numerous FeS clusters and flavin mononucleotide (FMN). As part of the Wood-Ljungdahl pathway, CODH/ACS catalyzes the reduction and fixation of CO_2 to acetyl-CoA. At resolutions of 3.25 Å for FDH and 2.1 Å for CODH/ACS, the difference maps between the oxidized and reduced states of the enzymes show qualitatively negative charges on the electron accepting atoms in the electron transfer chain [2]. The setup of the microscope, imaging conditions, and applications are discussed.

[1] Bick, T., Dominiak, P.M., Wendler, P., BBA Advances 5 (2024)
[2] Radon, C., Mittelstädt, G., Duffus, B.R., Bürger, J., Hartmann, T., Mielke, T., Teutloff, C., Leimkühler, S. & Wendler, P. Nat Commun 11, 1912 (2020).

06 EasyGrid: a versatile platform for automated Cryo-EM sample preparation and quality control

Olivier Gemin[1], Victor Armijo[2], Michael Hons[2], Caroline Bissardon[2], Romain Linares[2], Matthew W. Bowler[2], Georg Wolff[1], Kirill Kovalev[2,3], Anastasiia Babenko[1], Veijo T. Salo[1], Sarah Schneider[2], Christopher Rossi[2], Léa Lecomte[2], Thibault Deckers[2], Kévin Lauzier[2], Robert Janocha[2], Franck Felisaz[2], Jérémy Sinoir[2], Wojciech Galej[2], Julia Mahamid[1,4], Christoph W. Müller[1], Sebastian Eustermann[1], Simone Mattei[1,5], Florent Cipriani[2], Gergely Papp[2]

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Imaging biological macromolecules in their native state with single-particle cryo-electron microscopy (cryo-EM) or in situ cryo-electron tomography (cryo-ET) requires optimized approaches for the preparation and vitrification of biological samples. Here, we present EasyGrid, a versatile technology enabling systematic, tailored and advanced sample preparation for cellular and structural biology. This automated, standalone platform combines in-line plasma treatment, microfluidic dispensing, blot-less sample spreading, jet-based vitrification and on-the-fly grid quality control using light interferometry to streamline cryo-EM sample optimization. With EasyGrid, we optimized grid preparation for different purified macromolecular complexes and subsequently determined their structure with cryo- EM. We also demonstrated how the platform allows better vitrification of large, mammalian cells compared to standard plunge-freezing. Automated sample preparation with EasyGrid establishes an advanced, high-throughput platform for both single-particle cryo-EM and cellular cryo-ET sample preparation.

07 Advances in the stream processing of single particles by CryoEM

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Cryo-electron microscopy (cryo-EM) is essential for structural biology, providing insights into the molecular machinery of life at near-atomic resolutions. However, the complexity and volume of data generated in cryo-EM studies necessitate sophisticated processing workflows to extract meaningful information efficiently. This project addressed these challenges by developing robust stream processing workflows for cryo-EM facilities, facilitating unattended operations from movie alignment to particle 2D classification, class selection, and extending towards initial 3D reconstruction. These processing steps are shown in the Figure below.

A primary objective of the project was to enhance processing speed while ensuring fully automatic operation, thereby minimizing the need for manual intervention, reducing the potential for errors and actually increasing the quality and the quantity of data being generated at the facilities. To achieve this, the project focused on automating critical imaging processing steps within the software integrator and workflow engine “Scipion”, including parameter estimation. The net results if that the processing workflow gains both efficiency and reliability, as it reduces the likelihood of failures associated with manual parameter tuning.

Another significant advancement introduced by this project is developing a fast alternative to the traditional 2D classification methods. This alternative approach is designed to be more time-efficient, making the overall workflow suitable for academic research environments and attractive to commercial users with different constraints and requirements.

Throughout the project, a key consideration was the robustness of the workflow.

By prioritizing speed and automation, the project team sought to create a workflow that performs efficiently under a wide range of conditions and is resilient against common points of failure. The automatic selection of parameters is pivotal, ensuring the workflow can adapt to various datasets without requiring extensive user input or customization.

Additionally, automatic resource allocation decisions have been implemented by configuring Scipion to utilize a queue management system like SLURM. This approach provides a more controlled and efficient method of managing computation resources. Users no longer need to manually assign resources such as GPUs to each step of the processing pipelines, reducing the risk of common mistakes such as assigning the same GPU to different protocols, which could overload memory and lead to workflow failures.

To benefit the wider community, enhance collaborative research efforts and facilitate knowledge sharing, the workflows developed within the project have been uploaded to public databases such as WorkflowHub allowing easy access and re-usability for other researchers and fostering innovation through open science.

In conclusion, this project represents a significant step in automating cryo-EM data processing. By developing a robust, unattended stream processing workflow that encompasses critical stages from movie alignment to initial 3D reconstruction, the project team has laid the groundwork for more efficient and reliable cryo-EM studies. This advancement holds the promise of accelerating scientific discoveries in structural biology by enabling faster, more accessible, and error-resistant processing of cryo-EM data.

08 Detecting protein-drug interactions in human cells by real-time ^{19}F NMR

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In-cell NMR spectroscopy is a unique approach to study the structure and function of biological macromolecules in their native cellular environment at atomic resolution.[1] A major limitation of in-cell NMR is the short lifetime of the cells once they are densely packed in a closed environment. NMR flow bioreactors can greatly extend the sample lifetime by providing the cells with fresh nutrients and oxygen. This makes possible to study intracellular processes in real time over the course of up to 72 hours.[2] Real-time in-cell NMR provides important information on protein-ligand interactions, such as intracellular ligand binding kinetics and thermodynamics, which are critical to optimize drug penetrance and potency.[3,4] Classical screening by ^{1}H and ^{1}H-^{15}N spectra is often limited by fact that signals from target proteins interacting with cellular components are broadened beyond detection. ^{19}F NMR spectroscopy is ideally suited for the purpose, thanks to the high-sensitivity and background-free nature of ^{19}F. We recently showed that fluorinated amino acids can be incorporated in proteins expressed in human cells.[5] This allows protein-observed screening on otherwise invisible targets. Finally, fluorinated ligands can be directly observed as they interact with their intracellular targets. We showed that ligand-based in-cell screening can be achieved by competition binding. In this setup, the displacement of a fluorinated spy ligand is monitored by time-resolved NMR in the bioreactor as a function of the concentration of a second non-fluorinated ligand (**Figure 1**). The binding affinity of the latter is then obtained relative to that of the spy ligand.[6] Such approaches hold great potential in the development of more effective drugs towards pharmacologically relevant targets.

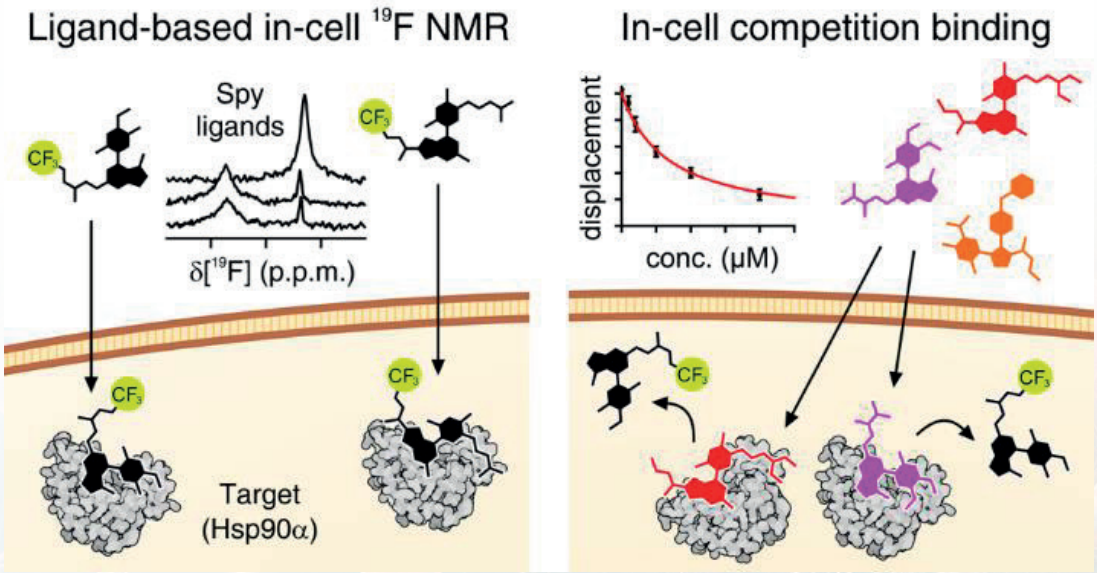

Figure 1. Ligand-based competition binding in human cells by in-cell ^{19}F NMR.

References
[1] Luchinat, E., Cremonini, M., & Banci, L. (2022). Chemical reviews, 122(10), 9267-9306.
[2] Luchinat, E., Barbieri, L., Campbell, T. F., & Banci, L. (2020). Analytical chemistry, 92(14), 9997-10006.
[3] Luchinat, E., Barbieri, L., Cremonini, M., Nocentini, A., Supuran, C. T., & Banci, L. (2020). Angewandte Chemie, 132(16), 6597-6601.
[4] Luchinat, E., Barbieri, L., Cremonini, M., Pennestri, M., Nocentini, A., Supuran, C. T., & Banci, L. (2021). Acta Crystallographica Section D: Structural Biology, 77(10), 1270-1281.
[5] Pham, L. B., Costantino, A., Barbieri, L., Calderone, V., Luchinat, E., & Banci, L. (2023). Journal of the American Chemical Society, 145(2), 1389-1399.
[6] Luchinat, E., Barbieri, L., Davis, B., Brough, P. A., Pennestri, M., & Banci, L. (2024). Journal of Medicinal Chemistry, 67(2), 1115–1126.

09 Light-NMR: what can we learn about photo-transformable fluorescent proteins?

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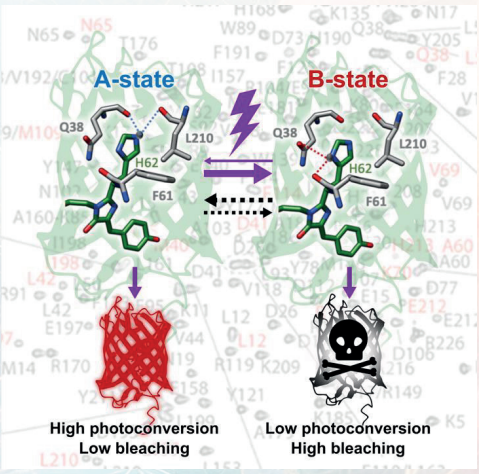
Fluorescent proteins (FPs) that undergo reversible or irreversible photo-transformations when exposed to light at specific wavelengths are of crucial importance for a wide range of applications in advanced fluorescence microscopy and biotechnology. The exact mechanisms behind these photo-induced conformational changes remain poorly understood, which makes engineering of improved variants based on rational protein design a difficult or even impossible task. At present, mechanistic information on FPs has been derived from crystallographic structures, complemented by data from optical spectroscopy and quantum-chemical calculations. Solution NMR spectroscopy, combined with in-situ sample illumination provides an unique tool to investigate at atomic resolution the conformational and dynamic properties of FPs in their different photo-stationary states [1]. It also allows to access the interconversion dynamics of various conformational states and to derive kinetic models underlying the observed photophysical properties. We have set up a portable in-situ illumination device that is compatible with high-field NMR spectrometers [2], and currently permits sample illumination at 3 wavelengths (405, 488, and 561 nm). Recently, we have added light detection capabilities to our setup in order to record simultaneously NMR and fluorescence emission data. This allows to correlate changes in the populations of conformational states (NMR) with their fluorescent properties (emitted light). Here we will illustrate the potential of in-situ light-NMR for FP research by recent results obtained for the green-to-red photoconvertible protein mEos4b [3]. We observed by NMR spectroscopy that mEos4b exhibits two distinct conformations in the green state that slowly interconvert. NMR also revealed that these conformations differ in the protonation states of two amino-acid side chains in the chromophore pocket, resulting in an altered hydrogen bond network. These subtle rearrangements were not visible in high-resolution x-ray structures of mEos4b. Importantly, only one of these conformations photoconverts efficiently to the red state, while the other one appears to be more susceptible to photobleaching. This study helps to explain the observed complex photophysical behavior of mEos4b and related PCFPs. More generally, it reveals how conformational dynamics of fluorescent proteins affect their photophysics, and in particular the photoconversion mechanisms of mEos-derived PCFPs. Finally, our results open the door for designing new PCFP variants with superior photoconversion efficiency.

[1] Christou et al. J. Am. Chem. Soc. (2021) 143, 7521-7530;
[2] Christou et al. Biophys. J. (2019), 117, 2087-2100;
[3] Maity et al. Adv. Sci. (2023) 2306272, 1–11.



10 Instrumentation development: Integrated multimodal microscopy of cryo-preserved samples

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The recent technological breakthrough in cryo-electron tomography (cryo-ET) opens the transforming possibility to image macromolecular complexes in their native cellular context at close to atomic resolution to directly reveal how different conformational states of protein complexes are linked to biological functions. At the same time, fluorescence imaging can now study fundamental cellular functions at the single molecule level in space and time in both cellular and organismal models. To link the structural and functional breakthroughs from both these fields, we must develop correlative multimodal microscopies for seamless switching from millimeter-scale multicellular specimens via micrometer scale cells to the nanometer-range of macromolecular complexes.
Our recent developments within the framework of iNext Discovery for automation of cryo-focused ion beam (FIB) thinning guided by 3D correlative fluorescence microscopy now allow routine structural analysis of frozen-hydrated single-cell specimens [1]. To enable cryo-ET to be performed in multicellular specimens, the key challenge is to fabricate a few hundred nanometer thin electron transparent window (lamella) with a focused ion beam, at the site of interest within an up to millimeter sized volume. Targeting at such precision within very large volumes is only possible by developing technology that allows to integrate multiple microscopy modalities for cryo-preserved specimen [2]. In order to bring about these advances, we combined a micromanipulator cryo-lift-out system (Omniprobe-350, Oxford Instruments) with an optimized sample holder design (Leica) incorporated into a FIB-SEM microscope (Zeiss, Crossbeam 550). Here, we will present the use of such instrumentation setup in the context of a cryo-correlative light and electron microscopy (CLEM) pipeline for the investigation of high pressure frozen multicellular specimens across scales, achieved via cryo-fluorescence confocal microscopy, cryo-FIB-SEM volume imaging, followed by extraction of vitrified specimen blocks for the preparation of cryo-FIB lamellae towards cryo-ET.

Acknowledgments: this work is supported by the EMBL and iNext Discovery, the EMBL electron microscopy core facility and our industry collaboration partners Leica Microsystems and Oxford Instruments.

[1] Klumpe S, Fung HKH, Goetz SK, Zagoriy I, Hampoelz B, Zhang X, Erdmann PS, Baumbach J, Müller CW, Beck M, Plitzko JM, Mahamid J, eLife, 2021
[2]D'Imprima, E, Garcia Montero M, Gawrzak S, Ronchi P, Zagoriy I, Schwab Y, Jechlinger M, Mahamid J, Developmental cell, 2023

11 Advancing Correlative Microscopy: Automated Alignment of cryo-3D-SIM and Soft X-ray Tomography Data – Strategies and Implementation

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Correlative microscopy, combining multiple imaging modalities, is a powerful tool for obtaining comprehensive insights into complex biological systems. This work presents a significant advancement in the field by fully automating the 3D alignment of cryo 3D Structured Illumination Microscopy (3D-SIM) data and Soft X-ray Tomography (SXT) data [1]. The proposed strategies and their implementation aim to enhance the accuracy and efficiency of this correlative imaging approach.

The workflow involves the integration of the 3D visible light fluorescence and absorption data, providing complementary information on cellular function and structure at high resolution. The challenge lies in precisely aligning these datasets, considering their differences in imaging modalities and 3D data reconstruction procedures. To address this, we have developed novel algorithms for the automatic alignment, paving the way for a more streamlined and accurate correlative process.

The key strategies employed in the automated alignment process include:

Feature-Based Matching: Leveraging distinctive features within the cryo 3D-SIM and SXT datasets, the algorithm performs feature size range-based matching to establish correspondences between the images. This approach ensures accurate alignment by identifying common structural landmarks in both datasets.

Two-Steps Alignment: The alignment strategy is divided into two processes. First, a coarse 2D alignment between a Soft X-ray mosaic view and a 2D projection of the 3D fluorescence data. Second, a fine iterative 3D angular and shift search refines the alignment between both 3D datasets.

Tomographic 3D-Correlation based on 2D-Correlation Backprojection: Given the sparsity of SXT 3D data in the Fourier domain (limited projections and missing wedge), a novel 3D correlation process is implemented. The 3D alignment is addressed by only considering effective data through tomographic backprojection reconstruction of 2D projection correlations.

Implementation of these strategies has yielded promising results in automating the alignment of cryo 3D-SIM and SXT data. The success of this automated alignment process relies on size range filters rather than specific landmarks, allowing for the effective fiducialization of various cellular structures, such as lipid droplets or mithochondrias.

In conclusion, the automated alignment of cryo 3D-SIM and SXT data represents a significant step forward in correlative microscopy. The strategies and algorithms presented in this work offer a robust and efficient solution for aligning datasets, facilitating more accurate and insightful investigations into the intricate world of cellular structures and their function.

References:

[1] Groen, J. et al. Correlative 3D cryo X-ray imaging reveals intracellular location and effect of designed antifibrotic protein-nanomaterial hybrids. Chemical Science, 12(45), 15090–15103 (2021).

12 Building a Fragment Screening Data Pilot Repository: Challenges and Opportunities

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A fragment screening experiment can be visualized as small probes being released onto a macromolecule, exploring its cavities and surfaces in search of potential interaction sites. A fragment screening experiment has complexity with experimental setup and observed outcomes and this creates challenges in making its data publicly **F**indable and **A**ccessible, as well as in a standard format to enable **I**nteroperability and **R**eusability – aka compliant with **FAIR** principles.[1] Among the difficulties in structuring a global repository for fragment screening is to effectively capture the relationships between the data generated by these experiments.

Fragment screening experiments yield knowledge that goes beyond analyzing a solitary fragment bound to a macromolecule. Consider structure-based fragment screening, the insights obtained come not only from an individual structure of a macromolecule with a fragment bound, but also come from considering this structure in the context of the other fragments in the screen , as well as the other structures with fragments bound, along with relevant data and metadata. The Worldwide Protein Data Bank (wwPDB, wwpdb.org) has a long established pipeline that processes individual structures determined by a variety of structural biology methods. This process is enabled by a data framework called Protein Data Bank Exchange (PDBx) / macromolecular Crystallographic Information Framework (mmCIF).[2] When one submits a structure it undergoes a semi-automated standardization and validation pipeline, where every structure has input and review by a wwPDB curator at one of the wwPDB sites.

In consultation with large-scale structure-based fragment screening facilities (e.g. FragMAX, EMBL Grenoble, DIAMOND, etc.), we have been exploring how to support the capture and archiving of the rich datasets generated at these facilities, leveraging the PDBx/mmCIF data framework. A key aspect is that there are multiple structures that need to be considered in context with each other and we have developed “investigation files” to capture this.

We have created a pilot repository using existing data from the wwPDB. This repository showcases the new data standards designed to facilitate the capture of the data and metadata generated from fragment screening experiments. This includes the chemical identity of all fragments screened, as well as whether they were hits or non-hits. This data structure can be expanded to capture additional metadata, (*e.g.* temperature of the experiment), as the repository evolves. Our “investigation files” demonstrate how this data structure supports fragment screening metadata with links to fragment bound structures deposited in the wwPDB, and has the capacity to link to different repositories or databases and other url locations. The fragment screening pilot repository is accessible at: https://ftp.ebi.ac.uk/pub/databases/msd/fragment_screening/

This fundamental development is a key step for enhancing data capture for fragment screening experiments, and will support enhancements in data deposition, data standardization, and data analysis, providing additional opportunities for discovery, especially for interactions between small molecules and macromolecules.

References:
[1] Wilkinson, Mark D et al. "The FAIR Guiding Principles for scientific data management and stewardship." Scientific data vol. 3 160018. 15 Mar. 2016, doi:10.1038/sdata.2016.18
[2] Westbrook, John D et al. "PDBx/mmCIF Ecosystem: Foundational Semantic Tools for Structural Biology." Journal of molecular biology vol. 434,11 (2022): 167599. doi:10.1016/j.jmb.2022.167599

13 Magnet Innovations, Liquid State NMR Probes and Software News from Bruker BioSpin

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Modern science relies on precision, advanced analytical techniques, and methods. For decades, Bruker Biospin has closely collaborated with scientists to develop state-of-the-art instrumentation that enables and supports cutting-edge research.
In this presentation, we will talk about recent innovations in magnet technology - the Ascend Evo magnet line; provide updates on the liquid-state probe-head portfolio - the latest additions to helium and nitrogen cooled cryoprobes; and inform you on the current status of the NMR in Functional Structural Biology project at Bruker BioSpin – Moving from Trained Experts to Integrated Workflows.

Acknowledgments: Dr. Rainer Kuemmerle, Applications and R&D teams.

14 Chemical evolution of the aggregation-prone regions by means of amyloid formation

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It has been proposed that short amyloidogenic oligopeptides were the first macromolecules under prebiotic Earth conditions through the formation of aggregated and solvent-separated nanosystems. Here, we provide experimental evidence that an aggregation-prone oligopeptide A, APR-A, can be transformed into APR-B via a mutational pathway that consists of either only insoluble amyloid-like oligopeptides or only water-soluble non-aggregating oligopeptides. Among the parallel transformation pathways linking the two APR end points, a set of strongly amyloidogenic (pro-amyloid) sequences and a set of weakly amyloidogenic (anti-amyloid) sequences were generated and examined. The pro-amyloid pathways were found to be inducing heterogeneous phase separation. We found that the order of the point mutations alone determines the physicochemical properties of the pathway elements. A number of amyloids have been crystallized and about a dozen polymorphs have been structurally characterized, including the recently identified class 3 topology. Establishing pro- and anti-amyloid pathways between two intrinsically aggregation-prone oligopeptides may help elucidate how water-soluble miniproteins and insoluble amyloid structures evolved to form the first self-organizing, phase-separated biosystems. It also provides deeper insight into the mechanism by which the amyloidicity of medically relevant oligopeptides is enhanced or inhibited.

15 Molecular mechanisms of MICAL signaling in cytoskeletal dynamics

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MICALs (Molecules Interacting with CasL) are a family of unique signaling molecules that directly bind and disassemble actin filaments and are known to play essential roles in cell processes requiring discrete changes in the cytoskeleton, such as axon growth and guidance, synapse development, dendrite morphology and neuronal cell migration. A growing number of studies implicate MICALs and their signaling pathways in a range of psychiatric and neurological disorders. The activity of MICALs in cells must be tightly controlled; however, the mechanism by which the MICALs activity is precisely turned on and off is unknown.

Our study unveils the cryoEM structure of full-length human MICAL1. The structural analysis highlights intermolecular interactions between the catalytic monooxygenase and C-terminal coiled-coil domains, elucidating a mechanism of pre-signaling autoinhibition in MICAL1. Experimental validation via actin depolymerization assay and TIRF microscopy further corroborates this autoinhibitory interaction, shedding light on the precise modulation of MICAL1 activity within cells.

16 A divide-and-conquer structural biology approach to elucidate the photocycle of light-regulated transcription factor EL222

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EL222 is a photocontrolled DNA-binding protein composed of a light-oxygen-voltage (LOV) sensor domain and a helix-turn-helix (HTH) effector domain. The protein is transcriptionally active only upon blue-light excitation of the flavin mononucleotide (FMN) chromophore embedded in the LOV domain. However, the structure-activity relationship of EL222 along the photocycle is not completely understood. To gain molecular-level insight into the photoinduced fold-switching of EL222, we applied an integrative structural biology approach combining nuclear magnetic resonance (NMR) spectroscopy, X-ray crystallography, optical spectroscopy, and molecular dynamics simulations. NMR provided evidence for two monomeric lit-state species of EL222 featuring differences in the local environment around certain residues in the absence of major changes of secondary structure and protein compactness. With the help of UV-visible and infrared spectroscopies, these states were assigned to covalently-bound semiquinone (lit1) and non-covalently-bound hydroquinone (lit2) forms of the FMN cofactor. Subtle atomic displacements were observed by comparing the 1.85 Å-resolution crystal structures of EL222 with and without back-illumination. Loss of α-helices upon irradiation was inferred from infrared difference spectroscopy, and was ascribed to the dimeric EL222 species. All three conformations (dark, lit1, lit2) can associate with DNA but only the lit states populate stable EL222:DNA (2:1) complexes. Overall, we propose a new model of EL222 regulation where the redox state of FMN and thioadduct formation, both dependent on blue-light intensity, dictate EL222 structure, dynamics, oligomerization, and interaction with DNA.

17 Using structural biology to transform nature's switches into our biotech tools and therapeutics

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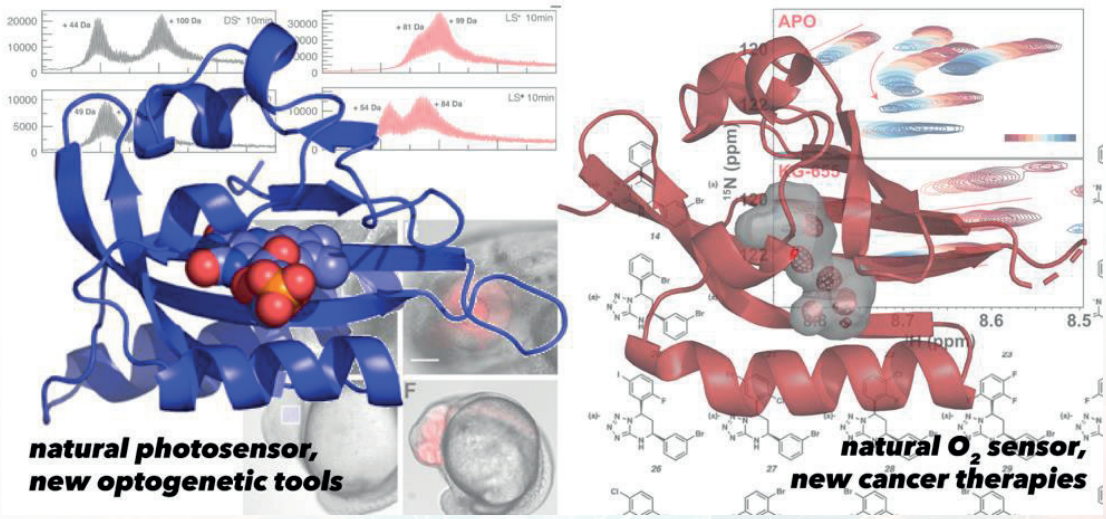
Environmental cues regulate many biological processes, coordinating cellular pathways to respond to changing conditions. Such regulation is often initiated by sensory protein domains which expand their chemical repertoire by using small molecule ligands to convert environmentally-triggered changes into altered protein/protein interactions. Using a combination of biophysics, biochemistry and synthetic chemistry, we study the mechanistic controls of such domains for both fundamental understanding and subsequent artificial control. Here I will discuss two examples of this principle, showing how such signaling proteins can be converted into novel biotech tools or targeted by small molecule therapeutics, including Merck's newly-approved belzutifan, a first in-class anti-cancer therapeutic targeting the Hypoxia Inducible Factor 2 (HIF-2) transcription factor. Future directions stemming from this work will also be discussed.

Figure 1. Examples of adapting environmental sensory proteins – blue light Light-Oxygen-Voltage (LOV) domains and Hypoxia Inducible Factors (HIFs) – into biotechnology tools and therapeutic targets with structural biology-rooted approaches.



18 Ultrastructural details of the bacterial spore revealed by in situ cryo-electron tomography

Elda Bauda[1], Benoit Gallet[1], Jana Moravcova[2], Gregory Effantin[1], Helena Chan[3], Jiri Novacek[2], Pierre-Henri Jouneau[4], Christopher D.A. Rodrigues[5], Guy Schoehn[1], Christine Moriscot[6], Cecile Morlot[1]

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Bacterial sporulation is a morphological differentiation process that leads to the formation of a dormant spore insensitive to antibiotics and resistant to extreme environmental conditions, including detergents, radiation, and high temperatures. This resilience makes spore-forming pathogens difficult to eliminate and contributes to their persistence. The bacterial spore's striking resistance capacities arise from protective molecular structures, including the compacted structure of the chromosome and the multi-protein extracellular shell known as the coat. The mechanisms underlying the formation of such complex protective structures are examples of the sophistication that the cell achieves in building subcellular assemblies with remarkable spatial and temporal precision. Despite their importance for cell survival, these mechanisms are not fully elucidated, mainly because they involve macromolecular complexes of nanometric dimensions, the assembly of which requires the cellular environment. As a result, the study of these processes requires high-resolution in situ observation methods. To address this challenge and analyze the ultrastructure of *Bacillus subtilis* spore, we have implemented in situ cryo-electron tomography (cryo-ET) on bacteria lamellae generated by cryo-focused ion beam milling (cryo-FIBM). Our tomograms reveal a wide variety of cellular structures with unprecedented levels of detail. Early during sporulation, the chromosome in the developing spore adopts a toroidal structure. At the same stage, coat proteins at the surface of the forespore form a complex stack of amorphous and structured layers with distinct electron density, dimensions, and organization. We investigated the nature of the nascent coat layers in various mutant strains using cryo-FIBM/ET and transmission electron microscopy (EM) on resin sections of freeze-substituted bacteria. Combining these two cellular EM approaches, we distinguish seven nascent coat regions with different molecular properties and propose a model for the contribution of the essential morphogenetic proteins SpoIVA, SpoVID, SafA and CotE.

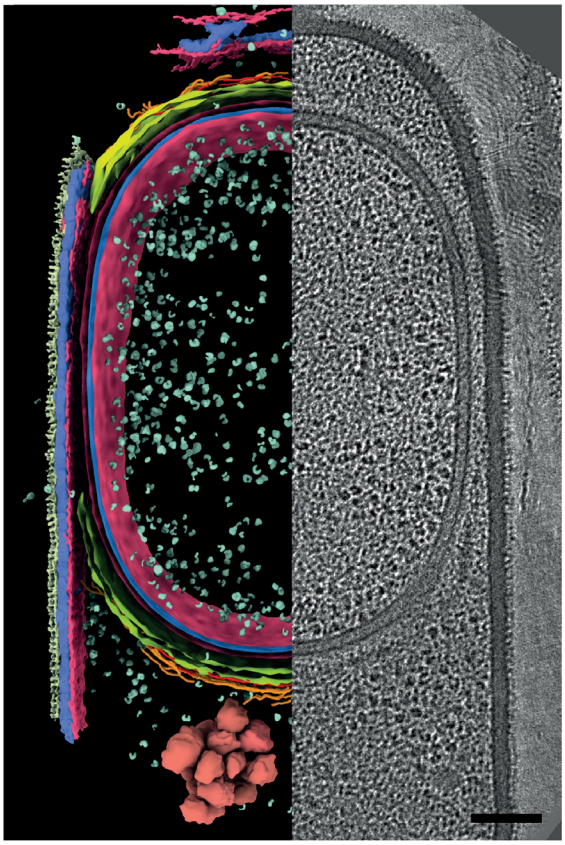

Figure 1. Tomogram reconstruction and segmentation of the cellular ultrastructures of B. subtilis sporangium. Scale bar, 100 nm

Bauda, E., Gallet, B., Moravcova, J., Effantin, G., Chan, H., Novacek, J., Jouneau, P.-H., Rodrigues, C. D. A., Schoehn, G., Moriscot, C., & Morlot, C. (2024). Ultrastructure of macromolecular assemblies contributing to bacterial spore resistance revealed by in situ cryo-electron tomography. Nature Communications, 15(1), Article 1. https://doi.org/10.1038/s41467-024-45770-6

19 Hybrid methods for analyzing conformational variability in cryo-EM and cryo-ET data

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The elucidation of different conformations of biomolecular complexes is the key to understand the molecular mechanisms behind the biological functions of the complexes and the key to novel drug discovery. Single-particle cryo electron microscopy (cryo-EM) allows 3D reconstruction of multiple conformations of purified biomolecular complexes from their 2D images. Cryo electron tomography (cryo-ET) allows obtaining information on the conformational variability of the complexes in their cellular environment. My group is developing hybrid methods for analyzing continuous conformational changes of biomolecules from cryo-EM and cryo-ET data, which integrate image processing, molecular dynamics simulations, and deep learning approaches. These methods are made available publicly via our open-source, ContinuousFlex software package (a plugin of Scipion, the software largely used in the cryo-EM/ET field). In this talk, I will present our recent work regarding these methodological developments.

20 What do microRNAs and ribosomes have in common? Dynamics!

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Many functions of RNA depend on rearrangements in secondary structure that are triggered by external factors, such as protein or small molecule binding. These transitions can feature on one hand localized structural changes in base pairs[1] or can be presented by a change in the chemical identity of e.g. a nucleo-tbase tautomer[2]. We use and develop $R_{1\rho}$-relaxation-dispersion NMR methods[3,4] for characterizing transient structures of RNA that exist in low abundance (populations <10%) and that are sampled on timescales spanning three orders of magnitude. We further analyse RNA complexes using RNA structural probing[5], Cryo-EM and functional assays of the trapped states.
Two different systems are going to be discussed: 1) A microRNA – mRNA complex changes conformation to activate the RISC complex[6] and how microRNAs in general select their targets[7]. 2) Flipping the 3D stabilizer A-minor motifs to regulate ribosome dynamics.
We will furthermore give an outlook on recent efforts to measure in-cell NMR of nucleic acids in functional complexes[8].

References:
[1] Dethoff, E. A., Petzold, K., Chugh, J., Casiano-Negroni, A. & Al-Hashimi, H. M. Visualizing transient low-populated structures of RNA. Nature 491, 724 728 (2012).
[2] Kimsey, I. J., Petzold, K., Sathyamoorthy, B., Stein, Z. W. & Al-Hashimi, H. M. Visualizing transient Watson–Crick-like mispairs in DNA and RNA duplexes. Nature 519, 315 320 (2015).
[3] Steiner, E., Schlagnitweit, J., Lundström, P. & Petzold, K. Capturing Excited States in the Fast-Intermediate Exchange Limit in Biological Systems Using 1H NMR Spectroscopy. Angewandte, (2016)
[4] Schlagnitweit, J., Steiner, E., Karlsson, H. & Petzold, K. Efficient Detection of Structure and Dynamics in Unlabeled RNAs: The SELOPE Approach. Chem European J 24, 6067–6070 (2018).
[5] Banijamali, E. et al. RNA:RNA Interaction in Ternary Complexes Resolved by Chemical Probing. RNA 29, rna.079190.122 (2022).
[6] Baronti, L. et al. Base-pair conformational switch modulates miR-34a targeting of Sirt1 mRNA. Nature 583, 139–144 (2020).
[7] Kosek, D. M., Banijamali, E., Becker, W., Petzold, K. & Andersson, E. R. Efficient 3'-pairing renders microRNA targeting less sensitive to mRNA seed accessibility. Nucleic Acids Res. 51, 11162–11177 (2023).
[8] Schlagnitweit, J. et al. Observing an Antisense Drug Complex in Intact Human Cells by in-Cell NMR Spectroscopy. Chembiochem 20, 2474–2478 (2019).

21 Impact of osteosarcoma cells differentiation on biomineralization and mitochondrial morphology

Francesca Rossi[1,2], Giovanna Picone[1], Andrea Sorrentino[3], Concettina Cappadone[1], Giovanna Farruggia[1], Martina Rossi[1], Iotti Stefano[1], Eva Pereiro[3], Emil Malucelli[1]

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OBJECTIVE: Osteosarcoma (OS) is the most common primary malignant bone tumor, and its etiology has been recently associated with osteogenic differentiation dysfunctions [1,2]. A deeper understanding of the relationship between defects in osteogenic differentiation and malignant bone tissue formation could help the comprehension of osteosarcoma tumorigenesis and the development of new treatment strategies. To characterize the defective biomineralization process that occurs in OS, we studied osteoblast-like SaOS-2 cells during the early stages of differentiation. Cryo-soft X-ray tomography (cryo-SXT) and cryo-XANES imaging at the CaL2,3 edges were combined to investigate the evolution of the Ca-depositions during differentiation, allowing to simultaneously track morphological variations in intracellular organelles at nanometric resolution.
MATERIALS and METHODS: SaOS-2 cells were grown on electron microscopy grids, treated for 4 and 10 days with a differentiating cocktail, plunge frozen in liquid ethane and finally imaged in a quasi-native state using the soft X-ray transmission microscope installed at the Mistral beamline of the Alba synchrotron.
RESULTS and DISCUSSION: Untreated SaOS-2 produced calcite depositions (Fig. 1) as previously observed in early differentiated bMSCs (bone mesenchymal stem cells) [2]. This confirms that the early phases of osteoblastic differentiation are similar in both bMSCs and SaOS-2 cells, supporting the hypothesis that OS cells arise from MSCs unable to undergo complete differentiation [3, 4]. The evolution of mineral deposits from calcium phosphate to hydroxyapatite (Fig. 1) induced by the differentiating treatment suggests a partial restoration of the physiological biomineralization process in SaOS-2 cells, as previously assessed in differentiating bMSCs [2]. At 4 days after induction (Fig. 1), mitochondria contain small Ca structures and are linked to vesicles containing calcium phosphate depositions highlighting calcium transfer between the two intracellular organelles (Fig. 1). At 10 days after induction, no Ca minerals are detected in the mitochondria, supporting the interplay between mitochondria and vesicles in calcium trafficking (Fig. 1). Interestingly, during differentiation, mitochondria show a change in morphology from elongated to rounded indicating a metabolic reprogramming of OS cells possibly linked to an increase in glycolysis contribution to energy metabolism. These findings contribute to the understanding of OS genesis giving new insights on the development of therapeutic strategies able to restore the physiological mineralization in OS cells.

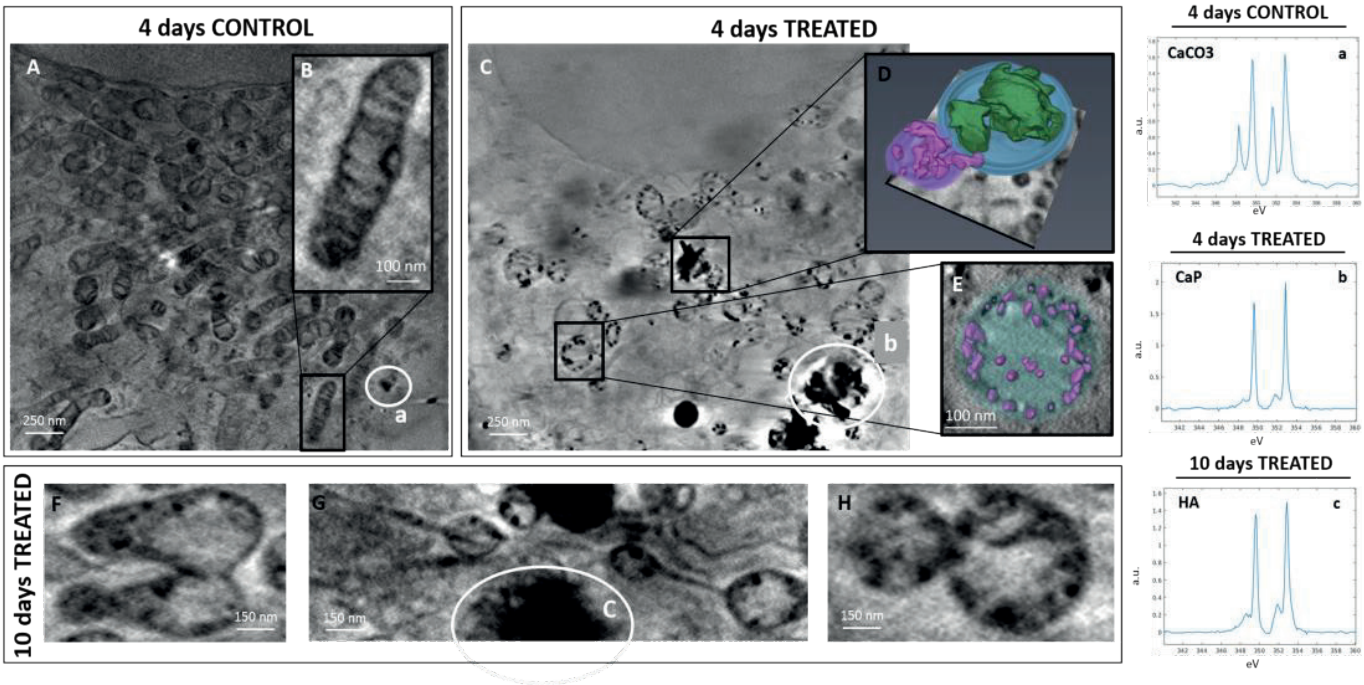

Figure1: A) Tomogram of a 4 days control cell, B) zoom on an elongated mitochondrion. White circle indicates CaCO3 deposition and a) reports the XANES spectrum. C) Tomogram of a 4 days treated cell, D) zoom on a mitochondrion containing Ca linked to a vesicle, E) 3D reconstruction of a mitochondrion. White circle indicates calcium phosphate deposition and b) reports the XANES spectrum. F), G) and H) zoom on mitochondria from a tomogram of a 10 days treated cell. White circle indicates HA depositions and c) reports the corresponding XANES spectrum.

[1] Brown HK, Schiavone K, Gouin F, Heymann MF, Heymann D. Calcif Tissue Int. 2018;102(2):174–95
[2] Sorrentino A, Malucelli E, Rossi F, Cappadone C, et al. Int J Mol Sci. 2021;22(9).1
[3] Lamplot J, Denduluri S, Qin J, Li R, Liu X, Zhang H, et al. Curr Cancer Ther Rev. 2013;9(1):55–77
[4] Wagner ER, He BC, Chen L, Zuo GW, Zhang W, Shi Q, et al. PPAR Res. 2010;2010:11–3

22 *In-situ* characterization of the bacterial outer membrane protein A

Johannes Thoma

Outer membranes of Gram-negative bacteria contain transmembrane proteins in a distinguished membrane environment of highly asymmetric architecture. Whereas the inner leaflet of outer membranes is composed of regular phospholipids, the outer leaflet consists almost exclusively of lipopolysaccharides (LPS). To date, no methods exist to experimentally mimic the complex native lipid environment of bacterial outer membranes. Studies characterizing the structure and function of outer membrane proteins rely on membrane-mimetic systems or artificially reconstituted bilayers. However, a growing number of studies indicate that the membrane composition directly impacts the structural and functional states of integral membrane proteins.
To address this crucial issue, we recently developed a method to manipulate the protein content of bacterial outer membrane vesicles (OMVs). These vesicles are prepared to contain selected outer membrane proteins at high density and circumvent the limitations of established methods. Using engineered OMVs, we here characterize the prototypic outer membrane protein A (OmpA) from *Escherichia coli* for the first time within its native asymmetric membrane environment at the atomic level using proton detected ultra-fast solid-state NMR spectroscopy. Our methodology allows extensive assignment and structure determination of the transmembrane part of OmpA and site-specific comparison with OmpA reconstituted in lipid bilayers. An increase in detectable resonances by > 50% as well as increased backbone order parameters indicate that OmpA has a different, more stable and extended fold within the native membrane environment compared to existing structures in membrane-mimetic systems. Our work thereby provides a blueprint for *in situ* structural biology of membrane proteins in the future.

23 Structural and functional characterisation of mechanisms controlling epithelial tissue integrity

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The Wnt3 cascade controls cell fate during tissue morphogenesis and homeostasis. Wnt activation stabilizes the main effector β-catenin and activates transcriptional programs for stemness maintenance. Seminal studies from the Nusse laboratory showed that localized Wnt3 ligands suffice to orient asymmetric divisions of stem cells and determine unequal daughter cell identities. However, the cross-talk between localized Wnt3 signals, division orientation, and fate inheritance is poorly understood.
I will present genetic dissection and biochemical reconstitution of molecular events instructing oriented cell divisions toward artificial niches created by coating magnetic nanospheres with purified Wnt3 ligands. Live imaging of cells dividing in contact with Wnt3-coated beads showed that the division orientation proteins NuMA, LGN and Gαi, as well as Lrp6, β-catenin, and Dvl2 are essential for Wnt3-mediated division orientation.
This indicates that the spindle orientation machinery, centered on cortical dynein/NuMA/LGN/Gαi complexes, enriches at the Wnt3a-coated bead to orient the division perpendicular to the source of Wnt3. Magnetic isolation revealed that Wnt3a-bead-engaged Lrp6 co-receptors recruit Dvl2, Axin1, CK1α, and NuMA. Proteomic analyses of Wnt3a-bead pulldowns revealed that not only canonical Wnt components, mitotic effectors, and actomyosin remodelers but also RNA-binding proteins and mitochondrial components accumulate at the bead contact site and will be segregated asymmetrically. I will also discuss the reconstitution from recombinant sources of the NuMA/β-catenin containing complexes responding to localized Wnt3 signals, and the characterization of their topology by cryo-EM and Cross-Linking Mass-Spectrometry.

Collectively, our findings uncover the fundamental principles of Wnt3a-driven division orientation, and provide insights into the molecular mechanisms orchestrating Wnt3-dependent fate asymmetry.

24 Characterizing biomolecular structure and interactions in living human cells synchronized in defined physiological states using NMR spectroscopy

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In-cell NMR spectroscopy is the sole technique for characterizing biomolecular structure, dynamics, and interactions in living human cells at atomic resolution. This approach has recently emerged as a source of valuable information for drug screening and development. However, its applications have been restricted to asynchronous single-cell suspensions. We adapted the original in-cell NMR technique to allow the acquisition of high-resolution NMR spectra of proteins and nucleic acids in living human cells synchronized in different defined physiological states. The approach can be directly transferred to other in-cell spectroscopic techniques such as in-cell EPR, solid-state NMR, and single-molecule FRET. I will discuss the possibilities for acquiring high-resolution in-cell NMR spectra of proteins in 3D models of human tissue.

References:
Víšková, P., Ištvánková, E., Ryneš, J. et al. In-cell NMR suggests that DNA i-motif levels are strongly depleted in living human cells. Nat. Commun. 15, 1992 (2024).
Ryneš, J., Ištvánková, E., Krafčíková, M. et al. Inducible protein expression in stably transfected cells paves the way toward in-cell NMR studies in defined physiological states and 3D tissue cultures. Submitted (2024).

25 Allostery of the Lac repressor

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Gene regulation is an essential step in the expression of DNA. A well-known gene regulator is the Lac repressor which regulates the expression of bacterial lactose genes. Thus far, structural understanding of its mode of action is still largely lacking. We analyzed the Lac repressor in distinct states related to gene activation using NMR and show that it exists in a dynamic equilibrium between two conformations.[1] In one conformation the Lac repressor can form a tight complex with DNA. In the other it can bind an inducer, causing destabilization of the helices connecting its core and DNA-binding domains, and thereby weakening DNA binding. Our observations favor a mechanism of activation via the well-known Monod-Wyman-Changeux model [2] as it relies on actively shifting defined structural states upon ligand binding.

[1] Romanuka et al, 2023, Proc Natl Acad Sci USA, 120 (49) e2311240120
[2] Monod et al, 1965, J Mol Biol, 12, 88-118

26 Advances and Frontiers in Cryo Electron Microscopy

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In the recent years, electron-cryo microscopy (cryo-EM) single-particle analysis (SPA) has greatly benefited from a multitude of technological advances cumulating in breaking the atomic-resolution barrier for biological macromolecules. Since then, several applications fields such as drug discovery and drug design have benefitted and accelerated due to high throughput and high resolution screening and data collection technologies. Here we show how the powerful tandem of the 200 kV Glacios and the 300 kV Krios cryo transmission electron microscope equipped with state-of- the-art hardware and software contribute to gaining more detailed insights in molecular mechanisms and design of new medical therapies.
Whereas single particle analysis (SPA) is focusing on unraveling purified proteins and protein complexes at the atomic resolution level, *in situ* Cryo-Electron Tomography (Cryo-ET) has emerged as the method of choice to investigate these structures in their native context. As such, cryo tomography can image the entire proteome of cells and tissues at increasingly higher resolutions thus providing a rich source of information and potentially contribute to finding cures for diseases.
Here we will shortly discuss the latest status of tomography and show how the next-generation cryo-plasmaFIB improve both lamellae quality and throughput. These initial results open a promising new direction for large cryo-ET datasets which will facilitate high throughput collaborative in situ structural biology.

27 Accelerating Cryo-TEM Sample Preparation with TESCAN plasma FIB-SEM

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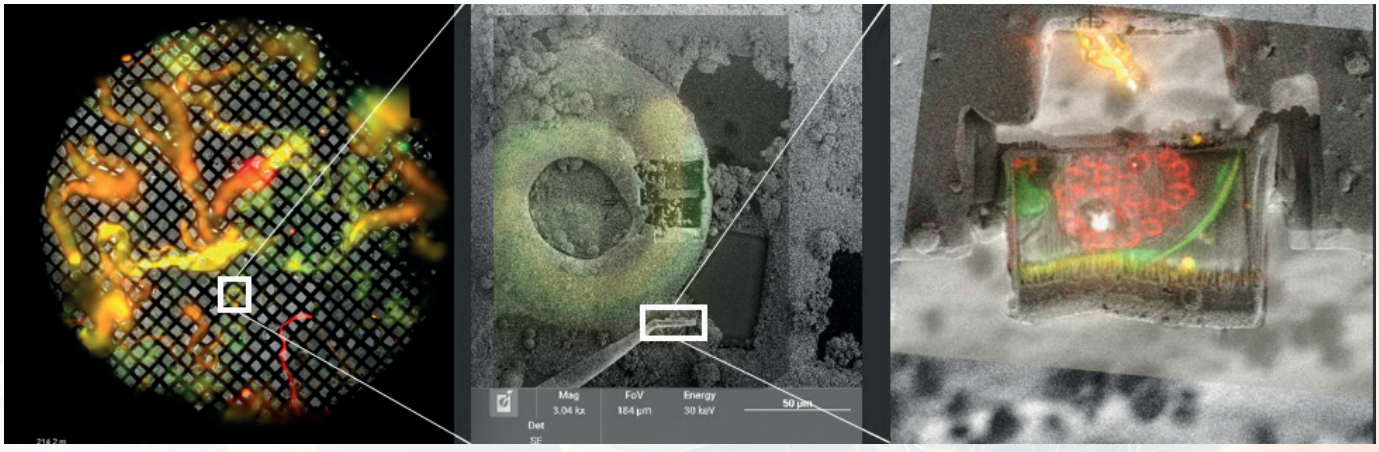
Cryo-electron tomography (cryo-ET) has revolutionized structural biology by enabling the observation and characterization of biological samples at a molecular level in their near-native state. This technique provides an unprecedented level of structural detail, but it necessitates specimens thin enough for electron beam transmission. Presently, the prevailing method for achieving such thinning involves Focused Ion Beam (FIB) milling. FIB instruments utilizing Gallium ion sources are widely employed, offering exceptional resolution and precise milling. However, the meticulousness of sample preparation becomes time-consuming, creating a bottleneck that compromises overall lab throughput. Recently, there has been a surge of interest in adopting FIBs based on plasma ion sources to expedite cryo transmission electron microscopy (TEM) lamella preparation. Among various ion species, Xenon stands out as the most suitable choice due to its highly focused beam profile, which facilitates precise lamella shaping and accelerates the milling rate. Presented results show the practical advantages of optimized workflows like on-grid lamella, waffle, and lift-out techniques, enhancing the speed and throughput of cryo-TEM lamella preparation. This work will explore the TESCAN AMBER X cryo-plasma FIB-SEM as a robust and versatile workstation that serves as an indispensable tool for not only routine cryo-TEM sample preparation. This instrument also offers a remarkable range of additional capabilities, making it a comprehensive solution for various research needs.



POSTERS

P01 Dynamics of Antifungal Disulfide Proteins: Thermal and Chemical Unfolding

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Small, cysteine-rich antifungal proteins (50-60 aa) like PAF[1] secreted by fungi (*P. chrysogenum*) are efficient antimicrobials with potential anti-Candida and/or anti-corona virus activity. Their conserved β-barrel tertiary structures are supported by 3-4 disulfide bonds. However, enhanced dynamics may persist in loop and terminal regions as shown by NMR relaxation, ^{15}N-CEST and MD calculations. Previous studies are now extended by combined stress induced unfolding experiments. In addition to heat/cold unfolding[2], we present DMSO induced unfolding of PAF and its inactive mutant PAF^{D19S} as monitored by NMR and DSC microcalorimetry[3]. Moreover, cryogenic 50 v/v % DMSO/H_2O solvent mixture was tested at subzero temperatures (down to 260K) for PAF unfolding, that was followed by ^{15}N/^{1}H HSQC and TROSY. Artificial intelligence AF2 predictions[4] of the protein structures in H_2O buffer were accurate and reliable including disulfide patterns. Partially unfolded states of antifungal disulfide proteins might be biologically relevant for conformational selection, disulfide shuffling or thiol related transitions. Practical applications may have implications on the validation of MD simulations and protein concentration measurements.

[1.] Batta, G. et al. (2009) Functional aspects of the solution structure and dynamics of PAF - a highly-stable antifungal protein from Penicillium chrysogenum, FEBS Journal 276, 2875-2890. https://doi.org/10.1111/j.1742-4658.2009.07011.x
[2.] Fizil, Á. et al. (2015) "Invisible" Conformers of an Antifungal Disulfide Protein Revealed by Constrained Cold and Heat Unfolding, CEST-NMR Experiments, and Molecular Dynamics Calculations, Chemistry-A European Journal, 21, 13, 5136-5144 https://doi.org/10.1002/chem.201404879
[3.] Czajlik, A. et al. (2023) DMSO-Induced Unfolding of the Antifungal Disulfide Protein PAF and Its Inactive Variant: A Combined NMR and DSC Study, International Journal of Molecular Sciences, 24, 2, https://doi.org/10.3390/ijms24021208
[4.] Jumper, J. et al. (2021) Highly accurate protein structure prediction with AlphaFold, Nature 596, 7873, 583, https://doi.org/10.1038/s41586-021-03819-2

P02 How Euro-BioImaging can support your research with access to the best imaging tools

Johanna Bischof

Advanced imaging technologies are a central technology platform driving research in most disciplines of the life sciences and correlating multiple imaging approaches has become an increased need in many research approaches.
Euro-BioImaging's role in the imaging revolution is to offers all scientists open access to imaging instruments, expertise, training opportunities, and data management services beyond what is available at their home institutions or among their collaborators.
The technologies offered by Euro-BioImaging can be accessed at Euro-BioImaging Nodes, which comprise more than 190 internationally renowned imaging facilities distributed across Europe. They cover the whole spectrum of biological and biomedical imaging, with a significant number offering Electron Microscopy approaches, from Cryo-ET in a cellular context to volumeEM and correlative imaging. In addition to access to cutting-edge instruments, the Euro-BioImaging Nodes provide expertise, guidance and training on all aspects of the imaging experiment - from experimental design and sample preparation to image analysis services, including on external data. Euro-BioImaging has a strong commitment to Open Science, working with image data repositories such as the BioImage Archive and EMPIAR, as well as offering image analysis and support with data FAIRification.
All scientists, regardless of affiliation, area of expertise, or field of activity, can benefit from Euro-BioImaging's pan-European open access services and funding for user access is available.
For the technical experts at imaging core facilities, Euro-BioImaging provides a cross-European network and platform for exchange of experience, as well as new training opportunities.

P03 Get funded access to structural biology services through Instruct-ERIC and canSERV

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Instruct-ERIC is a distributed European infrastructure providing open access to high-end structural biology techniques to researchers from all countries to promote innovation in biomedical science in Europe. Instruct also offers funding support towards access costs for researchers from its 17 member countries and institutions (instruct-eric.org). Furthermore, Instruct has a strong commitment to offer a range of training opportunities for European scientists, enabling researchers to expand their expertise in structural biology and implement new techniques in their research.
In addition to being an active partner in iNEXT-Discovery in which many Instruct centres provide access to their services, Instruct is a partner in multiple Horizon Europe projects, such as canSERV (canserv.eu). The canSERV project aims to provide a comprehensive approach to combat cancer, with partners translating findings from basic research to clinical trials. Insight from structural studies conducted in the facilities in Instruct are indispensable to support such interdisciplinary research. In this contribution, I will introduce how researchers can become involved in this multidisciplinary research project and highlight insights derived in this project.

P04 Structure and mode of action studies of glycopeptide antibiotics: eremomycin and oritavancin as seen by NMR

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Antibiotic resistance emerges as a worldwide public health problem. Therefore, development of new antibiotics[1] is a must. Oritavancin is a chloro-eremomycin derivative in clinical use that has been approved by FDA against Gram-positive bacteria, including methicillin-resistant Staphylococcus aureus (MRSA). Structure and mode of action studies of glycopeptide antibiotics are often supported by ^{1}H and ^{13}C NMR investigations. Here we show that ^{15}N labelling of eremomycin opens a new avenue for NMR studies.
^{15}N relaxation (T_1, T_2, and ^{15}N-{^{1}H} NOE) provides more insight into the internal dynamics[2] of peptides. Global correlation times derived from these data prove that adding cell-wall analogue peptide N-Ac-D-Ala-D-Ala[3] (DADA) to eremomycin, induces tetramers and octamers from the dimers of eremomycin. Diffusion NMR method (DOSY) led to the same conclusion. Oritavancin forms strong asymmetric dimers in aqueous solution, however adding DADA ligand causes precipitation, perhaps due to higher oligomerisation.
Recently, it was shown that new glycopeptide variants may have antiviral effects (e.g. against flue, HIV, COVID), however understanding of the new antimicrobial impacts awaits for explanation. In our experiments structure-activity studies of eremomycin and other glycopeptides (e.g. fluoroalkylated ones) were introduced. The sensitivity of ^{19}F NMR spectroscopy is promising for in-vitro and possibly in-vivo detection of the oligomerisation states.
Investigation of hypothetical supramolecular organisation of oritavancin as induced by cell wall analogue peptides is in progress.

[1] S. Solov'eva, S. Printsevskaia, E. Olsuf'eva, G. Batta, és M. Preobrazhenskaya, „New Derivatives of Eremomycin Containing N-15 or F Atoms for NMR Study", Russian Journal of Bioorganic Chemistry, 34, o. 831–9, 0 2008, doi: 10.1134/S1068162008060162.
[2] N. Tjandra, A. Szabo, és A. Bax, „Protein Backbone Dynamics and 15N Chemical Shift Anisotropy from Quantitative Measurement of Relaxation Interference Effects", J. Am. Chem. Soc.,. 118, sz. 29, o. 6986–6991, jan. 1996, doi: 10.1021/ja960510m.
[3] L. Izsépi et al., „Bacterial Cell Wall Analogue Peptides Control the Oligomeric States and Activity of the Glycopeptide Antibiotic Eremomycin: Solution NMR and Antimicrobial Studies", Pharm. Basel Switz., 14, sz. 2, o. 83, jan. 2021, doi: 10.3390/ph14020083.

P05 PDBe & PDBe-KB: a toolbox of options to help today's structural biologist.

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There are >200,000 experimentally determined structures in the PDB archive, and in many instances, there is more than one structure for a protein or a part of a protein molecule. As part of the Protein Data Bank in Europe Knowledge Base (PDBe-KB, https://pdbe-kb.org) [1], we've developed an advanced view that aggregates all the structures and associated information for a given protein or protein component (index by UniProt IDs) in one place. These pages feature (amongst other things):

- Conformational analysis capturing minor differences in shape and/or large movements of protein domains found between the set of structures for one protein.[2]
- A "*3D view of superposed ligands*" with ligands from all PDB entries for one protein superposed on representative conformational states.[2]
- 3D-structure data is combined with additional analysis / information as part of a collaborative consortium with multiple research group partners.[1]
- In addition, under the PDBe-KB umbrella, we have developed other tools to support better findability:
- 3D-Beacons Network (https://3d-beacons.org) provides united access for experimentally determined and predicted protein structure models (along with their confidence metric). [3]
- Covalently Linked Components (CLCs) as an additional class of reference molecules that make it easier to identify covalently linked multi-component ligands across the whole PDB archive.[4]
- Unique, persistent identifiers for macromolecular assemblies in the PDB.[5]

References:
[1] Varadi, M. et al. 2022 "PDBe and PDBe-KB : Providing high-quality, up-to-date and integrated resources of macromolecular structures to support basic and applied research and education". Protein Sci. 31(10):e4439.
[2] Ellaway, J. I. J. et al. 2023 "Automated Pipeline for Comparing Protein Conformational States in the PDB to AlphaFold2 Predictions." BioRxiv 545008 [Preprint]. Available from: http://biorxiv.org/lookup/doi/10.1101/2023.07.13.545008
[3] Varadi, M. et al. 2022 "3D-Beacons: decreasing the gap between protein sequences and structures through a federated network of protein structure data resources." GigaScience 11: giac118.
[4] Kunnakkattu, I. R. et al. 2023 "PDBe CCDUtils: an RDKit-based toolkit for handling and analysing small molecules in the Protein Data Bank." J Cheminform. 15(1):117
[5] Appasamy, S. D. et al. 2023 "Annotating Macromolecular Complexes in the Protein Data Bank: Improving the FAIRness of Structure Data" Sci Data. 10(1):853

P06 Exploring extremophiles' cell envelope by cryo-electron microscopy: the case of Deinococcus radiodurans

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The cell envelope of the poly-extremophile bacterium *Deinococcus radiodurans* presents a crystalline organization resulting from the highly regular distribution of at least three main multi-subunit protein complexes. Two of these complexes, the S-layer Deinoxanthin-Binding Complex (SDBC) and the Type-II Secretion System (T2SS), have been investigated by cryo-EM single-particle analysis revealing their atomic structure. The SDBC, a multifunctional complex with UV/ROS-protection and gating properties, showed a trimeric pore region made by a massive β-barrel organization, a stalk region consisting of a trimeric coiled coil, and a trimeric collar region below the pores. The pore and stalk regions are functionally involved in UV-protections and gating mechanisms across the cell envelope, while the collar region is a trimeric Cu-only superoxide dismutase offering protection against reactive oxygen species. The other complex, the T2SS, showed uncovering unique features like an unconventional protein belt and a separate cap subunit associated with a GspD having a novel N-terminal region. This complex appears to contribute to the cell envelope integrity, nucleic acid, and nutrient trafficking. The repetition of such types of complexes may highlight their significance in several cellular functions and adaptation mechanisms to extreme conditions. These findings shed a light into the intricate organization and functional roles of protein complexes within bacterial cell envelopes, shaping our understanding of bacterial survival strategies and evolutionary adaptations.

Farci D, Milenkovic S, Iesu L, Tanas M, Ceccarelli M, Piano D. Structural characterization and functional insights into the type II secretion system of the poly-extremophile Deinococcus radiodurans. J Biol Chem. 2024 Feb;300(2):105537. doi: 10.1016/j.jbc.2023.105537.

Farci D, Graça AT, Iesu L, de Sanctis D, Piano D. The SDBC is active in quenching oxidative conditions and bridges the cell envelope layers in Deinococcus radiodurans. J Biol Chem. 2023 Jan;299(1):102784. doi: 10.1016/j.jbc.2022.102784.

Farci D, Haniewicz P, de Sanctis D, Iesu L, Kereïche S, Winterhalter M, Piano D. The cryo-EM structure of the S-layer deinoxanthin-binding complex of Deinococcus radiodurans informs properties of its environmental interactions. J Biol Chem. 2022 Jun;298(6):102031. doi: 10.1016/j.jbc.2022.102031.

Farci D, Haniewicz P, Piano D. The structured organization of Deinococcus radiodurans' cell envelope. Proc Natl Acad Sci U S A. 2022 Nov 8;119(45):e2209111119. doi: 10.1073/pnas.2209111119.

Farci D, Kereïche S, Pangeni S, Haniewicz P, Bodrenko IV, Ceccarelli M, Winterhalter M, Piano D. Structural analysis of the architecture and in situ localization of the main S-layer complex in Deinococcus radiodurans. Structure. 2021 Nov 4;29(11):1279-1285.e3. doi: 10.1016/j.str.2021.06.014.

P07 Interaction of Aralkyl-Thiodigalactosides as Potentional Inhibitors of Human-Galectin-3 Investigated by Saturation Transfer Difference NMR and Molecular Docking Simulation

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Human Galectin-3 (*h*Gal-3) is a specific protein that belongs to the family of galectins and binds selectively the molecules containing free β-galactosides elements. This interaction process is implicated in numerous physiological and pathological processes, such as fibrosis, heart disease and tumor progression.[1] The development of small inhibitors that selectively block the activity of galectins has a significant role in the treatment strategy of pathological processes and one of the important research areas of chemical biology. The interaction of thiodigalactoside (TDG) with *h*Gal-3 is well known. This compound and its derivatives have been successfully used in the clinical treatment of diseases associated with this protein. [2] In this research, as a step towards developing novel *h*Gal-3 inhibitors, we investigated three derivatives of (TDG) modified with different alkyl-substituents: (naphthalen-2-yl) methyl, (quinolin-2-yl)methyl and benzyl) using Saturation Transfer Difference (STD) NMR spectroscopy and molecular docking calculations.[3] By competitive STD method we were able to determine the resonance signal changes occurring in the STD spectra during the titration experiment, and thus the strength of the interactions (K_d). The binding modes of these derivatives were confirmed by molecular docking calculations. Based on these, the binding forces, such as the cation-π interactions between the arginine residues in the binding pocket of the protein and the aromatic groups of the ligands, were established as significant features. Our results offer a molecular-level understanding of the varying affinities observed among the synthesized thiodigalactoside derivatives, which can be a key aspect in the future development of more effective ligands of *h*Gal-3.

References:
[1] Hisrich B. V., Young R. B., Sansone A. M., Bowens Z., Green L. J., Lessey B. A., Blenda A. V. Role of Human Galectins in Inflammation and Cancers Associated with Endometriosis. Biomolecules. 2020;10:230. doi: 10.3390/biom10020230.
[2] Mariño K.V., Cagnoni A.J., Croci D.O., Rabinovich G.A. Targeting galectin-driven regulatory circuits in cancer and fibrosis. Nat. Rev. Drug Discov. 2023;22:295–316. doi: 10.1038/s41573-023-00636-2.
[3] Hőgye F., Farkas L. B., Balogh Á. K., Szilágyi L., Alnukari S., Bajza I., Borbás A., Fehér K., Illyés T. Z., Timári I. Saturation Transfer Difference NMR and Molecular Docking Interaction Study of Aralkyl-Thiodigalactosides as Potential Inhibitors of the Human-Galectin-3 Protein. Int J Mol Sci. 2024 Feb 1;25(3):1742. doi: 10.3390/ijms25031742.

P08 Investigation and Assessment of a Machine Learning and Molecular Dynamics Assisted anti-SARS-CoV2 Nanobody Design Workflow

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In silico assisted protein binding optimization has gained a lot of attention in recent years. With the emergence of high computation power hardware and advanced machine learning algorithms this strategy became more approachable and favored, as in silico pre-filtering of strong binders is fast and cheap compared to in vitro library screening methods. This advancement has already left its mark on the worldwide response against pandemic threats. In this study, we proposed and tested a workflow for the design of nanobodies targeting the SARS-CoV-2 Spike protein receptor binding domain (S-RBD) using machine learning techniques and dynamics simulations. We assessed the viability of this workflow using a test set of three different nanobodies and two different S-RBD variants, from the bacterial expression to the binding assays of the designed nanobody variants. We successfully developed two nanobodies that bind to both the wild type (Wuhan-type) and delta variant S-RBD stronger than the wild type nanobody. We use this case study to describe the strengths and weaknesses of in silico assisted nanobody design strategies.

P09 Cryogenic Electron Microscopy at IOCB Prague

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We are the newly established Cryogenic Electron Microscopy (cryo-EM) Core Facility at the Institute of Organic Chemistry and Biochemistry (IOCB) in Prague. Our dedicated cryo-EM team offers a comprehensive range of services, including sample preparation and characterization, data collection, and single particle analysis (SPA) on High-Performance Computing (HPC) systems. In close cooperation with other groups, we can provide atomic modelling and quantum mechanics/molecular mechanics (QM/MM) molecular dynamics (MD) simulations for the structures we analyze.
Cryo-EM allows visualization of bound small-molecule ligands in the macromolecules at high resolution. These new structures provide beneficial insights into the molecular mechanisms of complex biochemical processes and a profound impact on drug discovery, defining the binding modes and mechanisms of action.
Our proficiency extends beyond cryo-EM, encompassing techniques at ambient temperatures and 3D electron diffraction. The Cryo-EM group is equipped with an in-house JEOL JEM2100plus screening electron microscope and collaborates with CEITEC in Brno to access high-resolution instrumentation. Data analysis is performed in collaboration with a high-performance computing (HPC) facility. The cluster consists of ~250 CPU nodes and ~40 GPU nodes.
Our facility will be equipped with state-of-the-art instrumentation (300kV Krios G4 and 200kV Glacios) for single particle analysis and 3D electron diffraction. Our goal is to establish a cutting-edge cryo-EM infrastructure that will meet the needs of IOCB users and potential external collaborators.

P10 The Structure of Dynamic Intermediates of the Catalytic Cycle of the Oncogenic KRAS G12C Protein and it's Wildtype Investigated by an NMR-Chemical-Shift-Driven Protocol

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In this work, catalytically significant states of the wildtype and the oncogenic G12C variant of KRAS, those of Mg^{2+}-free and Mg^{2+}-bound GDP-loaded forms, have been determined using CS-Rosetta software and NMR-data-driven molecular dynamics simulations. There are several Mg^{2+}-bound G12C KRAS/GDP structures deposited in the Protein Data Bank (PDB), so this system was used as a reference, while the structure of the Mg^{2+}-free but GDP-bound state of the RAS cycle has not been determined previously. Due to the high flexibility of the Switch-I and Switch-II regions, which also happen to be the catalytically most significant segments, only chemical shift information could be collected for the most important regions of both systems. CS-Rosetta was used to derive an "NMR ensemble" based on the measured chemical shifts, which, however, did not contain the nonprotein components of the complex. We developed a torsional restraint set for backbone torsions based on the CS-Rosetta ensembles for MD simulations, overriding the force-field-based parametrization in the presence of the reinserted cofactors. This protocol (csdMD) resulted in complete models for both systems that also retained the structural features and heterogeneity defined by the measured chemical shifts. This methodology allowed a detailed comparison of the Mg^{2+}-bound and Mg^{2+}-free states of the wildtype and the G12C KRAS/GDP.

References:
Pálfy, G. et al. The Importance of Mg $^{2+}$ -Free State in Nucleotide Exchange of Oncogenic K-Ras Mutants. Chemistry – A European Journal 28, (2022).

Gadanecz, M. et al. NMR-Chemical-Shift-Driven Protocol Reveals the Cofactor-Bound, Complete Structure of Dynamic Intermediates of the Catalytic Cycle of Oncogenic KRAS G12C Protein and the Significance of the Mg^{2+} Ion. Int J Mol Sci 24, 12101 (2023).

P11 *In vitro* and In-cell recognition of Quadruplex-duplex hybrids (QDH): conformation, folding, and recognition by drug-like ligand molecule

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G-quadruplexes (G4) represent one of the crucial non-canonical structures in the genome's non-coding part, such as promoters of (onco-)genes and telomeres having significant biological functions. A quadruplex-duplex hybrid (QDH hereafter) is designed upon the juxtaposition of a duplex stem-loop onto a G4 across the multiple edges of a quadruplex core. QDH shows diverse functionalities ranging from (patho)biological processes to nanotechnology. Under *in vitro* conditions, the individual conformations can be separated and characterized at the atomic level. However, the identity of the conformation, which is responsible for biological function *in vivo*, remains obscured due to the lack of suitable technology allowing high-resolution structural studies under physiological conditions *in vivo*. The biggest challenge is to develop a ligand specific to a bioactive genomic QDH sequence with minimal off-target binding.
This work showed the selective recognition and structural elucidation of distinct QDH conformation from the PIM1 gene by two Bis-quinolinium ligands with state-of-the-art solution NMR in conjugation with low-resolution spectroscopic techniques. The structural insights will help to design sequence and scaffold-specific ligands.
On the contrary, we customized "in-cell" NMR strategies to monitor *de-novo* QDH DNA folding and ligand binding in *Xenopus laevis* Oocytes (eukaryotic cell model) by using selectively labeled ^{19}F modified QDH sequences. The complexes retain their native folding inside the cellular environment with reduced off-target binding and refolding.
Our *in vitro* observation, coupled with in-cell NMR data, is well-suited for assessing the binding epitome and capability of promising drug-like candidates identified through *in vitro* screening assays, thereby modulating their further development.

P12 Structural investigation of *in vitro* recombinant tau protein filaments

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Tau protein, predominantly found in the central nervous system, plays diverse roles in neurons, including microtubule regulation, signal transduction, and fast axonal transport. However, misregulated tau forms pathological filaments, which are a hallmark of neurodegenerative diseases like Alzheimer's disease. Aggregation and accumulation of misregulated tau is toxic to the neurons, and the underlying mechanisms of tau pathology are not understood well [1].
Advances in amyloid cryo-EM helical reconstruction have revealed distinct structural differences in tau filaments among various diseases [2]. However, the mechanism of tau aggregation remains unknown, and the reason behind conformational diversity is not yet clear.
Currently, the only pathological-like tau filaments can be prepared *in vitro* using dGAE (297-391) tau fragment. It has a high aggregation propensity and readily forms filaments without aggregation inducers. Interestingly, their conformation highly depends on buffer conditions [3].
Our goal is to understand the factors influencing and driving tau aggregation and to prepare *in vitro* recombinant tau filaments. We are preparing tau filaments using a physiological tau isoform (2N4R) in different buffer conditions (salt content, pH). Filaments are analysed using AFM, negative stain EM, and cryo-EM. Here we present our preliminary results of the ongoing structural analysis of the filaments and discuss the morphological differences of the prepared filaments.

[1] Alonso, A. C., El Idrissi, A., Candia, R., Morozova, V., & Kleiman, F. E. Cytoskeleton, 81(1), 71–77, (2024).
[2] Goedert, M., Crowther, R. A., Scheres, S. H. W., & Spillantini, M. G. Cytoskeleton, 81(1), 95–102, (2024).
[3] Lövestam S., Koh F. A., van Knippenberg B., Kotecha A., Murzin A. G., Goedert M., Scheres S. H. W. eLife 11, (2022).

P13 Fine-tuning Biological Activity, Aggregation Propensity, and Thermo-stability of Tc5b Chimera Exenatide Derivatives through Trp-cage Compactness

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Exenatide/Exendin-4 (Ex4), a polypeptide derived from the saliva of Heloderma Suspectum, mimics the incretin effect of human GLP-1, making it an effective therapeutic agent for T2DM and obesity. Despite sequential differences, these peptides share a common G protein-coupled receptor binding scheme, but additionally, Ex4 has a unique C-terminal elongation, a Trp-cage motif, that transiently shields the receptor-binding surface. Here we explore salt-bridge and disulfide bond-reinforced derivatives, investigating how Trp-cage compactness influences thermal stability, aggregation, and insulin secretion enhancement. Based on NMR data, we describe the atomic-level sidechain interactions that are involved in the optimization of the Trp-cage. Fine-Tuned Trp-cage motifs display increased resilience against unfolding and aggregation but exhibit an inverse relationship between bioactivity and stability. Molecular dynamics simulations of the receptor coupled forms revealed that the fortified cage motifs remain intact within the complexes but interfere with one of the main stabilizing contacts and recognition loci of the extracellular side (W214 of extracellular loop 1) and dislodge the N-terminal activating region of the hormone mimetics, leading to the loss of the free movement of transmembrane helix 6 – the primary signal transduction pathway of GLP-1R.

P14 Structural studies of a novel, ultra-stable Carbonic Anhydrase - a promising biocatalyst for industrial CO2 capture

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In response to the Global Warming crisis caused by increased CO2 levels in the atmosphere, there is a growing scientific interest in eco-friendly CO2 Capture Technologies. Prompt adoption of sustainable technologies is crucial for industrial decarbonization, aligning with the European Commission's aim for net-zero CO2 emissions by 2025. Robust carbonic anhydrases (CAs), enzymes that accelerate CO2 hydration and withstand harsh industrial conditions, are a key focus for biotechnological advancement in CO2 capture. Here we present structural studies of CA-KR1, a novel ultra-stable CA identified through high-throughput metagenomics screening. CA-KR1 is one of the most robust CAs reported to date, exhibiting remarkable stability under extreme temperatures and alkalinity. Understanding CA-KR1's 3D structure enhances our grasp of this highly promising biocatalyst, while expanding our knowledge on industrial-relevant CAs. Crystallisation trials were performed using the sitting drop vapour diffusion method screening a large number of conditions with the aid of an OryxNano crystallization robot (Douglas Instruments Ltd, Hungerford Berkshire, UK) installed at INSTRUCT-EL hub/NHRF, using commercially available crystallization screens. The plates were incubated at 19 °C and crystal growth was monitored via an automated imaging system for protein crystallization Rock Imager (Formulatrix, USA) also installed at INSTRUCT-EL hub/NHRF that captures high resolution images at selected time intervals. Single crystals of CA-KR1 were exposed to X-rays from synchrotron radiation at PETRA III, EMBL-Hamburg P13 at 100 K. Diffraction data were collected at 2.05 Å resolution and the CA-KR1 crystal structure was determined. The results obtained from the structure determination and preliminary analysis will be presented.

Acknowledgements
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P15 Structural heterogenicity of AAP investigated by cryo-EM

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Cryo-EM has revealed the structure of the tetrameric mammalian acylaminoacyl peptidase (AAP) and was further elucidated by MD simulations.[1] AAP acts as an upstream regulator of the proteasome[2] and removes terminal N-acetylated residues from its protein substrates.[3] It modulates the ubiquitin/proteasome degradation system and is involved in a drug-drug interaction between the antipsychotic valproate (specifically its glucuronated metabolite, VPA-G) and carbapenem antibiotics.[4–8] We present the first structure of AAP forming a covalent complex with Meropenem, a β-lactam antibiotic.[9] This underscores AAP's potential as a target for cancer therapy, given its impact on the proteasome system, localization, and function of oncogenic K-Ras variants, alongside its esterase activity.[10]

Tetramerization, facilitated by interaction-prone beta-edges and unique inserts, drives self-compartmentalization, endowing AAP with a unique substrate-selection system. The active site, featuring a Pro insertion within the central beta-sheet of the hydrolase domain, grants conformational flexibility to the catalytic Ser587, leading to alternating active and inactive conformations of the classical serine protease catalytic triad. This flexibility underscores a novel mechanism whereby substrate entrance is governed by flexible loops of a double-gated channel system, with substrate binding stabilizing the catalytic apparatus as a secondary filter before hydrolysis. Meropenem, by specifically inhibiting AAP's serine-protease function through binding to the active site, takes advantage of AAP's susceptibility to association due to sheltered active pockets and flexible catalytic triads.

These structural findings provide insight into AAP's function and possible new roles in therapy, as well as the mechanisms by which antibiotics may produce side effects in human physiology.

References:
[1] Kiss-Szemán A.J. et al. Chem. Sci. 2022, 13, 7132-7142.
[2] Palmieri G. et al. PLoS ONE. 2011, 6(10), 25888.
[3] Hwang C.S. et al. Science. 2010, 327, 973–977.
[4] Mori H. et al. Drug. Metab. Rev. 2007, 39(4), 647-57.
[5] Deshayes S. et al. Drug Saf. 2017, 40(12), 1171-1198.
[6] Cunningham D. et al. Am. J. Emerg. Med. 2022, 53, 284.e1-284.e3.
[7] Dreucean D. et al. Am. J. Emerg. Med. 2019, 37(11), 2120.e5-2120.e7.
[8] Thomas C. et al. Am. J. Emerg. Med. 2020, 38(3), 690.e1-690.e2.
[9] Kiss-Szemán A.J. et al. Chem. Sci. 2022, 13, 14264-14276.
[10] Tan L. et al. J. Cell. Sci. 2019, 132(15), 232132.

P16 Structural and functional studies of a eukaryotic phosphate transporter

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Inorganic phosphate (Pi) is one of the most important macronutrients especially in actively growing and dividing cells. Inappropriate cellular Pi concentration is associated with a range of deleterious effects in both unicellular and multicellular organisms. Appropriate Pi concentration across plasma membrane is maintained through phosphate homeostasis by the action of transmembrane Pi transporters and signaling proteins.
X-ray structure of isolated amino-terminal domain (NTD) of different eukaryotic Pi transporters including fungi, plant as well as human has been determined. So far, however, no information on structural studies of full-length Pi transporter is available.
My project is aimed at solving the structure of full-length transmembrane eukaryotic Pi transporter using high-resolution single particle cryogenic electron microscopy (cryo-EM). At the current stage of the project, I am actively screening different sample and grid preparation conditions; while at the same time processing collected cryo-EM data. I intend to present a poster showing our current effort towards determining a high-resolution cryo-EM structure of a eukaryotic Pi transporter; including major challenges recently faced. I hope to get a good contribution from the protein cryo-EM community that will help to progress in the project.

P17 Investigation of the kinetic properties of the -AsnGly- isomerization in peptides as function of the molecular environment by NMR spectroscopy and ab initio calculations

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Our scientific goal is better understanding of the isomerization of -AsnGly- (-NG-) containing polypeptides and protein's subunits, and to investigate the kinetic properties of this reaction as function of the molecular environment. To follow the isomerization reaction, time-dependent (t = 5, 10, 30 min etc.) ^{1}H-NMR signals of Ac-NGXA-NH_2 (X=$E^{(-)}$: glutamate - sidechain negatively charged, A: alanine) were recorded at 37 and 55 °C, at pH 7.4 in protonated and at pH 7.0 in deuterated buffers. As NG deamidation - which is the reaction rate determining step - is a pseudo-first-order reaction, the reaction rate coefficients (k_1) and half-lifes ($t_{1/2}$) of the isomerization can be calculated from integral values (proportional to the concentration) – which is decreasing over time (Asn)[1]. An H/D exchange allowed the determination of the kinetic isotope effect for the succinimide ring closure in order to distinguish whether reaction goes either by classical and/or quantum tunneling mechanism. From the examination of deuterated peptides, it can be concluded that the reaction takes place with a mixed mechanism at this temperature range, but in the case of AcNGEANH_2, it goes mainly via quantum tunneling.
The deamidation process was theoretically studied through IRC path and NBO calculations. These were carried out at B3LYP/6-31G(d) level of theory in vacuum. Frequency calculation was completed and ΔG values were determined at each point of the IRC paths. Endpoints of the IRC paths and transition states were further optimized at DFT B3LYP/6-31++G(d,p) level of theory in vacuum and using IEFPCM water model.

[1] Láng, A., Jákli, I., Enyedi, K. N., Mező, G., Menyhárd, D. K., & Perczel, A. (2020). Off-pathway 3D-structure provides protection against spontaneous Asn/Asp isomerization: shielding proteins Achilles heel. Quarterly Reviews of Biophysics, 53, e2.

P18 Peptidomimetics with full antagonistic effect stabilize insulin receptor in novel conformation

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The human insulin receptor (IR) is one of the most studied receptor tyrosine kinases (RTKs). Binding of the signal molecule to the extracellular part of RTKs initiates receptor dimerization which triggers autophosphorylation on the intracellular part of the receptor. Unlike most RTKs, IR forms a disulfide bond stabilized dimer already in the apo-form. Therefore, an autoinhibitory mechanism must exist to prevent non-receptor activation of the signaling pathway.
We have studied the structural changes of the insulin receptor ectodomain *in vitro* caused by 3 peptidomimetics: adamantane-derived mimetic (Ada), trimesic acid-derived mimetic (Trim) and S661, which were shown to be full insulin antagonists. We have used single particle electron cryo-microcopy (cryo-EM) to describe the mechanism of insulin receptor inhibition by these compounds. Our cryo-EM structures reveal that the studied antagonists cross-link L1 and FnIII-1' domains of the insulin receptor, therefore stabilizing the Π shape structure in which membrane proximal regions of FnIII-3 and FnIII-3' are separated by 115 Å, thus preventing autophosphorylation in the intracellular domains of the receptor. The head of the dimer (L1 to FnIII-1' and L1' to FnIII-1) is wider than in the case of insulin-IR and forms a shape resembling goggles. Remarkably, we do not observe α-CT helix densities in our cryo-EM maps. In addition, multiple interaction interfaces are observed between L1 and FnIII-2 domains which provide indication for elucidation of receptor activation mechanism.

P19 PhiKZ baseplate structure

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PhiKZ is a bacteriophage that infects *Pseudomonas aeruginosa*, an opportunistic human pathogen. The phage phiKZ is known for its large genome and complex structure, making it a notable subject for structural biology studies. Among all its parts, the baseplate is the most complex. It adheres to the phage prey and triggers the genome ejection. The signal that triggers the ejection travels from the tail fibres to the tail through the baseplate. Here, we used cryo-Electron Microscopy to visualise the structure of the phiKZ tail and the baseplate at high resolution, the first one of a jumbo bacteriophage. Our results reveal an intricate network of proteins organised in six-fold symmetry. Structural comparisons with related systems highlight the universal conservation observed in contractile injection systems. This sheds light on phiKZ's baseplate specificities and suggests a potential mode of action. Its structural analysis enhances our understanding of phiKZ and contributes with valuable knowledge to the broader field of myovirus biology.

P20 Spontaneous isomerization of -AsnGly- amino acid pairs in dependence of sequence and structure

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Peptides and protein sequences with -AsnGly- (-NG-) amino acid pairs show spontaneous isomerization under physiological conditions. The backbone atoms of the -NG- dipeptide will rearrange themselves first through succinimide ring closure, leading then to both β-Asp and α-Asp formation with hydrolysis. However, the kinetics of this process shows striking variability, as it may take place within just a few hours, or can last up to several days or even weeks. Previously, we demonstrated on a row of short model peptides the pH, charge and temperature dependence of this process [1]. However, these data still do not fully explain the wide range of conversion rates known so far. In our present work, we aim to shed light on the influencing effect of neighboring amino acids, flanking sequences, as well as their inherent structural rigidity and/or flexibility with NMR spectroscopy. Therefore, we performed the kinetic analysis of naturally occurring proteins with -NG- dipeptides, such as protein PEP-19, compared to the analysis of a row of peptides of the same sequence, but different lengths.

[1] Láng, A., Jákli, I., Enyedi, K. N., Mező, G., Menyhárd, D. K., & Perczel, A. (2020). Off-pathway 3D-structure provides protection against spontaneous Asn/Asp isomerization: shielding proteins Achilles heel. Quarterly Reviews of Biophysics, 53, e2.

P21 Developing a Fragment Screening pilot repository

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There is limited accessibility of comprehensive datasets for fragment-based screening (FBS) information in the public domain to the scientific community despite its potential value. While some data are deposited in public databases like the Protein Data Bank (PDB), most remain inaccessible or stored in non-standardized formats at individual facilities. These datasets are crucial for developing experimental methods and training artificial intelligence (AI) models. Additionally, FBS projects can reveal insights into the druggability of biological targets, even challenging ones, but if the data are not published, these insights remain hidden from the broader scientific community.

Fundamental to standardising structural biology data in the wwPDB core archives is the data dictionary containing a complete set of semantic definitions and descriptions[1]. A data dictionary facilitates better data quality and integrity by validating semantic relationships between data items.

In consultation with the large-scale fragment screening facilities (e.g. FragMAX, EMBL Grenoble, DIAMOND, etc.), we have been exploring how to support the capture and archiving of the rich datasets generated at the facilities leveraging the PDBx/mmCIF data framework for dissemination. The new data standards will underpin a fragment-screening pilot repository as an extension to the core PDBx/mmCIF dictionary.

Several fragment screening datasets are represented using the newly developed data categories as part of the fragment screening pilot repository and are made available at: https://ftp.ebi.ac.uk/pub/databases/msd/fragment_screening/

References:

[1] Westbrook, J.D. et al. (2022) PDBx/mmCIF Ecosystem: Foundational Semantic Tools for Structural Biology.
JMB 434(11). 10.1016/j.jmb.2022.167599

P22 Functional flexibility versus stability in human ileal bile acid-binding protein

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Human ileal bile acid-binding protein (hI-BABP), a 14.2-kDa member of the family of intracellular lipid-binding proteins (iLBP) is expressed in the absorptive enterocytes of the distal small intestine and has a key role in the transcellular trafficking of bile salts. Flexibility and function are intimaly related in hI-BABP and other members of the iLBP family. As we have shown previously for hI-BABP, a ms-timescale conformational fluctuation involving an opening/closing motion of the ligand portal region mediates ligand entry via a conformational selection mechanism, in which the protonation state of histidines has a key role. A joint analysis of NMR thermal melts and relaxation dispersion measurements suggests a connection between the main unfolding transition of the protein (60 °C) and the functionally related global conformational motion. Further, MD simulations indicate that non-native conformation of specific beta-strands, overlapping with the portal region, could be responsible for an onset of self-association, suggesting a delicate balance between flexibility required for ligand binding and aggregation risk in the protein. In the current study, the determinants of stability in hI-BABP are further explored by investigating the effect of bound ligand. In addition to thermal unfolding, high-pressure NMR measurements are used to obtain insight into the relationship between internal dynamics and stability in hI-BABP at the amino acid level.

[1] Toke O. Structural and Dynamic Determinants of Molecular Recognition in Bile Acid-Binding Proteins. Int J Mol Sci. 2022, 23:505.
[2] Horváth G, Egyed O, Tang C, Kovács M, Micsonai A, Kardos J, Toke O. Ligand entry in human ileal bile acid-binding protein is mediated by histidine protonation. Sci Rep. 2019, 9:4825.
[3] Horváth G, Bencsura Á, Simon Á, Tochtrop GP, DeKoster GT, Covey DF, Cistola DP, Toke O. Structural determinants of ligand binding in the ternary complex of human ileal bile acid binding protein with glycocholate and glycochenodeoxycholate obtained from solution NMR. FEBS J. 2016, 283:541-55.

P23 Structural and dynamic determinants of site-selectivity in human ileal bile acid-binding protein

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Disorders in bile acid transport and metabolism is related to a number of disease states. Human ileal bile acid-binding protein (hI-BABP) has a key role in the cellular trafficking and metabolic targeting of bile salts. Its two internal binding sites exhibit positive cooperativity accompanied by a site-selectivity of glycocholate (GCA) and glycochenodeoxycholate (GCDA), the two most abundant bile salts in humans. To improve our understanding of the structural and dynamic determinants of ligand binding, NMR chemical shift and spin relaxation analysis was carried out on wild-type and functionally impaired mutant hI-BABP-bile salt complexes. According to our analysis, while the homotypic GCDA (3α, 7α)-complex of wild-type hI-BABP shows a high degree of similarity to the thermodynamically most stable heterotypic hI-BABP:GCA:GCDA complex, GCA (3α, 7α, 12α) instead of GCDA at site 1 increases the flexibility of the protein backbone in both the EFGH portal region and the helical cap. Mutation Q51A, resulting in the loss of site preference of di- and trihydroxy bile salts, affects the flexibility of the complexed forms on both the ps-ns and the µs-ms timescales. In particular, the increased local flexibility of key amino acids in GCDA-bound Q51A hI-BABP appears to interfere with the formation of stabilizing interactions in the binding pocket. Taken together, site-selectivity in hI-BABP is governed by a fine balance of enthalpic and entropic contributions, where the affinity of bile salts is affected by long-range dynamic effects.

[1] Toke O. Structural and Dynamic Determinants of Molecular Recognition in Bile Acid-Binding Proteins. Int J Mol Sci. 2022, 23:505.
[2] Horváth G, Egyed O, Tang C, Kovács M, Micsonai A, Kardos J, Toke O. Ligand entry in human ileal bile acid-binding protein is mediated by histidine protonation. Sci Rep. 2019, 9:4825.
[3] Horváth G, Bencsura Á, Simon Á, Tochtrop GP, DeKoster GT, Covey DF, Cistola DP, Toke O. Structural determinants of ligand binding in the ternary complex of human ileal bile acid binding protein with glycocholate and glycochenodeoxycholate obtained from solution NMR. FEBS J. 2016, 283:541-55.

P24 Toward in-cell NMR studies in defined physiological states and 3D tissue cultures

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Atomically resolved studies of proteins within the intracellular milieu of living cells, achieved exclusively through in-cell NMR spectroscopy, provide unique insights into protein folding, maturation, and dynamics in their native context. More recently, these studies have emerged as a valuable source of quantitative data pertaining to drug-protein interactions, offering significant applications in drug screening and development [1].
Since its adaptation to human cells in 2009, in-cell NMR spectroscopy applications have been confined to asynchronous cells in 2D cell suspension. As a result, the in-cell NMR data has provided ensemble-averaged information regarding protein structure and interactions across populations of cells in distinct physiological states. Restricting its scope to 2D cell cultures, it has also been impossible to confront in-cell NMR structural information with functional biological data obtained from the widely utilized 3D cell culture models of tissues. Here, we report on the development of methodology allowing for the first time acquisition of atomically-resolved in-cell NMR spectral information on protein structure and interactions in living human cells synchronized in the defined physiological states and basic human tissue models [2].

[1] Theillet, F.-X. & Luchinat, E. In-cell NMR: Why and how? Prog. Nucl. Magn. Reson. Spectrosc. 132–133, 1–112 (2022).
[2] Rynes et al. Inducible protein expression in stably transfected cells paves the way toward in-cell NMR studies in defined physiological states and 3D tissue cultures. ChemRxiv. doi:10.26434/chemrxiv-2024-jqd66-v2 (2024).

P25 Diving deep into the regulation of inflammatory and growth factor signalling: Structural studies of intramembrane pseudoprotease iRhom and metalloprotease ADAM17

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The pseudoprotease Inactive rhomboid protein 2 (iRhom2), a member of the rhomboid superfamily, regulates inflammatory and growth factor signalling in mice and humans. The iRhom acts as a trafficking and activating cofactor of the metalloprotease A disintegrin and metalloprotease 17 (ADAM17) that regulates the inflammatory response by secreting Tumour necrosis factor α (TNFα), a potent pro-inflammatory cytokine. Previous studies have identified the domains and moieties of iRhom2 essential for different stages of its interaction with ADAM17, and structure predictions in AlphaFold2 suggested the likely overall conformation of the ADAM17-iRhom2 complex. However, direct structural and biophysical evidence underlying the regulation of ADAM17 proteolytic activity and substrate selectivity by iRhom2 has been missing. Here we use cryogenic electron microscopy to investigate the structure of the ADAM17-iRhom2 complex in various states including the interaction with ADAM17 substrates to provide a mechanistic explanation for the function of the complex. Such insight provides a structural basis for the design of new anti-inflammatory drugs with low off-target effects.

P26 Enhancing small molecule drug discovery through a robust

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Chemical compounds exerting specific biological effects on cellular targets serve as versatile tools in fundamental research. They facilitate the exploration of diseases at the molecular and cellular levels and aid in the validation of novel drug targets. Concurrently, bioactive compounds represent starting points for the development of new effective therapeutics, with the majority of marketed drugs today being small molecules. However, despite their advantages, discovering these compounds demands significant resources, including advanced facilities, specialized expertise (such as in assay development or medicinal chemistry), and access to compound collections, which are often inaccessible to many academic researchers.
The European Research Infrastructure for Chemical Biology, EU-OPENSCREEN ERIC aids researchers in expediting drug discovery endeavors within an open-access framework. This is achieved through collaborative initiatives involving academic and industrial partners. The consortium comprises over 30 partner sites across 10 European nations.
EU-OPENSCREEN uses various types of compound collections: a collection of ca. 2,500 known bioactives and a 2,500-compound diversity collection, both for assay validation and pilot screening experiments; a diversity collection with ca. 100,000 commercial compounds; a growing collection of compounds which have been submitted by academic chemists; and a fragment-library with 968 fragments and 88 so-called “minifrags”.
The fragment library is utilized in joint fragment-based screening campaigns by EU-OPENSCREEN partners and structural biology groups to enable researchers to conduct structural screens and leverage medicinal chemistry expertise to advance fragment hits.
Here, we present
EU-OPENSCREEN's screening and medicinal chemistry services, its compound collections, and the collaborative, multidisciplinary approach to discovering novel therapeutics.

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