



The European Research Infrastructure
for Integrative Structural Biology

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Instruct Biennial Structural Biology Conference
Brussels, Belgium 27 – 29th May 2026

Abstract Booklet

Instruct Biennial Structural Biology Conference

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27 – 29 May 2026



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Speakers

Integrated Structural Biology in Instruct

Harald Schwalbe

Goethe-University Frankfurt, Instruct-ERIC, Oxford.

Instruct-ERIC is a leading infrastructure offering service to European researchers. In the contribution, the position of Instruct within integrated structural biology will be discussed and challenges for the future will be highlighted.

In addition, unique NMR contributions in addressing RNA structural biology and 4D structural biology will be highlighted.

Structural and functional studies of m3C tRNA methyltransferases

Iven Hollmer, Philipp Throll, Laura Tengo, **Eva Kowalinski**

Transfer RNA (tRNA) molecules play a pivotal role in translation, ensuring accurate protein synthesis by decoding the genetic information encoded in messenger RNA (mRNA). To attain their functional maturity, tRNAs undergo post-transcriptional modifications that fine-tune their functionality. The dysregulation of tRNA modification enzymes has been implicated in numerous diseases, such as cancers, metabolic disorders, and neurodevelopmental defects. However, tRNA modification enzymes face the challenging task of distinguishing subtle differences among structurally similar substrates. Therefore, a comprehensive understanding of the molecular mechanisms underlying their function necessitates resolving substrate-bound molecular structures.

We will present our proceedings toward understanding the molecular mechanism of methyltransferases that mediate the 3-methyl-cytosine (m3C) modification of position 32 in the anticodon loop of specific sets of tRNAs. Specifically, we have revealed the cryo-EM structure of the m3C tRNA methyltransferase METTL6 in complex with seryl tRNA synthetase (SerRS) complex and serine tRNA. Our data show that the association of the two enzymes with their common substrate significantly boosts METTL6 methylation activity.

With METTL6 as an identified oncogene in liver and breast cancer, our study holds promise in enabling targeted therapeutic interventions in the future.

De novo design of β -barrels nanopores with defined size and function

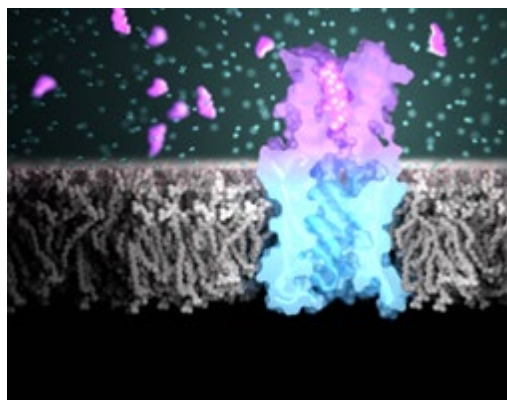
Anastassia A. Vorobieva^{1,2}

¹ Structural Biology Brussel, Vrije Universiteit Brussels, Brussels, Belgium.

² VUB-VIB Center for Structural Biology, Brussels. Belgium.

Anastassia.andreevna.vorobieva@vub.be

The field of de novo protein design has undergone major transformations in recent years, largely driven by advances in artificial intelligence. However, most progress has focused on proteins that fold in aqueous environments, while de novo design of membrane proteins (MPs) is only beginning to emerge. In this talk, I will discuss how both classical and modern design approaches can be applied to the design of transmembrane β -barrels (TMBs)—a class of multi-pass β -sheet MPs that form water-accessible pores across lipid membranes. Using de novo design, we generated TMBs with varying sizes, strand numbers, and lumen shapes [1,2]. Structural characterization by X-ray crystallography and NMR confirmed that these designs folded into the intended architectures. The most stable designs were shown to integrate into planar lipid bilayers and form stable nanopores. By fusing a de novo-designed binder to the cholic acid small molecule, we demonstrated the first proof-of-principle for the fully de novo design of nanopore sensors [3]. Finally, I will discuss current limitations in de novo design methods—particularly their difficulty in modeling unfolded states (which are critical for TMB design [4]) and non-globular architectures—and present our ongoing efforts to address these challenges through the development of new design models.



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Deciphering the Tau/14-3-3 Interaction via Artificial Intelligence and Chemical Cross-Linking

Lucia Il'kovičová^{1,2}, Vendula Milotová², Jozef Hritz^{1,2}

1 Central European Institute of Technology, Masaryk University, Kamenice 5, Brno, 625 00, Czechia

2 National Centre for Biomolecular Research, Masaryk University, Kamenice 5, Brno, 625 00, Czechia

Lucia.Ilkovicova@ceitec.muni.cz, jozef.hritz@ceitec.muni.cz

The aggregation of tau protein plays a crucial role in several neurodegenerative diseases, including Alzheimer's disease, Chronic traumatic encephalopathy and others. The diseases are characterized by the presence of tau fibrils with specific morphologies. Different phosphorylation patterns have been observed in diseases, indicating that morphology is dependent on the posttranslational modifications. With the release of AlphaFold3 allowing the structure prediction of posttranslationally modified proteins, we examined the differences in the predicted structures of several tau constructs with different phosphorylations.

Still, the exact cause and mechanism of tau aggregation remain unknown. However, the interaction with 14-3-3 proteins modulates the aggregation properties of tau protein. On this poster, we also evaluate models of tau/14-3-3 interactions validated by chemical cross-linking.

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The discovery of novel class of disaccharide-based ligands of Galectin-3

Anastasia S. Tsagkarakoua, Jakub Zýkab, Antonio Molinaroa, Kamil Parkanb, Alba Silipoa

Department of Chemical Sciences, University of Naples Federico II, Via Cintia 4, 80126, Naples, Italy,
anastasia.tsagkarakou@unina.it

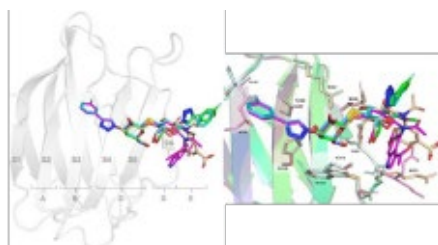
Department of Chemistry of Natural Compounds, University of Chemistry and Technology Prague,

Technická 5, 166 28 Prague, Czech Republic

Galectins are a family of proteins that bind β -galactosides [1,2]. The family of galectins is divided into three subclasses according to their carbohydrate recognition domain (CRD) distribution. The first subclass contains the monomeric galectins, the second class consists of only the chimera-type galectin, and the third subclass comprises the tandem repeat galectins with two different CRDs [1]. Despite their high homology, each galectin shows a different affinity for saccharides.

Galectin-3 (Gal3) is the sole member of chimera-type subclass. It is unique in having an extra-long and flexible N-terminal domain consisting of 100-150 residues, rich in Pro, Gly, Tyr, and Gln, which contains sites for phosphorylation [3,4]. The C-terminus is composed of about 135 residues which form a globular structure and accommodates the carbohydrate-binding site. CRD is composed by 5-stranded (F1-F5) and six stranded (S1-S6a/6b) β -sheets which associate in a β -sandwich arrangement. In all galectins the carbohydrate-binding site (CBS) is in a groove of the S-sheet side of the sandwich and the β -galactoside recognition core motif is mediated by sheets S4, S5 and S6 [5,6]. Gal3 is frequently overexpressed and contributes to multiple hallmarks of malignancies. It fosters immune evasion by inhibiting T cell activation and promoting regulatory T cell function, while also enhancing metastasis through tumor-endothelium adhesion and extracellular matrix interactions. Gal-3 overexpression has been associated with poor prognosis in several cancers, including nonsmall-cell lung cancer, melanoma, and colorectal carcinoma. Most of the inhibitor development studies involve mono and disaccharides with β -D-galactopyranoside being the basic scaffold [7]. The most potent ones reported thus far, are thiodigalactoside derivatives carrying aryl-triazolyl moieties attached to C-3 of both galactose units with affinities in the low nanomolar range. One compound of this class, GB0139, is in an unsuccessful phase 2b clinical trials (NCT03832946) against idiopathic pulmonary fibrosis, however it recently showed promise in a phase 1b/2a clinical trial for COVID-19 pneumonitis while another one, GB1211 is currently in phase 2a clinical trials (NCT04473053) as a potential therapy for liver cirrhosis and cancer [8].

To investigate the bioactivity of disaccharide-based inhibitors against Gal3, we employed a multidisciplinary approach utilizing X-ray crystallography, ligand-based NMR studies, and ITC to characterize the binding interactions and the thermodynamic parameters. Comparative structural



analysis across the galectin family provided key insights into ligands specificities. These findings provide a structural framework for the future design of selective, high-affinity galectin inhibitors.

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Modular protein design for novel folds and programmable cell regulation**Roman Jerala**

Department of Synthetic Biology and Immunology, National Institute of Chemistry

Modular protein design provides a powerful strategy for constructing protein architectures beyond those found in nature. We have developed a design framework based on orthogonal coiled-coil (CC) modules that enables the rational assembly of new protein folds with defined topology and geometry. By concatenating CC modules and encoding the folding pathway into the sequence, complex polyhedral assemblies can be generated using a reduced module set. Chemical and proteolytic control elements allow regulation of assembly and disassembly. Extension from dimeric CCs to higher-order helical bundles further expands the accessible topological space.

Crystal and cryo-EM structures of designed assemblies, including cages and knotted architectures, experimentally validate the underlying design principles. The same CC modules can be repurposed as regulatory elements in mammalian cells by inserting them into target proteins, enabling allosteric control through programmable heterodimerization. This modular strategy links protein structure, folding, and regulation within a unified design paradigm, providing versatile building blocks for synthetic biology and biomedical applications.

**NMR reveals dynamics and unfolded conformations in the
regulation of protein-RNA interactions**

Philipp Mayer^{1,2}, Christoph Mueller-Hermes^{1,2}, Valerio Piomponi³, Stefan Hilber⁴, Sam Asami¹, Xavier Blanchet⁵, James Henderson⁵, Donato Santovito⁵, Christian Weber⁵, Christoph Kreutz⁴, Giovanni Bussi³, **Michael Sattler**^{1,2*}

¹ Technical University of Munich, Bavarian NMR Center, 85748 Garching, Germany

² Helmholtz Munich, Molecular Targets and Therapeutics Center, 85764 Neuherberg, Germany

³ Scuola Internazionale Superiore di Studi Avanzati, via Bonomea 265, Trieste, 34136, Italy

⁴ University of Innsbruck, Institute of Organic Chemistry and Center for Molecular Biosciences (CMBI), 6020, Austria

⁵ Ludwig-Maximilians-Universität (LMU), Institute for Cardiovascular Prevention (IPEK), 80336 Munich, Germany.

The talk will discuss two examples how conformational dynamics can specifically regulate protein-RNA interactions linked to important biological functions. (i) NMR reveals how hyper-editing of dsRNA converts a canonical A-form duplex into a highly dynamic ensemble comprising non-canonical conformations. The specific recognition of such hyper-edited RNAs by the RNA binding protein Endonuclease V involves a complex binding mechanism. (ii) The tandem KH domains of Mex3a mediate RNA binding and contribute to ternary Ago2/MEX3A/miRNA-126-5p interactions. Using NMR methods to monitor conformational exchange we characterize a folding equilibrium in the Mex3a KH1 domain, where the presence of an unfolded KH domain population enables phosphorylation and modulation of RNA binding and function.

Bringing protein structure into cellular context through advances in cryo-electron tomography

Itziar Serna-Martin

Thermo Fisher Scientific, 5651 Eindhoven, NL

itziarserna.martin@thermofisher.com

Cryo-electron microscopy has transformed our ability to resolve biological structures at near-atomic resolution, yet many fundamental cellular processes remain difficult to interpret when molecular assemblies are studied outside their native environment. Cryo-electron tomography (cryo-ET) addresses this challenge by enabling three-dimensional, *in situ* visualization of macromolecular complexes within intact cells and tissues, providing a direct link between molecular structure and cellular context.

Recent technological advances are rapidly expanding the accessibility, throughput, and reliability of cryoET workflows. Innovations in cryogenic sample handling and transfer now preserve vitrified specimens under ultraclean, stable vacuum conditions, significantly reducing contamination and improving data quality. This presentation will highlight an integrated, next generation cryoET workflow, from optimized cryogenic sample transfer and automated specimen handling through to high resolution tiltseries acquisition on advanced 200–300 kV transmission electron microscopes. We will discuss how these advances collectively lower the barrier to *in situ* structural biology, supporting correlative and multiscale approaches that bridge cellular ultrastructure and molecular detail. Together, these technologies are reshaping cryoelectron tomography into a robust, scalable tool for exploring the molecular architecture of cells in their native state.

Charting the dark corners of the protein function space in the era of AI

Joana Pereira

VIB Center for AI and Computational Biology, Leuven, Belgium
Dept. Cellular and Molecular Medicine, KU Leuven, Leuven, Belgium

Life is largely driven by proteins, yet despite more than 250 million known protein-coding sequences, a substantial fraction of the protein universe remains functionally uncharacterized. But now, recent advances in artificial intelligence are enabling systematic exploration of these unknown regions and the evolutionary principles shaping protein diversity.

We have shown that a large-scale computational framework based on sequence similarity networks allows modeling the landscape of the natural protein universe and the identification of “dark clusters” enriched in uncharacterized proteins. These regions represent a major source of biological novelty and have led to the discovery of hundreds of new protein families across the tree of life. Examples include TumE, a superfamily of translation-targeting prokaryotic toxins, and KreTA, a novel type II toxin–antitoxin system. Furthermore, structural analyses revealed unusual protein folds such as the bacterial β -flowers.

These approaches lay the foundation for next-generation models that integrate sequence, structure, evolutionary, and genomic context to bridge the gap between protein sequence and function. By learning the global organization of protein space, such frameworks aim to enable large-scale functional classification and accelerate the discovery of novel biological systems.

Short Biography

Joana Pereira leads the Protein Evolution and Function Models group at the VIB Center for AI and Computational Biology in Leuven and is an Assistant Professor at KU Leuven. Trained as a biochemist, she holds a PhD in Chemistry from the University of Hamburg and EMBL and completed postdoctoral research at the Max Planck Institute for Developmental Biology with Andrei Lupas and the Biozentrum of the University of Basel with Torsten Schwede. Her research combines structural bioinformatics, complex network analysis, and deep learning to study protein evolution and function at a large scale. Her group focuses on mapping and understanding the diversity of the protein universe, working closely with experimental collaborators to investigate biological systems across the tree of life.

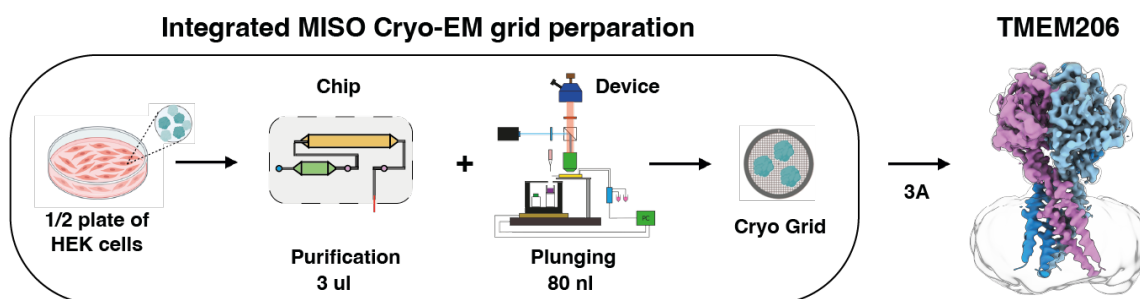
MISO: Microfluidic protein isolation enables cryo-EM structure determination of single dish of cells

Gangadhar Eluru^{1,2}, **Steven De Gieter**^{1,2}, Stephan Schenck^{1,2}, Annelore Stroobants^{1,2}, Binesh Shrestha³, Paul Erbel³, Janine D. Brunner^{1,2} & Rouslan G. Efremov

The study of protein structures is essential for understanding biological principles as well as applied research in drug development and other biotechnology advancements. Here, the field of cryo-electron microscopy (cryo-EM) has fundamentally transformed scientific research by enabling high-resolution structural analysis of complex and medical relevant proteins. However, despite its advancements, sample preparation remains a bottleneck, particularly for proteins with low abundance or challenging expression patterns. Traditional methods often demand substantial starting materials and may still yield unsuccessful structure determinations.

Introducing Micro ISolation (MISO) – our groundbreaking technology to revolutionize cryo-EM sample preparation. MISO integrates on-chip purification with cryo-EM sample preparation, allowing subsequent structure determination by starting from just a few micrograms of protein. Using our developed microfluidic chips, we perform small-scale protein purifications on the μ l scale. From here, only nanoliters of purified protein are directly plunged onto a Cryo-EM grid. This innovative technology eliminates the need for large starting materials, paving the way for more accessible structural studies both in terms of cost and time.

1. Center for Structural Biology, Vlaams Instituut voor Biotechnologie, Brussels, Belgium
2. Structural Biology Brussels, Department of Bioengineering Sciences, Vrije Universiteit Brussel, Brussels, Belgium
3. Protein Sciences, DSc, Biomedical Research, Novartis, Basel, Switzerland



Resolving anti-cancer drug binding to tubulin using 4th-generation synchrotron and XFEL sources

Shourav Saha^{1,2}, Michal Wawrzyniec Kepa¹, Joerg Standfuss^{1,2}

¹Laboratory of Biomolecular Research, Center for Life Science, PSI Paul Scherrer Institute

²Department of Chemistry, University of Zurich

Drug discovery is a complex endeavor which relies on understanding the interactions between a ligand and its cognate receptor. However, most structural biology approaches capture only the final conformation of ligand–receptor complexes, overlooking transient intermediate states that occur during the binding process. Time-resolved serial crystallography, enabled by mix-and-inject and laser-based pump-probe methods at 4th-generation synchrotrons and X-ray free electron lasers, has emerged as a powerful approach for resolving transient structural states that occur during biomolecular processes across multiple timescales.

Tubulin, a heterodimer composed of α - and β -subunits, assembles into cytoskeletal microtubules in eukaryotic cells and plays key roles in processes such as cell division. Its biological importance makes it a valuable target for anticancer therapeutics, particularly compounds that bind the intra-subunit colchicine binding site. Time-resolved fluorescence stopped-flow experiments with colchicine-like ligands suggest a two-step binding mechanism: an initial low-affinity association with the binding pocket followed by a slower conformational transition to the final bound state. Notably, modifications to the colchicine scaffold can substantially accelerate binding speed while preserving this two-step scheme.

In this study, we aim to characterize the binding of several anticancer colchicine-site ligands to tubulin using time-resolved crystallography. By capturing transient structural intermediates during ligand binding and correlating them with kinetic measurements, we seek to better understand how molecular structure influences binding kinetics. Here, we present recent progress in this effort, including initial results from single-crystal cryo-trapping experiments and mix-and-inject experiments using solid supports at Diamond Light Source, as well as future plans for this endeavor.

Integrative Modelling in the Era of AlphaFold

Maya Topf

AI-based structure predictors have transformed structural modelling, but their impressive accuracy for many proteins should not obscure persistent limitations in reliability, particularly for dynamic macromolecules, nucleic acid-containing complexes, and large assemblies. In this talk, I will discuss how AlphaFold models can be evaluated, refined, and selected using complementary structural restraints from electron cryo-microscopy (cryo-EM) and crosslinking mass spectrometry (XL-MS).

Drawing on recent CASP rounds, I will show how refinement of predictions yielded structures closer to their reference targets, including regions with improved density fit, and how local model-to-map agreement pinpoints where flexibility or uncertainty limits interpretability. I will also present our work combining AlphaFold ensemble generation with XL-MS-based conformer selection for proteins with multiple states. Using residue-depth-based monolink and crosslink probability scores, this work illustrates how experimental XL-MS data can select conformations that AlphaFold confidence scores alone can miss. Together, these examples demonstrate how experimental data can improve the interpretation and validation of AI-based models, with cryo-EM and XL-MS providing complementary information on model accuracy, uncertainty, and conformational state.

Generating high quality data sets to help decision making in AI-informed drug discovery

Philip Gribbon

High-quality, decision-ready data are a key limiting factor for the successful adoption of AI methods in early drug discovery. While modern machine learning can accelerate hit finding, hit expansion, and lead optimization, many models still suffer from biased training sets, incomplete metadata, lack of negative results, limited assay context, and poor interoperability between screening sites. We will describe an integrated strategy for generating and structuring high-quality datasets that directly

support robust decision making in AI-informed drug discovery, building on activities across the EU-OPENSOURCE research infrastructure EU-OPENSOURCE provides access to relevant compound resources and high-capacity screening platforms, coupled to systematic assay development and standardized reporting. Central to this approach is the European Chemical Biology Database (ECBD), designed according to FAIR data principles and structured to capture both positive and negative screening outcomes, primary and orthogonal confirmation assays, and detailed experimental metadata. We highlight how curated chemical libraries (including fragment and screening collections), multi-parametric profiling (e.g., morphological cell painting datasets), and standardized bioprofiling workflows provide rich, machine-readable evidence for AI pipelines. In parallel, complementary information sources such as literature and patent-derived knowledge graphs support target, disease, and mechanism-of-action contextualization—bridging experimental screening data and translational insights. Finally, we outline how quality control, annotation, provenance tracking, and interoperable formats can be operationalized as routine infrastructure services. This framework supports objective benchmarking of AI models, and ultimately improves decision confidence in selecting compounds, assays, and progression criteria in early-stage drug discovery.

Principles of human pre-60S assembly

Arnaud Vanden Broeck^{1,2*} & Sebastian Klinge^{2*}

¹ Laboratory of RNA structural biology and Biochemistry, CIP-InBioS, University of Liège, 4000 Liège, Belgium

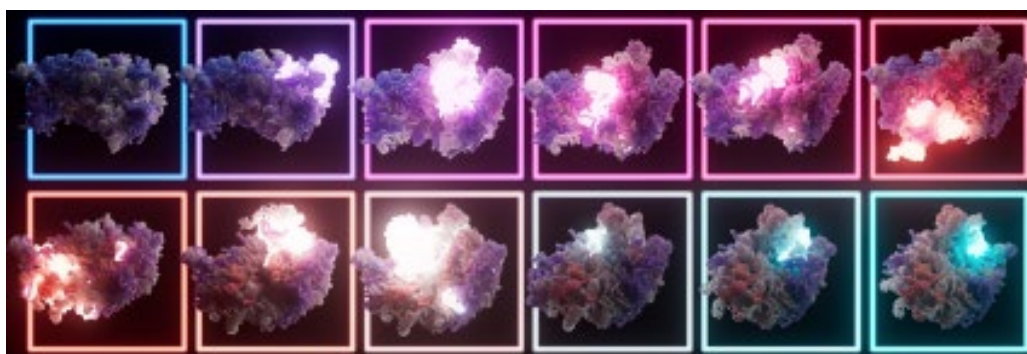
² Laboratory of Protein and Nucleic Acid Chemistry, The Rockefeller University, New York, New York 10065, USA

*co-corresponding author

During early stages of human large ribosomal subunit (60S) biogenesis, an ensemble of assembly factors establishes the foundation of essential RNA functional centers and catalyzes the processing of pre-ribosomal RNA by unknown mechanisms.

Here, we report a series of 24 cryogenic electron microscopy structures of endogenous human nucleolar and nuclear pre-60S assembly intermediates at resolutions of 2.5-3.0 Angstroms. This unprecedented structural landscape now enables an in-depth mechanistic understanding of the early human large ribosomal subunit assembly. In the nucleolus, the pre-60S structures highlight how protein interaction hubs tether assembly factor complexes to the maturing particles and how GTPases and ATPases, such as DEAD-box RNA helicases, can couple irreversible nucleotide hydrolysis steps to the installation of functional centers. In the nucleus, the progressing pre-60S particles illuminate how the structural plasticity of the pre-rRNA is interrogated by assembly factors and by large multiprotein complexes, such as the rixosome, to coordinate RNA cleavage with RNA exosome-mediated degradation of a pre-rRNA spacer; and how assembly factors interchange is used to drive irreversible fine-tuning of functional centers.

Altogether, our work rationalizes decades of existing data from bacterial and eukaryotic systems, reveals new principles of eukaryotic ribosome assembly and lays the foundation for a mechanistic understanding of human diseases in the context of ribosome assembly.



Investigating the viral-host membrane fusion events of Nipah Virus

Joseph W. Thrush^{1,3}, Luke Jones², Irene Carlon-Andres³, Lauren Eyssen¹, Leah McCaffrey¹, Hannah Campaigne¹, David Williamson³, Miles Carroll², Sergi Padilla-Parra³, Raymond J. Owens^{1,2}

[1] Rosalind Franklin Institute, Didcot, UK, [2] University of Oxford, Oxford, UK, [3] King's College London, London, UK

Nipah Virus (NiV) is a highly-pathogenic emerging henipavirus with outbreaks on the Indian subcontinent and Malaysian peninsula regions. It has a high fatality rate causing death due to severe encephalitis or respiratory associated symptomology, and with no approved vaccines or therapeutics, requires increased research focus. Investigations into the molecular mechanism of viral-host membrane fusion of NiV have been previously undertaken, specifically focusing on the coordination between the receptor binding NiV Glycoprotein (NiVG) and the fusogen NiV Fusion (NiVF) proteins. Two proposed mechanisms describe the interaction; the clamp model stipulates NiVG stabilises the pre-fusion NiVF and the provocateur model stipulates NiVG activates the pre-fusion NiVF.

We present highly-potent neutralising single domain antibodies (sdAb) that bind to a quaternary crypt region of the NiVF protein. sdAbs were obtained through NiVF immunisation of a llama, generation of a library from peripheral blood mononuclear cells and enrichment via two rounds of phage display. They were characterised using bio-layer interferometry and cryo-electron microscopy single particle analysis revealing low nanomolar affinity to the NiVF pre-fusion ectodomain, and binding to a quaternary crypt. Fusion inhibition and pseudovirus neutralisation studies revealed low nanomolar IC₅₀ values.

Furthermore, we have used cryo-electron tomography and fluorescence energy resonance transfer to investigate the interactions of NiVF and NiVG before and after receptor binding, and particularly their distributions on the viral membranes. This has revealed a NiVF oligomerisation network that occurs on the surface of both NiV pseudovirions and virus-like particles, which partially occludes the fusion peptide, suggesting a self-stabilisation mechanism.

Structures of the 26S proteasome in complex with the Hsp70 co-chaperone Bag1 reveal a mechanism for direct substrate transfer

Moisés Maestro-López¹, Tat Cheung Cheng^{2,3}, Jimena Muntaner¹, Margarita Menéndez⁴, Melissa Alonso¹, Andreas Schweitzer^{2,3}, Masato Ishizaka², Robert J. Tomko Jr.⁵, **Jorge Cuéllar**^{1, *}, José María Valpuesta^{1, *} and Eri Sakata^{2,3, *}

¹Centro Nacional de Biotecnología (CNB-CSIC). Darwin, 3. 28049 Madrid, Spain. ²Institute for Auditory Neuroscience, University Medical Center Göttingen, 37077, Göttingen, Germany. ³Multiscale Bioimaging: from Molecular Machines to Networks of Excitable Cells (MBExC), University of Goettingen, 37073 Göttingen, Germany ⁴Instituto de Química-Física Rocasolano (IQFR-CSIC), Serrano, 119, 28006 Madrid, Spain. ⁵Department of Biomedical Sciences, Florida State University College of Medicine, FL 32306, USA

*Corresponding authors

Abstract:

Coupling between the chaperone and degradation systems, particularly under stress, is essential for eliminating unfolded proteins¹. The co-chaperone Bag1 links Hsp70 to the 26S proteasome, recruiting Hsp70-bound clients for proteasomal degradation. Here, we present cryo-electron microscopy structures of the Bag1-bound 26S proteasome (Fig. 1a), revealing unprecedented conformational rearrangements within the 19S regulatory particle (Fig. 1a-d). Bag1 binding to the Rpn1 induces a marked reconfiguration of AAA+ adenosine triphosphatase (ATPase) ring, disrupting its canonical spiral staircase and remodeling the central channel architecture. This reconfiguration generates a large cavity above the substrate entry gate of the 20S core particle. The conserved pore-2 loops of ATPases Rpt2 and Rpt5 play critical roles in opening of the 20S gate, enabling substrate entry into proteolytic chamber independently of ubiquitination (Fig. 1e). These findings suggest a previously unknown mechanism of the proteasomal degradation, by which remodeling the central cavity and 20S gate in the presence of Bag1, possibly bypassing the need for ubiquitination².

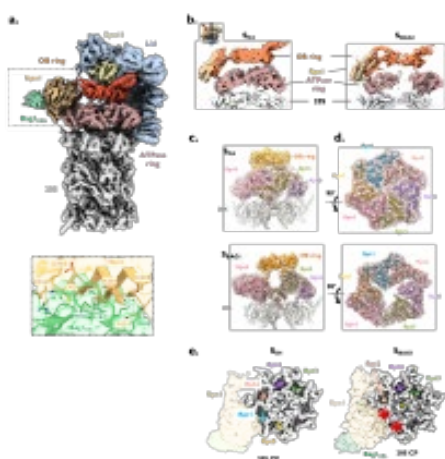


Figure 1. Structural Reorganization of the 26S Proteasome Induced by Bag1 Binding. (a) Cryo-EM reconstruction of the 26S proteasome in the SBAG1 conformation (EMDB:52097) at 3.8 Å resolution, revealing only the UBL domain of Bag1 (Bag1UBL). The inset highlights the interaction interface between Rpn1 and Bag1UBL. (b) Cross-sectional view of the cryo-EM maps of SBAG1 and SD4 (EMDB: 32282; PDB: 7W3K) shows a distinct alignment of the OB, ATPase, and 20S rings in SBAG1, indicating structural rearrangements upon Bag1 binding. (c, d) Segmentation of the OB-ATPase interface (c) and ATPase ring (d) reveals that Bag1 disrupts the ATPase ring symmetry, generating a large central cavity and inducing conformational shifts in Rpt3 (green) and Rpt4 (pink). These changes suggest an alternative substrate entry pathway (red

arrow in (c)) into the 20S proteasome. (e) Differences in 20S gate opening between SD4 and SBAG1. In SBAG1, only Rpt2, Rpt3, and Rpt6 insert into the α -ring, yet the 20S gate remains open, suggesting a non-canonical activation mechanism. The SD4 conformation serves as a reference due to its structural similarity to SBAG1 in both 19S and 20S.

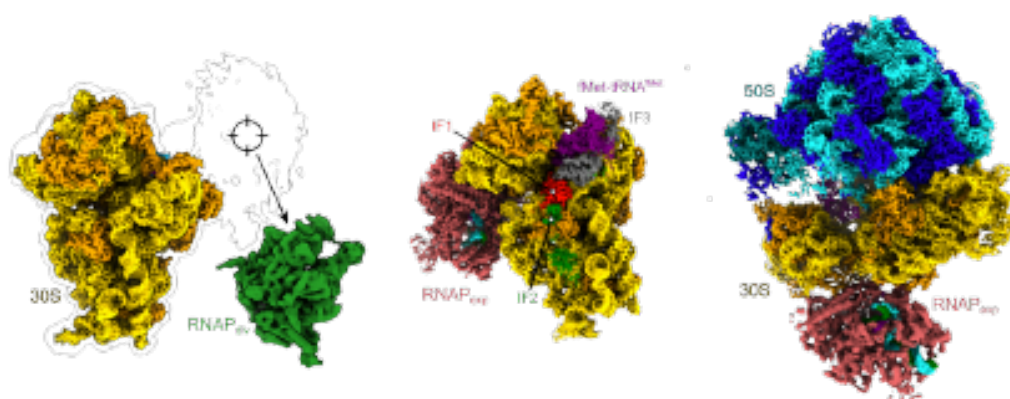
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Supramolecular assemblies in gene expression: Cooperation of RNA polymerase and the ribosome.

Huma RAHIL¹, Maria TAKACS¹, Charlotte SAINT-ANDRÉ¹, and **Albert WEIXLBAUMER¹**

¹IGBMC, Department of Integrated Structural Biology, Strasbourg, France

In prokaryotes, transcription and translation are coupled. This supramolecular assembly line enables mutual regulation and affects gene expression on several levels. Recent evidence suggests that the cooperation between RNA polymerase (RNAP) and the ribosome is not restricted to their elongation phases. Instead, it can start earlier because RNAP can also stimulate ribosome recruitment to nascent mRNA, yet the molecular mechanisms governing the transition from initial recruitment to an active, coupled transcription-translation complex remain poorly defined. We identified an "mRNA delivery state," where ribosomal protein bS1 channels nascent mRNA to the small ribosomal subunit (30S). However, that work excluded translation initiation factors (IF1, IF2, and IF3) and utilized inactive 30S subunits to capture early binding events. Here, we use cryo-electron microscopy to investigate how IF1, IF2, and IF3 influence 30S subunit activation and the sequence of events during co-transcriptional translation initiation. By incorporating initiation factors and the transcription factors NusA and NusG into the assembly, we observe the stepwise transition from the mRNA delivery state to a fully accommodated, coupled expressome. Our results reveal that IFs are sufficient to activate the 30S population and play an unexpected role in monitoring mRNA accommodation. These findings provide a structural framework for understanding how IFs regulate the assembly and function of the coupled transcription-translation machi



Simplifying NMR of RNA and Proteins

Daniel Mathieu

Bruker BioSpin

“CryoID as a forward genetic tool in the study of bacterial cell surfaces”**Han Remaut**

VIB-VUB Center for Structural biology, Pleinlaan 2, 1050 Brussels

Bacterial cell surfaces are often decorated with micrometer-long protein appendages—pili, flagella, and functional amyloids—that power motility, enable the secretion or uptake of proteins and DNA, support biofilm formation and adhesion, or mediate competition and defense. Our understanding of these pathways is largely based on genetic, functional, and structural studies in a handful of model systems. Today, cryo-electron microscopy is turning this classical discovery pipeline upside down, uncovering surprising diversity in bacterial surface components. By combining direct, on-cell imaging with *de novo* 3D reconstruction, we can obtain structures first, that then pinpoint the molecular identity of previously orphan surface assemblies. In this seminar, I will highlight how cryoID turns these structural observations into a powerful forward-genetic route to discover novel biosynthetic pathways involved in cell-envelope maintenance and cell–cell interactions. As a case study, we present a structure-guided proteomics map of *Bacillus* spores, offering new insight into spore biology and their exceptional chemical robustness, and uncovering self-assembling proteins with promising material properties.

Nanobodies as Molecular Tools to Link Cell-Surface Architecture to Tissue Function

Maria Carrasquero-Ordaz^{1,2,3}, Miguel Berbeira-Santana^{1,2}, Claudia Peregrina³, Céline Delloye-Bourgeois⁴, Els Pardon^{5,6}, Jan Steyaert^{5,6}, Raymond Owens⁷, Valérie Castellani⁴, Daniel del Toro³, Elena Seiradake^{1,2}

1. Department of Biochemistry, University of Oxford, OX1 3QU, UK.

2. Kavli Institute for Nanoscience Discovery, University of Oxford, OX1 3QU, UK.

3. Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Institute of Neurosciences, IDIBAPS, CIBERNED, University of Barcelona, Barcelona, Spain.

4. MeLis, University of Lyon, Université Claude Bernard Lyon 1, CNRS UMR 5284, INSERM U1314, Institut NeuroMyoGène, 8 avenue Rockefeller 69008 Lyon, Lyon, France.

5. Structural Biology Brussels, Vrije Universiteit Brussel (VUB), Brussels, Belgium.

6. VIB-VUB Centre for Structural Biology, VIB, Brussels, Belgium.

7. Structural Biology, The Rosalind Franklin Institute, Harwell Science Campus, Didcot, UK; Division of Structural Biology, University of Oxford, Oxford, UK.

Abstract

We use nanobodies as integrative structural biology tools to examine protein mechanisms *in vitro* and *in vivo*. Here we present two applications harnessing the properties of nanobodies as high-affinity labels for sparsely expressed proteins and as modulators of specific molecular interactions.

Cancer cell migration depends on transient receptor-environment interactions. Using the GPC3-Unc5D guidance system as a model, we combined structure-guided mutagenesis and biophysical analyses to reveal an octameric complex organised by glycan-mediated contacts. Engineered anti-GPC3 nanobodies were then used to strengthen (NanoGlue) or weaken (NanoBreak) GPC3-Unc5 binding *in vitro*. Xenografting human neuroblastoma cells into chick embryos expressing NanoGlue or NanoBreak showed that enhancing this interaction promotes hyper-migratory, cohesive tumourigenic behaviour, whereas competitive inhibition disrupts cell cohesion, impairing targeting to the primary tumour site.

To extend the use of nanobodies to additional cell guidance systems in the developing cortex, we generated a nanobody that selectively labels Teneurin4, a receptor guiding migrating neurons during gestation. Its precise localisation was previously unclear. In embryonic mouse cortical sections immunostained with tetramerised anti-Ten4 nanobody, Tau-STED imaging revealed Ten4 enrichment along radial glial fibres, which act like migratory scaffolds for neurons. Ten4 expression increased as neurons transitioned from the intermediate zone to the cortical plate. Integration with structural and functional data indicated that Ten4 uses a switching mechanism to dictate the balance of cell-adhesive and cell-repulsive signals.

Together, these findings position nanobodies as precise molecular markers and regulators that provide high-resolution readouts linking extracellular structure to *in vivo* function, enabling causal dissection of receptor-ligand systems governing neuronal migration in development and disease.

From Intact Proteoforms to Biological Identity

Michal Sharon

Proteins rarely exist as single defined entities but rather as ensembles of proteoforms generated by sequence variation, post-translational modifications, and interactions with cofactors and ligands. These diverse molecular states ultimately define biological function and cellular identity. Mass spectrometry has emerged as a powerful approach for capturing this complexity, particularly through native and top-down strategies that preserve intact protein assemblies and their interactions. In this talk, I will present our efforts to bridge proteoform-level information with biological context using direct mass spectrometry (direct-MS), an approach that enables the analysis of proteins directly from crude samples without prior purification. By exploiting the intrinsic properties of native mass spectrometry, direct-MS allows selective detection of highly expressed proteins while preserving their interactions and heterogeneity. I will demonstrate how this approach can be used to characterize protein structure, interactions and ligand binding, including applications in antibody engineering, disease-associated mutations, and drug target engagement. Together, these advances highlight how moving from purified proteins to intact proteoforms in their native environments provides a more accurate view of biological systems, offering new opportunities to define biological heterogeneity at the molecular level.

Reversal of roles in a new era of structure-based discovery in immunology

Savvas Savvides

Mammalian development and immune regulation rely on cytokine–receptor signalling at the cell surface, yet dysregulation of these pathways frequently drives inflammation, autoimmunity, and cancer. To date, structural biologists have contributed to our understanding of such signaling assemblies by harnessing what immunologists have been contributing over several years ahead of any structural biology undertakings. However, this is set to change in the nascent era of structural biology. We have recently discovered a new cytokine, termed XL-47, the first such discovery in a decade. XL47 is highly expressed in many forms of cancer and autoimmune diseases. My presentation will take us through our structure-based approaches in the discovery process, the formulation of a hypotheses towards the function of XL-47, the creation of critical tools and the elucidation of structure-function insights that are poised to add a major new chapter in our understanding of cytokine-driven signalling in physiology and disease

Posters

Instruct-ERIC: Get access to advanced structural biology services

Claudia Alen Amaro^{1*}, [John Dolan](#)^{1*}, Natalie Haley^{1*}, Harald Schwalbe¹

¹Instruct-ERIC, Oxford House, Parkway Court, John Smith Drive, Oxford, OX4 2JY, UK.

*john@instruct-eric.org

Instruct-ERIC is a pan-European distributed research infrastructure making high-end technologies and methods in structural biology available to users. Instruct-ERIC is comprised of 17 Member Countries and organisations: Belgium, Czech Republic, EMBL, Finland, France, Germany, Greece, Israel, Italy, Latvia, Lithuania, Netherlands, Portugal, Slovakia, Slovenia, Spain and United Kingdom. Through its 12 specialist research Centres in Europe, Instruct-ERIC offers funded research visits, training, internships and R&D awards. Instruct Centres have years of experience in providing world-class technology provision and expertise to users both in academia and industry. By promoting integrative methods, Instruct-ERIC enables excellent science and technological development for the benefit of all life scientists [1]. More on <https://instruct-eric.org/>

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Structure doi: 10.1016/j.str.2024.08.014

Weighing the evidence for intrinsic disorder: Structural mass spectrometry approaches for integrative biology of alpha-synuclein

Frank Sobott, School of Molecular and Cellular Biology, Astbury Centre, LS2 9JT Leeds, U.K.

In this contribution we highlight structural mass spectrometry (MS) approaches to characterize dynamic monomer ensembles, exemplified by Parkinson's disease-related and intrinsically disordered protein alpha-synuclein. Native MS and ion mobility are used to resolve complex and heterogeneous species distributions according to their mass, i.e. oligomeric state and noncovalent ligand interactions, as well as overall size and shape via their rotationally averaged collision cross section. Key advantages of this approach are that it can capture both structured and disordered states of wild-type as well as mutant and post-translationally modified protein isoforms alike with little bias, and represent entire species distributions across size and mass scales. We use ensemble modelling approaches to interpret these complex equilibria and complement with chemical shift perturbations from NMR spectroscopy as well as associated aggregation kinetics from fluorescence assays. Recently we developed a new approach to study protein conformational dynamics directly in complex and high-salt buffers, and investigate how physiological Ca^{2+} ligands as well as Zn^{2+} ions commonly associated with pathological protein states remodel these structural ensembles.

Key questions remain unanswered about aggregation pathways of alpha-synuclein and other proteins implicated in neurodegenerative disorders. What are the molecular pathways which link disordered or misfolded monomers to fibril polymorphs observed by cryo-electron microscopy (EM) and tomography, and are believed to be uniquely associated with disease progression? What are the intermediate species that determine the outcomes of these pathways and could they be targeted by drugs? What associated factors in the cellular microenvironment, such as salt, metal ions, lipids, other proteins etc. might be causative factors or correlate with disease state? The structural MS toolbox enables integration of orthogonal low-resolution data with other structural and biophysical data as well as computational modelling to address these questions.

We used hydrogen/deuterium exchange MS to investigate soluble alpha-synuclein oligomers and their interactions with peptides designed to inhibit further aggregation. Limited proteolysis coupled with MS is a powerful technique to study dynamic regions of soluble species and insoluble fibrils alike, revealing insights into those protein sequences which are not sufficiently ordered to be visible in high-resolution structural techniques such as cryo-EM. Lastly, we present recent work using spatially resolved Secondary Ion Mass Spectrometry directly in thin frozen sections of tissue which was previously analysed by cryo-electron tomography, thus opening up the possibility of correlative imaging with orthogonal information on chemical composition and spatial organization of amyloid directly in native tissue.

Integrative structural biology of protein folding on the ribosome

Julian O. Streit^{1,2*}, Sammy H.S. Chan^{1*}, Tomasz Włodarski¹, Alkistis N. Mitropoulou¹, Anaïs M.E. Cassaignau¹, Ivana V. Bukvin¹, Christopher A. Waudby^{1,3}, Lisa D. Cabrita¹ & John Christodoulou^{1,4}

¹Institute of Structural and Molecular Biology, Department of Structural and Molecular Biology, University College London, London, UK

²Present address: Linderstrøm-Lang Centre for Protein Science, Department of Biology, University of Copenhagen, Copenhagen, Denmark

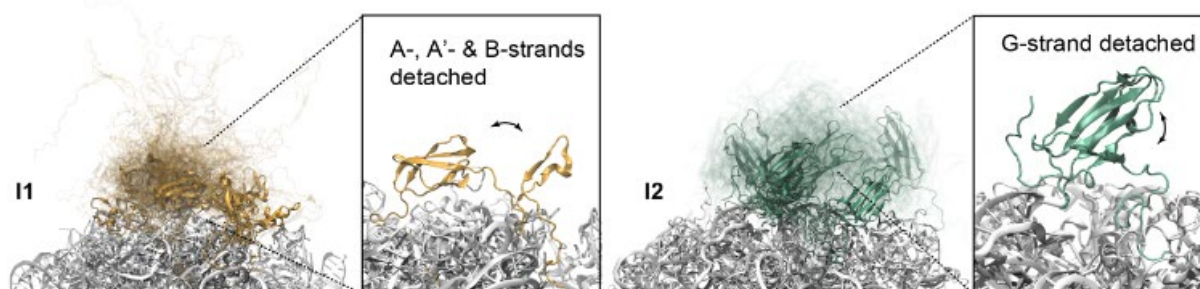
³Department of Pharmaceutical and Biological Chemistry, School of Pharmacy, University College London, London, UK

⁴School of Natural Sciences, Birkbeck College, London, UK

*These authors contributed equally to this work.

Abstract

The ribosome biases the conformations sampled by nascent polypeptide chains along folding pathways towards biologically active states. A hallmark of the co-translational folding (coTF) of many proteins are highly stable folding intermediates that are absent or only transiently populated off the ribosome yet persist during translation well-beyond complete emergence of the domain from the ribosome exit tunnel. By integrating NMR experiments with all-atom, explicit solvent molecular dynamics (MD) simulations of ribosome-nascent chain complexes, we have provided the first quantitative and high-resolution insights into these fundamental features of coTF. The ribosome favours partially folded coTF intermediates by lowering the entropic penalty of folding¹, while simultaneously destabilising natively structured states². Thus, coTF intermediates are a common feature for many proteins¹ and important for folding fidelity and post-translational protein function¹. We have structurally characterised two coTF intermediates of an immunoglobulin-like domain by developing comprehensive ¹⁹F NMR analyses using chemical shifts³, paramagnetic relaxation enhancement (PRE), and protein engineering. We integrated these experimental data with extensive MD simulations to obtain the first experimental, atomistic structures of the folding intermediates on the ribosome⁴. The resulting structures provide unprecedented insights into completely novel conformational states and the mechanism of de novo folding in the cell. Moreover, these intermediates are structurally conserved within the protein domain family, suggesting the ribosome promotes folding along common pathways defined by the topology of the native state. This may allow the ribosome to ensure efficient folding, regulate protein assembly and coordinate targeting by auxiliary factors to maintain cellular proteostasis.



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Title: Cryo-EM of an RNA origami nanopore for synthetic cells**Abstract:**

The RNA origami technique allows for the self-assembly of custom-designed nanostructures from a single RNA strand that folds co-transcriptionally. As a fundamental step toward the bottom-up construction of an artificial cell, our group has developed RNA-based nanopores, which, unlike DNA origami pores, can be produced by the synthetic cell itself. These structures are designed using PyFuRNAce, an RNA origami design engine developed in-house (Monari, 2025). The nanopore incorporates short RNA sequences that bind biotin molecules (aptamers) that enable attachment to vesicles composed of biotin-functionalized membranes.

Here, the RNA nanopore was structurally characterized using cryo-electron microscopy (cryo-EM) at a resolution of 6.7 Å. The reconstructed density reveals a well-folded and uniform architecture, confirming the successful assembly of the designed RNA origami structure. Due to the intrinsic properties of RNA, we further investigated the dynamic flexibility of the nanopore. Further computational analysis allowed visualization of the pore's internal tunnel geometry, revealing a constriction radius of approximately 11 Å. Additionally, negative-stain electron microscopy demonstrates efficient insertion of the RNA nanopore into lipid vesicles.

Together, these structural and functional insights advance our understanding of RNA origami-based architectures and provide valuable feedback for improving future RNA designs. The presented RNA nanopore represents a key component for synthetic cell systems, enabling transport of nutrients and small molecules across artificial membranes.

Authors: **Fenja Murken**^{1,2,3}, Tim Karrasch^{1,3}, Luca Monari¹, Erik Poppleton^{1,4}, Cody Geary¹, Sebastian Eustermann², Kerstin Göpfrich¹

Authors affiliations: ¹Biophysical Engineering Group, Center for Molecular Biology of Heidelberg University (ZMBH), ²Molecular Systems Biology, European Molecular Biology Laboratory, ³Max Planck School Matter to Life, ⁴MPI for polymer research

How does the SPRTN protease keep pace with the structural dynamics of the AAA+ ATPase p97

Primož Bembič¹, Matic Kisovec¹, Marta Popović², **Marjetka Podobnik**¹

¹Department of Molecular Biology and Nanobiotechnology, National Institute of Chemistry, Hajdrihova ulica 19, Ljubljana, Slovenia

²Laboratory for molecular ecotoxicology, Ruđer Bošković Institute, Bijenička cesta 54, Zagreb, Croatia

Covalent DNA-protein crosslinks (DPCs) are DNA lesions that disrupt all DNA-related processes. Unsuccessful repair of DPCs (DPCR) can lead to cancer, premature ageing, and neurodegenerative diseases. Cells possess several DPCR mechanisms, one of which involves the metalloprotease SPRTN, which degrades the protein component of DPCs, while the remaining DNA-bound peptides are subsequently resolved by other downstream mechanisms. SPRTN is supported by the hexameric AAA+ ATPase p97, especially in the case of bulky DPCs, which are first unfolded by p97. SPRTN is a member of the family of p97 protein adaptors. Its polypeptide chain is largely unstructured, with only the N-terminal protease domain adopting a globular fold. The C-terminal disordered region of SPRTN contains several interaction motifs, including an SHP motif that mediates the p97:SPRTN interactions. Using biochemical and biophysical approaches, we have analysed SPRTN binding to p97 and its pathogenic mutant. Our results show that, regardless of the nucleotide status bound to p97 or its pathogenic mutant *in vitro*, SPRTN binds to p97 with affinity in the low micromolar range, with the SHP peptide solely responsible for this interaction. This was further confirmed by cryo-EM analysis of various p97:SPRTN complexes, revealing how SPRTN adapts to different conformational states of p97. *In vivo*, several other adaptors of p97, in addition to SPRTN, are required for successful DPCR, and we are now exploring how they affect the structural dynamics of p97:SPRTN interactions.

Lipids play a role in the structure and dictate the stoichiometry of actinoporin pores

Gašper Šolinc, Marija Srnko, Ana Crnković, **Marjetka Podobnik**, **Gregor Anderluh**

Department of Molecular Biology and Nanobiotechnology, National Institute of Chemistry, Ljubljana,
Slovenia

Pore-forming toxins (PFTs) constitute a widespread class of proteins that permeabilize cellular membranes and play central roles in processes ranging from microbial virulence to defense and predation. These proteins disrupt lipid bilayers by assembling into oligomeric pores, typically formed by protomers arranged in circular arrays. Increasing structural evidence indicates that lipids are not merely a passive environment for pore formation but can act as integral structural components that contribute to pore architecture and stability. Actinoporins are a family of α -PFTs produced by cnidarians that specifically target membranes containing sphingomyelin and have become an important model for studying protein–lipid cooperation in membrane pore formation. Until recently, the only structurally characterised actinoporin pore was octameric, although alternative stoichiometries had been proposed. Here, we investigated the structure of the actinoporin Fav from the mountainous star coral (*Orbicella faveolata*), from its soluble monomer to multiple pore assemblies, using X-ray crystallography and cryo-electron microscopy. Our findings reveal that Fav forms funnel-shaped pores associated with numerous lipids, some of which are not only crucial for membrane binding but also play important roles in pore architecture. By varying the lipid composition used during pore preparation, we obtained and characterised pores with different stoichiometries, namely heptamers, octamers, and nonamers. High-resolution cryo-EM maps reveal a conserved pattern of lipid arrangement and protomer fold across the three assemblies. Our study shows that actinoporin pores are structurally versatile and stabilised by specific lipid interactions, underscoring the dynamic interplay between protein and membrane in pore formation

**Structure of a pH-sensitive pentameric ligand-gated ion channel
from the *Sarcoptes scabiei* mite****Jessica Matos Kleiz Ferreira**

Scabies is a skin infestation caused by the parasitic mite *Sarcoptes scabiei*, and remains a widespread public health concern. Control efforts are increasingly challenged by the emergence of resistance to frontline treatments such as ivermectin. Among the mite's ion channels, an anionic pentameric ligand-gated ion channel (pLGIC) termed SsCl exhibits sensitivity to pH and is strongly influenced by ivermectin. In this study, we combine cryo-electron microscopy (cryo-EM) with electrophysiological recordings to investigate the structural basis of SsCl gating and its modulation by ivermectin. Cryo-EM structures obtained under different pH conditions captured distinct functional states of the channel, including a closed conformation at pH 6.5 and a desensitized conformation at pH 9, together with ivermectin-bound states. Notably, the desensitized structure displays an unexpected "hourglass"-shaped architecture, suggesting gating mechanism closer related to cation-selective channels. Structural analysis and mutagenesis identify extracellular histidine and glutamic acid residues that impact the pH-sensitivity, likely contributing to a broader pH-sensing network.

Ivermectin was found to bind at the transmembrane subunit interfaces, where it stabilizes an open-pore conformation. Structural data further indicate that ivermectin's effects are modulated by pH, enhancing open-state prevalence at pH 9 and enabling atypical activation at pH 6.5, in agreement with electrophysiological data. These findings offer initial insights into SsCl's pH-sensitivity and ivermectin's activity, informing next-generation antiparasitic design. Ongoing work aims to further characterize additional candidate pH-sensing residues and to define the broader pH-sensing network that potentially regulates channel gating

**Comprehensive insight into the dynamic behaviour of covalent
rNKR-P1B homodimer in solution**

Jan Bláha^{1,2}, Cy Jeffries², Jakub Jeníček¹, Ondřej Skořepa¹, Barbora Kalousková³, Tereza Skálová⁴, Jan Dohnálek⁴, Ondřej Vaněk¹

¹ Department of Biochemistry, Faculty of Science, Charles University, Hlavova 2030/8, 12840 Prague, Czech Republic

² EMBL, Hamburg Unit c/o DESY, Notkestrasse 85, 22607 Hamburg, Germany

³ Institute of Applied Physics - Biophysics, Technical University of Vienna, Getreidemarkt 9, 1060 Vienna, Austria

⁴ Institute of Biotechnology, Czech Academy of Sciences, BIOCEV Centre, Průmyslová 595, 25250 Vestec, Czech Republic

Receptors from NKR-P1 family are important signalization molecules on the surface of Natural Killer cells – a subset of granular lymphocytes that oversee the detection and elimination of malignantly transformed cells in the host organism. Detection of these cells is facilitated via integration of activating and inhibitory signals from the surface receptors.

In this study, we present the first structural description of the covalent homodimer of NKR-P1 molecule and its behaviour in solution, revealing unprecedented insights into its dynamic structural behaviour. Utilizing an integrative approach, we combined an incomplete X-ray crystallographic model with

Analytical Ultracentrifugation (AUC) and Small Angle X-ray Scattering coupled with Size Exclusion Chromatography (SEC-SAXS) to construct a comprehensive picture of rNKR-P1B homodimer structure and dynamics in solution. Recognizing the limitations of the acquired X-ray structure - missing flexible residues and N-linked glycosylation, we employed Molecular Dynamics (MD) simulations to complete and refine our X-ray structural model. Subsequent combination of Rigid Body Modelling (CORAL) and Normal Mode Analysis (SREFLEX) facilitated identification of partially glycosylated models that accurately fit the acquired SEC-SAXS data. Through multi-model fitting, we identified a set of models that represent the dynamic behaviour of rNKR-P1B in solution, demonstrating a predominant open state that contrasts with the closed dimer observed in crystallographic structures. These findings not only shed light on the structural dynamics of the NKR-P1 receptor family but also demonstrate the power of combining computational and experimental methods to explore the structural intricacies of flexible, glycosylated proteins in their natural milieu. This research contributes to our understanding of NKR-P1 biological roles and opens new avenues for therapeutic intervention.

This work has been supported by the Czech Science Foundation (23-08490L).

AI-guided competitive docking for virtual screening and compound efficacy prediction

Manon Mirgaux¹, Valeria Barcelli², Adeline C.Y. Chua³, Pablo Bifani^{2,3}, René Wintjens¹

¹ Unit of Microbiology, Bioorganic and Macromolecular Chemistry, Department of Research in Drug Development, Faculté de Pharmacie, Université Libre de Bruxelles, B-1050 Brussels, Belgium

² Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore.

³ A*STAR Infectious Diseases Laboratory, Agency for Science, Technology and Research (A*STAR), Singapore.

Abstract:

Machine learning has profoundly transformed the prediction of protein structures and interactions. In structure-based drug discovery, virtual docking has long been the standard approach, but a breakthrough emerged with the advent of co-folding generative models such as RoseTTAFold All-Atom and AlphaFold3 (AF3). These methods can model protein–ligand interactions across a wide and largely unrestricted diversity of ligand types.

A key limitation, however, is that machine-learning-based docking methods generally generate an interaction pose for any ligand, independent of whether it truly binds or is inactive. While many models offer scoring functions to rank predicted complexes, reliably estimating binding affinities remains challenging. This raises two fundamental questions: (i) how can true binding events be differentiated from false positives? and (ii) how can predicted poses be ranked in a manner that accurately reflects their expected binding strengths?

In this work, we demonstrate that AlphaFold3 and Boltz-1/2 achieve not only high accuracy in predicting protein–ligand interactions but also enable discrimination between active and inactive compounds. We propose a simple and efficient strategy, termed pairwise competitive docking, which ranks candidate molecules by directly comparing their relative binding at a protein's target site. When applied to 17 benchmark protein systems, this approach produced rankings consistent with experimental observations. Furthermore, we demonstrate that pairwise competitive docking can accelerate the identification of promising hits from large chemical libraries and facilitate the de novo design of inhibitors with improved predicted potency. Overall, these results illustrate how modern machine-learning models can render structure-based drug discovery faster, more robust, and more cost-effective than approaches relying solely on experimental workflows.

Molecular determinants for recognition of serotonylated chromatin

L. Pulido-Cortés¹, H. Gieling², V. Thijssen³, M. Liu³, R. Yoshisada³, L. Romão Soares³, S. Nizamuddin¹, F. Friedrich⁴, H. Greschik¹, L. Peng¹, R. Vargas Honorato², M. Jung⁴, A.M.J.J. Bonvin², M.L. Biniossek⁵, R. Schüle¹, S. Jongkees³, **H. van Ingen^{2*}**† and H.Th.M. Timmers^{1*}†.

¹German Cancer Consortium (DKTK), Medical Center-University of Freiburg, Germany.

²NMR Spectroscopy, Bijvoet Center for Biomolecular Research, Utrecht University, the Netherlands.

³Department of Chemistry and Pharmaceutical Sciences, Vrije Universiteit Amsterdam, the Netherlands. ⁴Institute of Pharmaceutical Sciences, University of Freiburg, Germany.

⁵Institute of Molecular Medicine and Cell Research, Medical Center-University of Freiburg, Germany.

Post-translational modifications of histone tails constitute a key epigenetic mechanism controlling chromatin environment and gene transcription. Serotonylation of histone H3Q5 (H3Q5ser) is a recently discovered mark associated with active transcription of RNA polymerase II (pol II)-transcribed genes. The direct link between H3Q5ser and the pol II transcription machinery relies on the TFIID subunit TAF3. The presence of H3Q5ser enhances TAF3 binding to H3K4me3, but the molecular determinants underlying this interaction remained unclear.

Here, we resolve the binding mode of TAF3-PHD to H3K4me3Q5ser identifying a novel binding surface for H3Q5ser using solution NMR spectroscopy. This reveals how H3Q5ser recognizes a conserved surface of the TAF3-PHD via CH- π interactions in an edge-face conformation involving a proline residue stabilised by a tryptophan. This combination of proline and tryptophan is unique to the PHD finger of TAF3 and conserved amongst TAF3 orthologues. Combination of NMR and ITC data show that the additional interactions afforded by the serotonin group boost affinity ca. 3-fold by prolonging the life time of the complex.

Our findings establish a framework for the molecular recognition of serotonylated chromatin, laying the foundation for developing epigenetic inhibitors targeting serotonylation-dependent transcriptional regulation in neuronal development. In addition, this work highlights the ongoing need for experimental structural biology in the age of protein structure prediction to solve fundamental molecular mechanisms in biology.

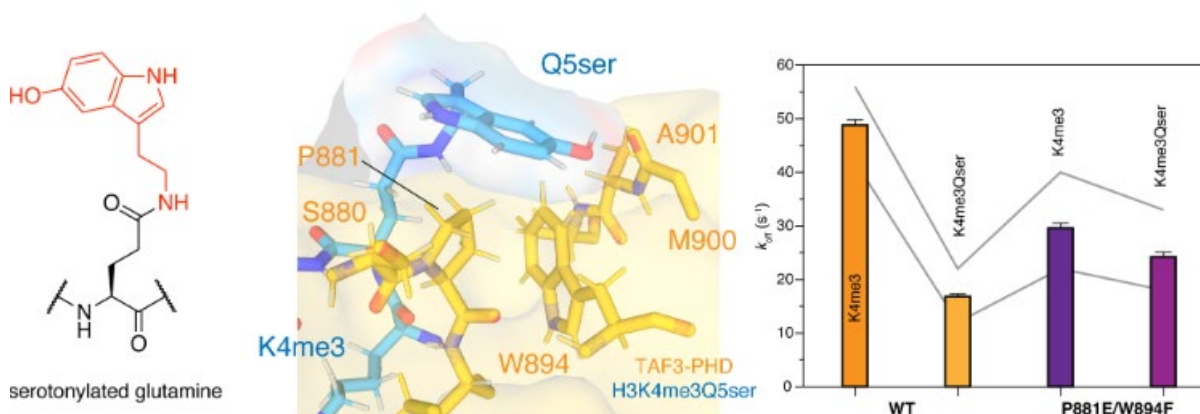


Figure. (left) structure of serotonylated glutamine; (middle) solution NMR based structure of a serotonylated histone tail peptide (H3K4me3Q5ser) bound to the TAF3-PHD finger; (right) complex

dissociation rates based on NMR signal line shape analysis for wild-type (WT) and mutant TAF3-PHD bound to histone peptides with or without serotonylation.

Pulido-Cortés et al, Nucleic Acids Research, 2025, 53, gkaf612, <https://doi.org/10.1093/nar/gkaf612>.
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Cryo-EM structure of the botulinum neurotoxin A/SV2B complex and its implications for translocation

Authors: Basavraj Khanppnavar; Oneda Leka; **Sushant K. Pal**; Volodymyr M. Korkhov; Richard A. Kammerer

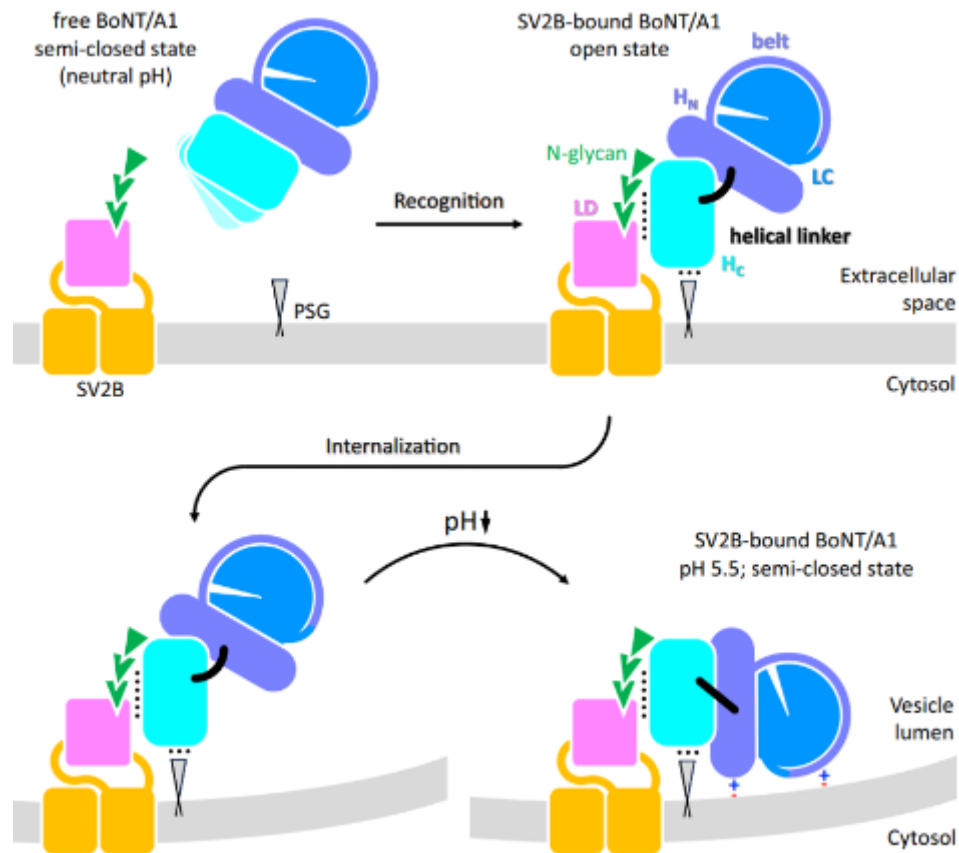
Affiliations: PSI Center for Life Sciences, Villigen, Switzerland; Institute of Molecular Biology and Biophysics, ETH Zurich, Zurich, Switzerland

Abstract: Botulinum neurotoxin A1 (BoNT/A1) is among the most potent biological toxins and is also the most widely used therapeutic, comprising three domains: a proteolytic domain (LC) that cleaves substrates responsible for neurotransmitter release, a translocation domain (HN), and a receptor-binding domain (HC). However, the structural transitions that prime translocation of its proteolytic domain across the synaptic vesicle membrane into the neuronal cytosol remain enigmatic. To elucidate early steps preceding translocation, cryo-EM structures were determined for full-length BoNT/A1 in solution and for full-length human synaptic vesicle glycoprotein 2B (SV2B) in complex with BoNT/A1 at neutral and acidic pH.

At physiological pH, BoNT/A1 adopts a predominant semi-closed conformation in solution. Unexpectedly, binding to full-length SV2B shifts BoNT/A1 into an open state in which LC and HN are positioned far from the membrane, suggesting a translocation-incompatible arrangement prior to endosomal acidification. At pH 5.5, mimicking the synaptic vesicle lumen, the SV2B-bound toxin undergoes a pronounced rearrangement back to a semi-closed architecture that places LC, HN, and membrane-proximal segments closer to the bilayer, consistent with acquisition of a translocation-competent state. The SV2B–BoNT/A1 interface combines protein–protein contacts with glycan-mediated recognition, and mutational disruption of either component abrogates SV2B binding in pull-down assays. Microscale thermophoresis measurements show high-affinity binding of SV2B to BoNT/A1, primarily through HC, with a nanomolar dissociation constant.

Together, these structures provide a mechanistic framework in which receptor binding and vesicular acidification act as sequential triggers that first prevent premature membrane engagement and then remodel the receptor-bound toxin into a membrane-proximal conformation poised for LC translocation.

Keywords: Botulinum neurotoxin; SV2; cryo-EM; conformational switching; translocation



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A pipeline for shortlisting and lead optimizing de novo designed protein binders towards CDKL5

Marco Orlando¹, Claudia D'Ercole, Marzia Calvanese², Lucia Cikatricisová, Maria Luisa Tutino, Ario de Marco

¹Lab of Environmental and Life Sciences, University of Nova Gorica, Vipavska cesta 13, Rožna Dolina, 5000 Nova Gorica, Slovenia

²Department Of Chemical Sciences, University Federico II, Napoli, Italy

Artificial Intelligence (AI) is now considered a mature technology for the **de novo** design of protein binders, offering a potentially more reliable option when working with poorly immunogenic or highly conserved target antigens, or when a specific conformational epitope must be targeted. Current benchmarks indicate that more than 10 designs typically must be characterized to yield binders with low nanomolar affinity and sufficient stability. However, most published successes have focused on biomarkers (e.g., **PD-L1**) for which structural data and targeting interfaces were already established via classical approaches. In contrast, there are very few documented cases where novel biomarkers and functionally relevant epitopes have been successfully targeted.

CDKL5 is an essential kinase whose dysfunction correlates with neurological conditions, including CDKL5 Deficiency Disorder. The sequence of its N-terminal kinase domain is highly conserved among CDKL enzymes, whereas its C-terminus is **disordered** and contains several evolutionary conserved sequences likely involved in functional regulation. These structural characteristics explain the historical difficulty in recovering highly specific antibodies for CDKL5 epitopes using classical discovery techniques. Consequently, the ability to quantify and precisely localize the protein in cells and **tissues** has been severely impaired.

Our project devised a customized approach to score **de novo** redesigned protein mini-binders targeting a specific, dynamically stable conformational epitope unique to the catalytic domain of CDKL5. The protocol integrated **AI-driven biophysical models** and physics-based simulations to shortlist the candidate pool to 15. The preliminary results reported here discuss the success rate of this pipeline. Furthermore, we demonstrate how these outputs can be exploited to optimize protein minibinders for other challenging biomarkers.

Experiment-guided AlphaFold3 resolves accurate protein ensembles

Advaith Maddipatla¹ Nadav Bojan Sella¹ Meital Bojan¹ Volodymyr Masalitin¹ Sanketh Vedula² Paul Schanda¹ Alex Bronstein^{1, 3} **Ailie Marx^{4, 5}**

¹Science, Institute of Science and Technology Austria, Austria

²Eric and Wendy Schmidt Center, Princeton University, USA

³Computer Science, Technion, Israel

⁴Faculty of Sciences and Technology, Tel Hai Academic College, Israel

⁵Biotechnology, Migal - Galilee Research Institute, Israel

AlphaFold3 predicts highly accurate protein structures from sequence, but tends to collapse to a single dominant conformation, even when the underlying structure is inherently heterogeneous. Moreover, its predictions are oblivious to experimental conditions that can alter local sequence conformation. In this work we show that AlphaFold3 can be guided to match data obtained by NMR spectroscopy, X-ray crystallography and cryo-EM experiments. We demonstrate that this methodology can generate ensembles of conformations having less distance restraint violations than traditionally resolved NMR structures and uncover unmodelled alternate conformations detectable in electron density. We show that AlphaFold3 can also be guided by cryo-electron microscopy maps and that doing so in combination with NMR parameters improves model quality. This methodology paves the way for the development of experimentally aware predictive models that capture the ensemble nature of protein structures.

Structural basis of the Themis-Grb2 complex in T cell signalling

Danielle M. Clancy^{1,2}, *, Alba Sanz-Sanjuan^{1,2}, Elisabeth Gilis^{2,3}, Peter Tougaard^{2,4,#}, Imke Velghe^{5,6}, Yana Van Droogenbroeck^{5,6}, Jan Felix^{1,2}, Yehudi Bloch^{1,2,#}, Álvaro Furones Cuadrado^{1,2,#}, Romain Merceron^{1,2,#}, Stephan Schenck^{7,8}, Peter Vandenaabeele^{2,4}, Janine D. Brunner^{7,8}, Tom Taghon^{5,6}, Dirk Elewaut^{2,3}, Savvas N. Savvides^{1,2,*}

¹Unit for Structural Biology, Department of Biochemistry and Microbiology, Ghent University, Ghent, Belgium.

²VIB Center for Inflammation Research, Ghent, Belgium

³Laboratory for Molecular Immunology and Inflammation, Department of Rheumatology, Ghent University Hospital, Ghent, Belgium.

⁴Department of Biomedical Molecular Biology, Ghent University, Ghent, Belgium

⁵Department of Diagnostic Sciences, Ghent University, Ghent, Belgium

⁶Cancer Research Institute Ghent (CRIG), Ghent, Belgium

⁷VIB-VUB Center for Structural Biology, VIB, Belgium

⁸Structural Biology Brussels, Vrije Universiteit Brussel, 1050, Brussels, Belgium

#Present addresses:

Peter Tougaard : Department of International Health, Immunology and Microbiology, University of Copenhagen, Denmark.

Alvaro Furones Cuadrado : VIB-UGent Center for Plant Systems Biology, Ghent, Belgium.

Yehudi Bloch : European Molecular Biology Laboratory, Hamburg Unit c/o DESY, Hamburg, Germany

Romain Merceron : Eurofins DiscoverX Products France, Celle-Lévescault, France.

*Correspondence: danielle.clancy@irc.vib-ugent.be ; savvas.savvides@ugent.be

Abstract

Positive selection of thymocytes is essential for establishing the mammalian T cell repertoire, maintaining self-tolerance and preventing autoimmunity. Themis, the archetypal member of a metazoan protein family featuring distinctive CABIT domains, crucially regulates this process by integrating signals from the T cell receptor (TCR). Intriguingly, Themis has been proposed to function via a constitutive complex with the multifunctional adaptor Grb2. Although poised to represent a paradigm shift in our understanding of TCR signalling, the structural and mechanistic basis of this assembly has remained enigmatic. Here, we present the cryo-EM structure of Themis in complex with Grb2, revealing how the tandem CABIT domains and proline-rich sequence of Themis cooperatively engulf the C-terminal SH3 domain of Grb2, while the remaining domains of Grb2 adopt multiple conformational states poised for interactions with additional binding partners. Structural insights of unbound Themis unmask the pronounced interdomain flexibility between the CABIT domains, which adopt a defined conformational state upon Grb2 binding. Importantly, structure-guided Themis mutants abrogated interactions with Grb2 and failed to regulate the tyrosine phosphatase SHP-1 after TCR stimulation, analogous to the phenotype of Themis-deficient cells. Collectively, our study draws the blueprint of the Themis-Grb2 complex as a dynamic structural hub in T cell signalling.

Integrating SPA and Flexibility Analysis in Cryo-EM: The FlexibilityHub Service at Instruct-ES

Gragera M., Mendonça DC., Herreros D., Melero R., Sorzano COS., Carazo JM.

Cryogenic electron microscopy (cryo-EM) is a well-established technique for determining the structures of macromolecular complexes at high resolution [1]. Single-particle analysis (SPA) enables the reconstruction of one or several structures by aligning and averaging thousands of individual projections of a biological specimen from a single cryo-EM dataset. However, these methods generally capture only discrete states of a protein's conformational landscape, even though cryo-EM data contain valuable information about molecular motions.

Recent advances in image processing have begun to exploit this information, allowing researchers to explore conformational flexibility and improve the resolution of mobile subregions within protein complexes. In response to this growing interest, Instruct-ES, the Spanish centre of Instruct, now offers FlexibilityHub [2], a new service designed to extract and analyze structural dynamics from cryo-EM data.

Users are encouraged to provide particle sets that have undergone prior 2D and 3D classification but still exhibit some degree of flexibility, whether compositional or conformational. We then conduct a comprehensive analysis of the dataset's flexibility using state-of-the-art software packages such as Zernike3D, hetSIREN, cryoDRGN, OpusDSD, cryoSPARC 3DVA and RECOVER. These tools are combined with flexible-reconstruction methods such as RELION 5-DynaMight, cryoSPARC 3DFlex, and Xmipp-ZART to correct for molecular motions.

This multi-algorithmic approach is facilitated by Scipion, our in-house image-processing platform for cryo-EM, which integrates software for both SPA and flexibility analysis within a single environment.

To illustrate the capabilities of FlexibilityHub, we present several key use cases highlighting its potential benefits for the structural biology community. FlexibilityHub is freely available to eligible users alongside our established SPA service within the Instruct catalogue.

[1] Saibil H. R. (2022). *Molecular cell*, 82(2), 274–284.

[2] Herreros, D., Krieger, J. M., Fonseca, Y., Conesa, P., Harastani, M., Vuillemot, R., Hamitouche, I., Serrano Gutiérrez, R., Gragera, M., Melero, R., Jonic, S., Carazo, J. M., & Sorzano, C. O. S. (2023). *Acta crystallographica. Section D, Structural biology*, 79(Pt 7), 569–584.

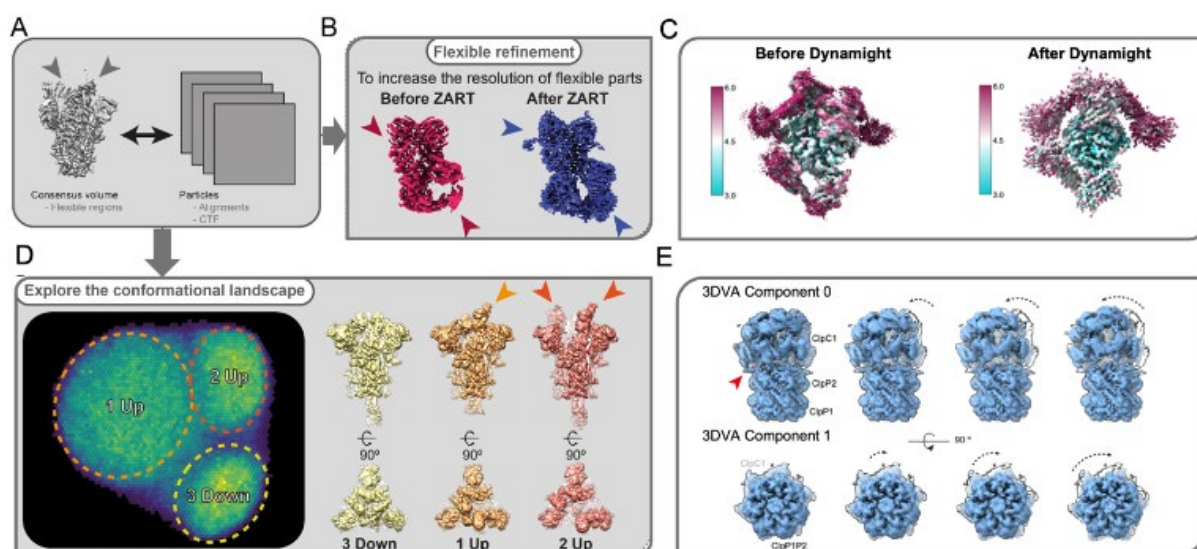


Figure 1. Use cases of the methods offered in the FlexibilityHub service. (A) The starting material consists of a set of cryo-EM particles (with alignments and CTF) and a consensus volume obtained from such particles. Note that some regions are at lower resolution due to its flexibility (grey arrowheads). (B) Flexible regions, solved at poorer resolution (red arrowheads), can undergo a flexible refinement (in the example, using the ZART reconstruction method) to improve their resolution locally (blue arrowheads) by leveraging the flexibility information contained in the particles. (C) Another example where local resolution improves after applying Dynamight (D) Alternatively, an extensive assessment of the sample conformational space can be performed to identify populations of particles displaying different, but coexisting conformations (dashed circles in left panel). Representative volumes of each cluster in the conformational space are shown in the right panel. Arrowheads point at Receptor Binding Domains (RBDs) in "Up" conformation. (E) Additional example of a multiprotein complex where the top subcomplex rotates and tilts relative to the bottom one.

**Affinity maturation of a single-domain antibody via rational
mutagenesis informed by artificial intelligence**Gillian Dekkers¹, Sebastiaan Tetteroo¹, Raimond Heukers^{1,2}, **Matti F. Pronker**^{1,3}

1: QVQ Holding B.V., Utrecht, the Netherlands

2: Vrije Universiteit Amsterdam, Amsterdam, the Netherlands

3: Sanquin Research, Amsterdam, the Netherlands (current affiliation)

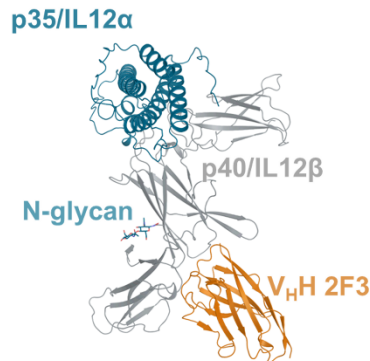
Abstract:

Antibodies can nowadays originate from various sources such as animal immunizations, patient material (both B-cell genetic material and directly at the protein level, e.g. from serum/plasma), DNA/RNA libraries and (AI-based) *de novo* design. Pre-made (e.g. synthetic) DNA libraries have substantial advantages over traditional immunization campaigns in terms of speed, cost, animal welfare, amount of required antigen and the possibility to select against otherwise problematic (e.g. toxic) antigens. The main downside is that selections from these non-immune libraries often result in binders with relatively low affinity due to the absence of *in vivo* affinity maturation. Now that *in silico* structure predictions tools are coming of age, the resulting models could conceivably be used to rationally design mutations, e.g. to enhance binding affinity.

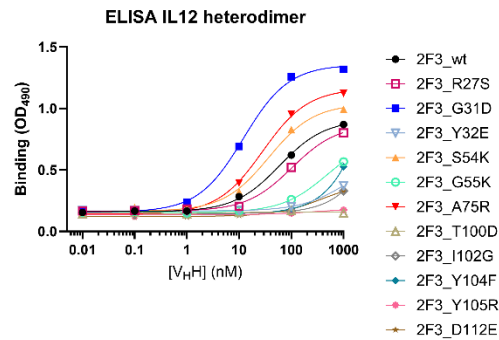
Here we introduce our synthetic single-domain antibody (sdAb) library QSiLy and use it to select new binders against IL12, a cytokine involved in differentiation of T cells into Th1 cells via the Th1 pathway. One of the hits with a low apparent binding affinity was therefore subjected to affinity maturation. The selected sdAb was predicted by AlphaFold3 to bind to the p40 subunit in a manner that would compete with IL12R β 1 binding for both IL12 and IL23. Interestingly, this is the same epitope that is targeted by the clinically approved antibodies Briakinumab and Ustekinumab for a variety of inflammation disorders (arthritis, psoriasis, inflammatory bowel disease *etc.*).

The predicted structure was used to rationally design twelve point mutations, of which three appeared to enhance binding. By combining these mutations, we could enhance the affinity by more than 8-fold compared to the original hit. This shows that AI models, if correct, can be used for rational mutagenesis to enhance binding affinity to relevant drug targets

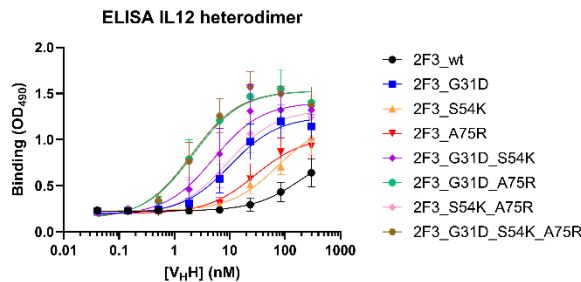
1: Predict/determine structure of complex



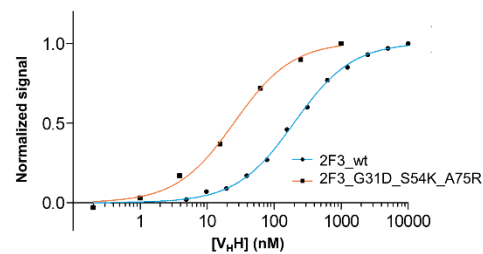
2: Design and test point mutations



3: Combine succesful point mutations



4: Validate by SPR



Exploring Structural Heterogeneity in Cryo-EM Through Flexibility Analysis and Workflow Integration in Scipion

Deborah Cezar Mendonca¹, Luis Alberto Valverde Fernandez², Marcos Gragera¹, David Herreros¹, Richard Garratt², Carlos-Oscar Sorzano¹, Jose-Maria Carazo¹
¹Biocomputing Unit, Centro Nacional de Biotecnología-CSIC, Madrid, Spain
²Instituto de Física de Sao Carlos, Universidade de Sao Paulo, Sao Carlos, Brazil

Biological macromolecules are highly dynamic entities whose functions depend on their ability to change conformation and respond to their environment. Although single particle Cryo-electron microscopy (Cryo-EM) has revolutionized structural biology by providing near-atomic resolution maps, traditional reconstruction methods often struggle with structural heterogeneity. 3D classification methods usually solve discrete states but frequently fails in cases where there are flexible regions or a continuum of transitory states, resulting in smeared reconstructions of these moving parts. In this study, we investigated the conformational landscape of a septin octamer from *Magnaporthe oryzae*. Septins are a class of GTP-binding proteins essential for several cellular processes, including cytokinesis, and their intrinsic flexibility has been proposed to play a role in membrane recognition. Notably, human septins located in the central region of octamers have been shown to undergo conformational changes that expose a polybasic α -helix normally buried within the complex.

Using a cryo-EM dataset of *M. oryzae* septin octamers, we employed the Scipion framework, which integrates multiple tools for flexibility analysis, to characterize the possible conformational states. Our analysis not only captured the expected bending motions but also revealed a continuous transition between the open and closed conformations in the central subunits of the octamer. Moreover, the use of the flexibility algorithms enabled the recovery of densities in peripheral regions typically blurred by the motion.

Together, these results provide a detailed view of septin structural dynamics and highlight the advantages of integrative cryo-EM flexibility analysis for resolving heterogeneity and continuous conformational states in dynamic macromolecular assemblies.

The Ybbr domains of CdaR as key sensors for bacterial pH adaptation

Katharina Weinhäupl^a, Rajkumar Singh^b, Teresa Megre Lousada^a, Arantza Muguruza Monteroc^a, Isabel Matasa^a, Hugo Fragaa^a, Paul Schandab^a, João Morais Cabrala^a

^a i3S - Instituto de Investigação e Inovação em Saúde da Universidade do Porto, Porto, Portugal

^b Institute of Science and Technology Austria (ISTA), Klosterneuburg, Austria

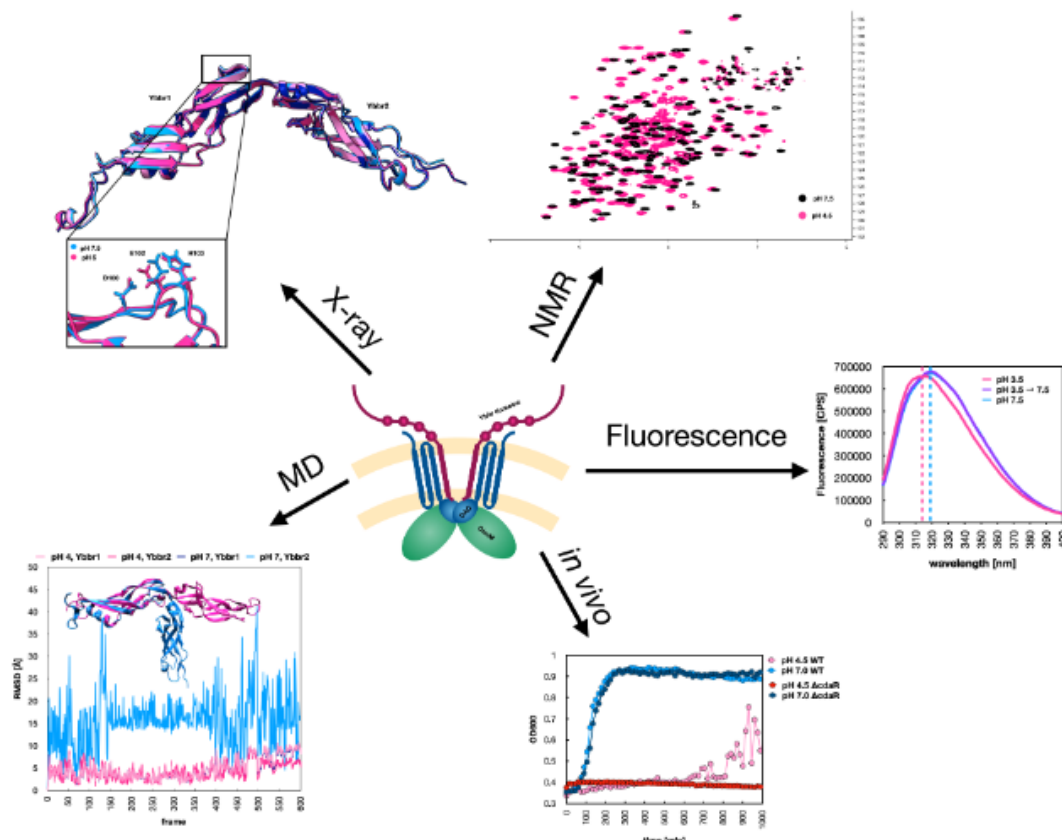
^c University of the Basque Country, Bizkaia, Spain

@ kweinhaeupl@i3s.up.pt

Environmental adaption is crucial for bacterial survival. The essential second messenger cyclic-di-AMP has been implicated in many adaption mechanisms such as potassium homeostasis, cell turgor regulation or biofilm formation.

Using fluorescence assays, X-ray crystallography, NMR and MD simulations we elucidated the molecular mechanism behind *Bacillus subtilis* low pH adaptation. We show that the Ybbr domains of the CdaR protein, an important regulator of the cyclic-di-AMP producing enzyme CdaA, sense low pH through a conformational change. Additionally, we have located the sensor to a conserved hinge region between Ybbr domain 1 and 2.

Our findings have important implications for understanding bacterial survival under low pH contexts such as during infection, in biofilms or during fermentation.



Dynamics of a *de novo* designed inactive soluble GPCR analogue

Flavio von Philipsborn¹, Matthias Schuster¹, Renato Valsecchi¹ and Oliver Zerbe^{1,c}

(1) University of Zurich, Department of Chemistry, 8057 Zurich, Switzerland, (c) corresponding author

G protein-coupled receptors (GPCRs) are pharmacologically important membrane proteins that undergo conformational exchange between inactive, intermediate, and active states, accompanied by fast side chain dynamics. Nuclear magnetic resonance (NMR) spectroscopy is uniquely suited to characterize such motions at atomic resolution. However, the production and stability of GPCRs often limit their investigation by solution NMR. Recently, *de novo* protein design has enabled the generation of soluble GPCR analogues retaining the overall fold architecture and the G-protein binding site [1].

We investigated pico-to-nanosecond side chain dynamics based on methyl order parameters as well as conformational dynamics of an inactive soluble GPCR analogue using methyl-based solution NMR spectroscopy. The receptor was expressed in *E. coli*, enabling perdeuteration and selective ¹³C,¹H labeling of the δ-methyl groups of Ile and Leu, and the γ-methyl groups of Val (ILV) residues. Methyl group assignments allowed us to gain insights into site-specific dynamics.

The findings reveal that most methyl groups are less well-ordered than in other soluble proteins, which was previously only observed in membrane proteins [2]. This suggests that enhanced side chain mobility in membrane proteins may be linked to the fold architecture rather than the membrane environment. Importantly, the observed fast side chain dynamics correlate with a substantial reservoir of residual conformational entropy, which favors the stability of the folded state and may facilitate allosteric communication. In addition, the receptor sampled different conformational states as probed via ¹³C relaxation dispersion experiments. Together, these results highlight the presence of dynamics similar to native-like systems, making such *de novo* designed soluble GPCR analogues promising tools in structural biology.

Acknowledgements:

We thank Bruno Correia for providing the protein construct used in this work.

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**A novel nanobody-based approach for targeting heterogeneous
Acinetobacter baumannii isolates****Charles Van der Henst**

Flanders Institute for Biotechnology (VIB)

Acinetobacter baumannii, a top-priority ESKAPE pathogen, presents significant challenges to human health due to its extensive antibiotic resistance. Additionally, its high strain heterogeneity and protective polysaccharide capsule hinder bacterial cell enrichment, rapid detection, and targeted therapy by limiting access to conserved outermembrane targets. We developed and characterized a new biotechnological tool, the nanobody H7 (NbH7), along with its conserved target, Omp25, and identified the bound epitope using structural biology. NbH7-functionalized magnetic beads enable selective enrichment of *A. baumannii* from complex mixtures containing nonpathogenic intestinal bacteria. This work demonstrates a new targeting system that remains effective across diverse clinical isolates and capsule types, providing proof of concept for its use in diagnostic cell enrichment and targeted therapies.

Characterisation of the ammonium assimilation pathway at the soybean symbiosome membrane.

Sander E. Van der Verren

Affiliation

VIB-UGent Center of Plant Systems Biology, Vlaams Instituut voor Biotechnologie - Universiteit Gent

Abstract

Biological nitrogen fixation is crucial to the cultivation of leguminous crops, where bacteria capture atmospheric nitrogen and deliver nitrogen compounds to the plant.

Soybean forms a symbiosis with *Bradyrhizobium* species, where bacteria enter specific root organs ("nodules") and transform into nitrogen-fixing organelles, the "symbiosomes". The symbiosome membrane forms the interface between plant and bacteria and features a vast proteome enabling nutrient exchange and communication. However, nutrient exchange and -control processes at the symbiotic interface remain poorly understood. We combine plant cell biology, electron microscopy and biophysics to structurally characterise the nitrogen assimilation pathway, starting from the export of ammonium from the symbiosome to its assimilation in infected cells. Candidate transporters and enzymes are identified from a single nuclei transcriptomic atlas and transiently expressed in soybean nodules. Purified target protein complexes are subsequently used for AP-MS to identify novel interaction partners and single particle cryo-EM for structural analysis. At the same time I am optimising the isolation of infected cells and symbiosomes from nodules in order to prepare them for cryo-electron tomography in order to visualise the nitrogen assimilating supercomplexes in their native environment. Together, these approaches will yield unprecedented detail of the enzyme cascade at the symbiosome interface offering the foundation to guide future efforts in optimising biological nitrogen fixation in soybean.

Structural and Dynamic NMR Characterization of a VLP–Alpha-Synuclein Conjugate in Vaccine Prototypes

Jana Petkus

Abstract:

Alpha-synuclein (α -syn) is a 140-residue intrinsically disordered protein (IDP) linked to Parkinson's disease. Existing treatments primarily focus on symptomatic management but fail to prevent the progression of the disease. One promising therapeutic strategy is immunotherapy, particularly vaccines designed to trigger an immune response against toxic α -syn aggregates.

Virus-like particles (VLPs) are highly immunogenic assemblies that mimic viral structures without being infectious. These structures can be modified via attachment of peptide antigens to induce targeted immune response. Structural characterization of VLP-antigen conjugates is essential but remains challenging due to disordered nature of α -syn, which limits the applicability of conventional structural analysis methods, including X-ray crystallography and cryo-electron microscopy.

In this work, we produced the VLP and α -syn conjugate and describe the structural properties and dynamics at atomic level using multidimensional nuclear magnetic resonance (NMR) spectroscopy. We applied both solution- and solid-state NMR to cover structural segments with different mobility. We managed to achieve up to 90% of the backbone resonances of α -syn in the VLP conjugate, using multidimensional solution-state ^1H and ^{13}C detection NMR experiments. Furthermore, ^{15}N relaxation measurements (T_1 and T_2), combined with ^1H - ^{15}N NOE experiments, were performed to characterize residue-specific backbone dynamics in solution.

To probe the VLP component of the conjugate, we employed solid-state NMR experiment at ultra-fast magic angle spinning regime (100 kHz). The 2D hNH INEPT-based solid-state NMR experiments were used to detect flexible regions, while cross-polarization (CP) experiments (2D hNH) were applied to observe more rigid parts.

Our results demonstrate that combined solution and solid-state NMR provide a powerful approach for atomic-level characterization of VLP- α -synuclein conjugate in vaccine formulations.

Pathogenic serpin polymerisation elucidated using orthogonal structural approaches

S.M. Lowen^{1,2,*}, I. Aldobiyani^{1,2,*}, S. Vickers^{2,3,*}, I. Serangeli⁴, G. Galliciotti⁵, A.M. Jagger^{1,2}, E.L.K. Elliston^{1,2}, N. Heyer-Chauhan¹, J. Christodoulou^{2,3,+,}, K. Thalassinou^{2,3,+,}, C.A. Waudby^{2,6,+,}, E.V. Orlova^{2,7,+,}, E. Miranda^{4,+,}, D.A. Lomas^{1,2,#}, **J.A. Irving^{1,2,#}**

1UCL Respiratory, University College London, London, United Kingdom, 2Institute of Structural and Molecular Biology, University College London, London, United Kingdom, 3Department of Structural and Molecular Biology, University College London, London, United Kingdom, 4Sapienza University of Rome, Rome, Italy, 5Institute of Neuropathology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany, 6UCL School of Pharmacy, University College London, London, United Kingdom, 7Birkbeck College, London, United Kingdom

*,+,# These authors contributed equally

Serpins are a family of protease inhibitors whose mechanism of action involves a protease 'bait' sequence in an exposed loop and the ability to undergo a thermodynamically-driven structural change. We have used single-particle EM, biomolecular NMR, X-ray crystallography, structural ion mobility mass spectrometry and conformationally selective monoclonal antibodies to study serpin protein deposits that have been isolated from tissue. This has allowed us to structurally elucidate disease-associated protein-protein interactions that lead to tissue accumulation of alpha-1-antitrypsin (associated with a genetic cause of liver disease and emphysema) and of neuroserpin (associated with a rare hereditary dementia). These aggregates are the consequence of a non-amyloid ordered aggregation that yields unbranched protein polymers, that are both extremely stable and functionally inactive.

Using orthogonal structural and biophysical techniques applied to protein extracted from human and transgenic mouse tissue, we have defined molecular details of the serpin polymerisation pathway. Our data support a mechanism whereby there is a progression through distinct states as it transitions from a mildly-perturbed monomeric intermediate to a hyper-stable polymeric form whose subunits have undergone an intra- and inter-molecular reconfiguration. This has allowed us to draw conclusions regarding transient species on the pathway and the likely mechanism by which the barrier to conformational change is circumvented. The commonalities between mechanistic function and pathological malfunction reveal a molecule that is poised between these opposing outcomes

Mapping substrate-dependent conformational changes in the short PIN8 auxin transporter by HDX-MS

Assia Khalid, Inès Benhammouche, Bjørn Panyella Pedersen, Chloé Martens

Amid the growing need to finely manipulate plant growth for agriculture and resilience, auxin transport represents a central regulatory process. PIN transporters mediate the directional redistribution of auxin within cells and are key regulators of hormone-driven development. How they accommodate chemically diverse compounds, from endogenous auxins to synthetic herbicidal analogues and inhibitors, is essential for understanding and controlling auxin distribution.

Our study focuses on the auxin transporter PIN8, a membrane protein mediating auxin export and interacting with the endogenous auxin indole-3-acetic acid (IAA), herbicidal analogues such as 2,4-D, and transport inhibitors including NPA. This ligand diversity provides a framework to examine how different substrate classes influence the conformational behavior of an auxin transporter at the molecular level. We build on recent structural characterization of PIN8 capturing distinct conformational states in the presence and absence of these compounds. Using differential HDX-MS (Δ HDX-MS), we compare ligand-dependent HDX signatures and associate specific fingerprints with structural, conformational, and functional effects.

We first compared HDX patterns in the presence of IAA and the inhibitor NPA, which stabilize distinct conformations. This analysis identified regions reporting on global conformational changes and provided a reference framework to assess the effects of other ligands.

We then analyzed the HDX fingerprint of 2,4-D. Although 2,4-D and IAA show similar affinity and comparable in vitro transport behavior, 2,4-D displays slower transport kinetics and acts as an herbicide. While IAA induces overall protection consistent with conformational stabilization, 2,4-D causes global deprotection. These differences are particularly evident on transmembrane helices TM4 and TM9, which form the binding site, and extend to distal regions such as TM10, not directly contacting the substrate. Together, these results indicate that subtle changes in the conformational landscape, not captured by static structures, underlie distinct functional outcomes.

Structural dynamics and functional expansion of the Sin3L transcriptional regulatory complex

Jasper Van de Veire¹, Neesha Rajesh Shewakramani¹ Joseph Cesare^{2,3}, Jules Nde³, Marc Vidal^{4,5}, Michael P. Washburn³, Julien Olivet^{1,@}, Kalyan Das^{1,@}

¹ Structural Biology Lab, Molecular, Structural and Translational Virology Research Group, Rega Institute for Medical Research, Department of Microbiology, Immunology and Transplantation, Katholieke Universiteit Leuven, Leuven, Belgium

² Stowers Institute for Medical Research, Kansas City, MO, USA

³ Department of Cancer Biology, University of Kansas Medical Center, Kansas City, KS, USA

⁴ Center for Cancer Systems Biology, Dana-Farber Cancer Institute, Boston, MA, USA

⁵ Department of Genetics, Blavatnik Institute, Harvard Medical School, Boston, MA, USA

⁶ Virology lab, Molecular, Structural and Translational Virology Research Group, Rega Institute for Medical Research, Department of Microbiology, Immunology and Transplantation, Katholieke Universiteit Leuven, Leuven, Belgium

Emails: julien.olivet@kuleuven.be, kalyan.das@kuleuven.be

Transcriptional regulation is orchestrated by histone deacetylase (HDAC) complexes, among which the evolutionarily conserved Sin3 large (Sin3L) complex acts as a major regulatory hub involved in various biological processes and diseases. While multiple cryogenic electron microscopy (cryo-EM) structures of the core complex assembly have been published in yeast, many transient interactions with key subunits such as histone readers or transcription factors have not been determined. This prevents fundamental understanding of the structure-function relationships that govern gene transcription in specific biological contexts. Moreover, due to the limited understanding of context-specific Sin3L interfaces, the design of new generations of highly specific HDAC complex inhibitors remains challenging.

Here, we will present the results of an integrative structural biology approach combining cryo-EM and crosslinking mass spectrometry (XL-MS) which will elucidate context-dependent Sin3L functions. We will show how XL-MS data can reveal robust interactions between various Sin3L proteins and the uncharacterized Fun19 protein that has been predicted to contain a chromatin-interacting SWIRM domain. Cryo-EM analyses of endogenously purified Sin3L complexes resulted in heterogeneous assemblies, containing previously unresolved densities. The core Sin3L complex was refined to create a density map with a global resolution of 4.1 Å. Remarkably, this refined volume contains a flexible density patch matching the overall positioning of the histone reader Cti6 modeled in a recently published manuscript. Overall, the characterization of Fun19 and the development of alternative purification strategies will result in multiple context-dependent Sin3L assemblies that will provide novel druggable target sites.

Structure of an oxygen-induced tubular nanocompartment in *Pyrococcus furiosus*

Ioannis Skalidis^{1,*}, Wenfei Song^{1†}, Jan Fiala^{2,3,*}, Pascal Albanese^{2,3§}, Constantinos Patinios⁴, Marten L. Chaillet¹, Servé W.M. Kengen⁴, Richard A. Scheltema^{2,3}, Stuart C. Howes¹, Albert J.R. Heck^{2,3}, Friedrich Förster^{1,*}

1. Structural Biochemistry, Bijvoet Centre for Biomolecular Research, Utrecht University, Universiteitsweg 99, 3584CG Utrecht, the Netherlands.
2. Netherlands Proteomics Center, Utrecht University, Utrecht University, 3584 CH Utrecht, The Netherlands
3. Biomolecular Mass Spectrometry and Proteomics Group, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, 3584 CH Utrecht, The Netherlands
4. Laboratory of Microbiology, Wageningen University and Research, Stippeneng 4, 6708WE, Wageningen, The Netherlands.
- 5.

* Corresponding author: f.g.forster@uu.nl.

§ Present address: Univ. Grenoble Alpes, CNRS, INRAE, CEA, LPCV, INSERM, UMR BioSanté U1292, 38000 Grenoble, France

*: Equally contributed

†: Leading author

Summary

Reactive oxygen species (ROS) pose a significant threat to biological molecules, prompting organisms to develop systems that buffer oxidative stress and contain iron, which otherwise amplifies ROS production. Understanding oxidative stress responses requires identifying the key proteins involved and their cellular organization. Here, we combined proteomics and cryo-EM to investigate the response of the anaerobic hyperthermophilic archaeon *Pyrococcus furiosus* to oxygen exposure. Proteome analysis revealed a significant upregulation of the oxidoreductase Rubrerythrin (Rbr) under oxidative stress. Cryo-electron tomograms showed the formation of prominent oxidative stress-induced tubules (OSITs). Single-particle cryo-EM and mass spectrometry of enriched OSITs identified them as stacked rings of Rbr homotetramers. The 3.3 Å structure demonstrates that rubredoxin-like domains mediate homotetramer assembly, suggesting that their oxidation drives OSIT formation. Within OSITs, we discovered virus-like particles formed by a ferritin-like/encapsulin fusion protein with iron hydroxide cores, uncovering a sophisticated organelle that protects *P. furiosus* from ROS through advanced compartmentalization.

**Structural basis of signal peptide recognition by the signal
peptidase complex**

Dimitrios Vismpas^{1,6}, A. Manuel Liaci^{1,6}, Lisbeth R. Kjølbye², Ioannis Skolidis¹, Gilberto P. Pereira^{2,3,4}, Adrian Fujiet Koh⁵, Mariska Gröllers-Mulderij¹, Abhay Kotecha⁵, Paulo C. T. Souza^{2,3,4}, Friedrich Förster^{1*}

Affiliations:

1: Structural Biochemistry, Bijvoet Centre for Biomolecular Research, Utrecht University, Universiteitsweg 99, 3584 CG, Utrecht, The Netherlands.

2: Molecular Microbiology and Structural Biochemistry, CNRS UMR 5086 and Université Claude Bernard Lyon 1, Lyon, France.

3: Laboratoire de Biologie et Modélisation de la Cellule, CNRS, UMR 5239, Inserm, U1293, Université Claude Bernard Lyon 1, Ecole Normale Supérieure de Lyon, Lyon, France.

4: Centre Blaise Pascal de Simulation et de Modélisation Numérique, Ecole Normale Supérieure de Lyon, Lyon, France

5: Thermo Fisher Scientific, Achtseweg Noord, 5651 GG, Eindhoven, The Netherlands

6: These authors contributed equally

*Correspondence to: f.g.forster@uu.nl

Abstract

The signal peptidase complex (SPC) is responsible for cleaving signal peptides (SPs) from approximately 10% of the human proteome. SPs are characterized by a tripartite structure, consisting of an N-terminal n-region, a central helical h-region and a C-terminal c-region, each defined by broad physicochemical preferences. Despite their sequence diversity, SPC recognizes and processes SPs with exquisite specificity. Here, we present a 2.6 Å cryo-EM map of the human SPC-A, one of two SPC paralogs, bound to a model SP. The c-region binds to a hydrophobic binding groove near the active site, a narrow gate marks the transition from c- to h-region, and the h-region localizes in a transmembrane (TM) window. Substrate engagement stabilizes N- and C-terminal helices of Sec11A, which frame the SP and are unresolved in the apo structure. Molecular dynamics (MD) simulations confirm a stable hydrogen-bonding network at the c-region and indicate dynamic interactions within a thinned lipid environment at the TM window. AlphaFold modeling supports this binding mode across physiological SPs. Collectively, our structural and computational analyses explain how the SPC achieves its specificity by combining the selectivity of the luminal binding groove and of the transmembrane window.

Crystallization chaperone to enable an integrated structure- and AI-guided pipeline for VHH humanization and affinity tuning

Senne Sienaert, Onno Akkermans, Dmytro Guzenko, Cristina Úbeda Nicolau, Sine Vaneerdewegh, Ali H. Munawar, Stephen D. Weeks

¹. *Pledge Therapeutics, Leuven, Belgium*

Single-domain antibodies (SdAb or VHH), derived from camelid heavy-chain-only antibodies, offer advantages as therapeutics due to their small size, high stability, and modular integration into multi-specific biologics. Yet their translation into clinical candidates typically requires modifying the sequence to acquire the desired properties, such as reduced immunogenicity or improved affinity. These engineering steps heavily rely on iterative labour-intensive efforts to validate the desired properties. Modifications to the framework residues offer the simplest route for gains in affinity and humanization. However, structural analyses have demonstrated that VHH paratopes often utilize framework residues to mediate interactions with the antigen or act as a scaffold for optimal presentation of the CDRs. Therefore, integrating structural information into the protein engineering process can significantly reduce the number of possible novel sequences thus accelerating the optimization steps.

While AI-based structure prediction has improved significantly in recent years, these techniques still struggle with antibody–target complexes. To address this, we present the use of a crystallization chaperone that binds to the VHH in complex with its target, aiding structure determination. The crystallization chaperones recognize a region of the VHH that is not utilized in antigen recognition, allowing for broad application. Because the crystallization chaperone dominates the crystal packing, it has enabled structural determination of difficult targets, such as highly glycosylated proteins.

The structural information is integrated into an in-house developed AI-driven antibody design platform called SaNBox, which proposes humanized framework variants and affinity-modulating mutations in a single, cohesive workflow.

Molecular basis of Fab-dependent IgA antibody recognition by gut-bacterial metalloproteases

Ana Martínez Gascuña^{1,5}, María Ángeles Márquez-Moñino^{1,5}, Tala Azzam², Andrea Persson³, Aitor Manzanares-Gomez¹, Marina Aguillo-Urarte¹, Rolf Lood³, Andreas Naegeli³, Diego E Sastre², Eric J Sundberg² & Beatriz Trastoy^{1,4}.

¹Structural Glycoimmunology Laboratory, Biobizkaia Health Research Institute, Barakaldo, Spain.

²Department of Biochemistry, Emory University School of Medicine, Atlanta, GA, USA.

³Genovis AB, Box 790, Lund 22007, Sweden.

⁴Ikerbasque, Basque Foundation for Science, Bilbao 48009, Spain.

⁵These authors contributed equally: María Ángeles Márquez-Moñino, Ana Martínez Gascuña.

Abstract:

Immunoglobulin A (IgA) is essential for mucosal immunity and has been implicated in autoimmune diseases, such as IgA nephropathy. Certain pathogenic and commensal bacteria produce IgA proteases that selectively cleave IgA, potentially aiding bacterial colonization as well as suggesting therapeutic avenues for IgA nephropathy. Here, we investigate the substrate specificities of two enzymes of the M64 metalloprotease family, the IgA protease ThomasA from *Thomasclavelia ramosa* and BF3526 from *Bacteroides fragilis*. Our structural, biochemical, and mutagenesis analyses demonstrate that ThomasA cleaves IgA through exclusive recognition of the Fab region. This mechanism is distinct from that of other antibody-specific proteases, which typically require engagement of the Fc region. In contrast, X-ray crystal structures of BF3526 in complex with substrate and product peptides, combined with enzymology assays, show that this enzyme targets the N-terminus of predigested proteins, but does not act on intact IgA. These findings reveal divergent substrate recognition strategies between M64 family members, while providing new structural insights into their conserved catalytic mechanism.

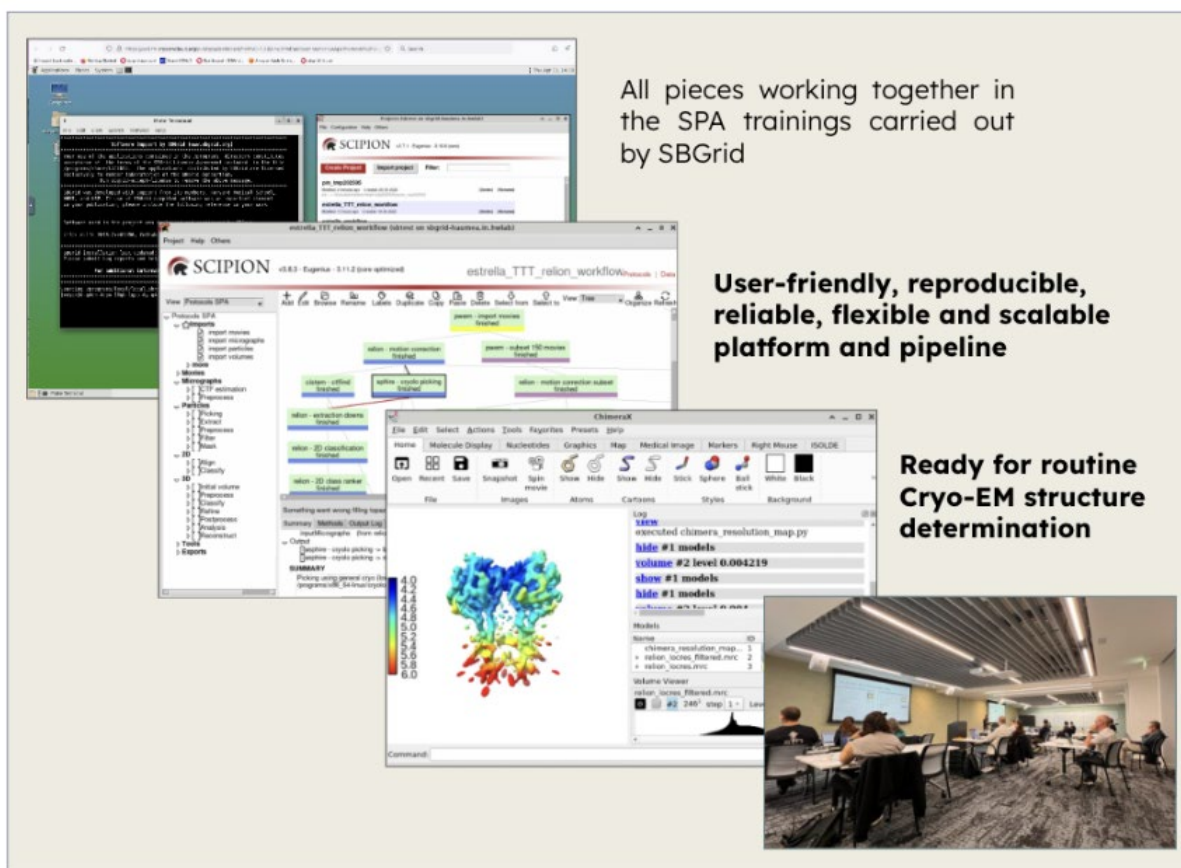
Scipion Meets SBGrid: Expanding Cloud-Based Cryo-EM Training Workflows

Author: **Estrella Fernández-Giménez**

Author affiliations: SBGrid Consortium (BCMP, Harvard Medical School, Boston),
Instruct-ERIC (CNB-CSIC, Madrid)

Abstract:

Cloud-based platforms are increasingly important for broadening access to cryo-electron microscopy (Cryo-EM) training without requiring local high-performance computing. SBGrid provides a stable environment for structural biology software, but its Cryo-EM workflows have traditionally been limited to standalone applications. To expand these capabilities, we integrated the latest version of Scipion—an open-source framework that unifies image-processing tools into a reproducible, user-friendly workflow system—directly into SBGrid. This integration allows trainees to run a full single-particle analysis (SPA) workflow within SBGrid while exploring Scipion's plugins and workflow-based approach. We outline the technical challenges, solutions, and training outcomes of this migration, and show how Scipion improves reproducibility, streamlines workflows, and positions SBGrid to support evolving Cryo-EM methods and more routine structure-determination tasks.



All pieces working together in the SPA trainings carried out by SBGrid

User-friendly, reproducible, reliable, flexible and scalable platform and pipeline

Ready for routine Cryo-EM structure determination

Enabling High Throughput Electron Cryo-microscopy for Structure-Based Design

Yibo Jin on behalf of Astex Pharmaceuticals

Astex Pharmaceuticals, 436 Cambridge Science Park, Milton Road, Cambridge CB4 0QA, United Kingdom

Access to high resolution structural data on protein–ligand complexes is a prerequisite for structure-based drug design. For proteins refractory to X-ray crystallography, high throughput structure determination by electron cryo-microscopy (cryo-EM) has the potential to be transformational for drug discovery. This poster will describe a workflow, from protein production through to end-user accessible high resolution structural data, applied to a biologically important ion channel TRPML1 in complex with a chemically diverse range of ligands. The depth of structural data generated provides important insights into protein-ligand structure-function relationships and demonstrates the potential of the workflow to support iterative compound design cycles.

**Capturing transient states of heterodimeric ABC transporter
TM287/288 by Time-Resolved Small-Angle X-ray Scattering**

Lea Schröder^{1,2}, Dario De Vecchis^{3,7}, Andrey Gruzinov⁴, Lars V. Schäfer³, Clement E. Blanchet⁴, Markus A. Seeger⁵, Henning Tidow^{1,2*}, and Inokentij Josts^{1,2,6*}

¹ The Hamburg Advanced Research Centre for Bioorganic Chemistry (HARBOR), Luruper Chaussee 149, D-22761 Hamburg, Germany

² Department of Chemistry, University of Hamburg, Luruper Chaussee 149, D-22761 Hamburg, Germany

³ Center for Theoretical Chemistry, Ruhr University Bochum, Universitätsstr. 150, D-44801 Bochum, Germany

⁴ European Molecular Biology Laboratory Hamburg Outstation c/o DESY, Notkestrasse 85, D-22607 Hamburg, Germany

⁵ Institute of Medical Microbiology, University of Zurich, Zürich, Switzerland

⁶ Newcastle University, Faculty of Medical Sciences, Newcastle University Biosciences Institute (NUBI), Framlington Place, Newcastle upon Tyne NE2 4HH, UK

⁷ Present address: Univ. Grenoble Alpes, CNRS, CEA, IBS, France

* Corresponding Authors

ATP-binding cassette (ABC) transporters are essential molecular machines that use the power of ATP binding and hydrolysis to move substances across cellular membranes. Structures of the heterodimeric ABC transporter TM287/288 have been previously determined in several states, indicating large conformational changes during its reaction cycle. However, for a complete description of the cycle, transient states such as an occluded state are still missing, as they are difficult to capture with static structural biology techniques. We investigated the kinetics and accompanying structural changes in the course of the reaction cycle of full-length TM287/288 using time-resolved small-angle X-ray scattering (SAXS), initiated by stopped-flow mixing. By observing changes in the radius of gyration (R_g) of the transporter, we can track transitions between its catalytic states on the second-to-minute timescale. The use of an active site mutant and state-specific sybodies/nanobodies enabled us to dissect the temporal events involved in the ATP-driven conformational cycle of TM287/288 and revealed a transient occluded state of this ABC transporter. Our results demonstrate that time-resolved stopped-flow SAXS can be a broadly applicable method for dissecting the different molecular states in membrane proteins

Gallic acid-induced tetramerisation of SuhB abolishes its extragenic suppressor activities

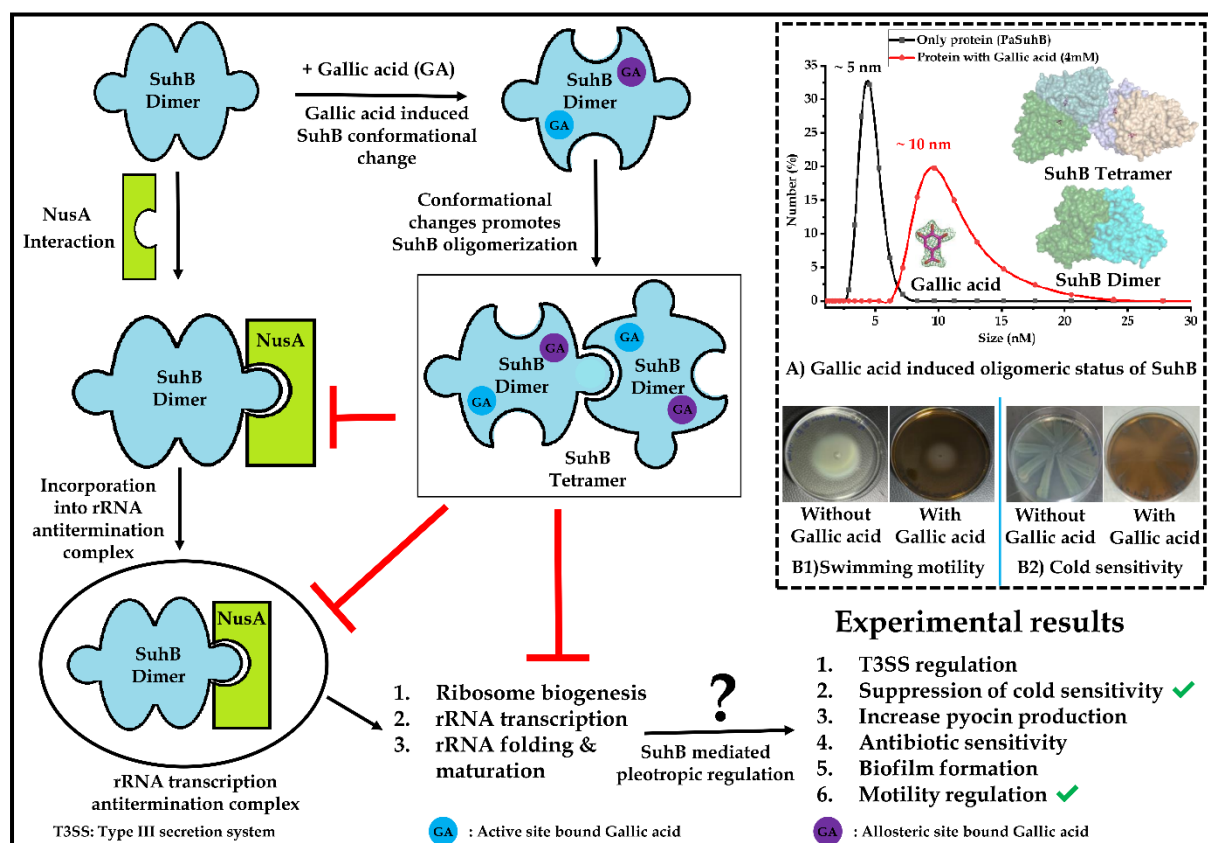
Vinay K. Yadav,¹ Abinash Jena,¹ Mitali Mukerji,¹ Sudipta Bhattacharyya^{*1}

¹ - Department of Bioscience and Bioengineering, Indian Institute of Technology, Jodhpur, Rajasthan (342037), India.

Presenting author: Vinay Kumar Yadav, PhD scholar, Department of Bioscience and Bioengineering, Indian Institute of Technology, Jodhpur, Rajasthan (342037), India. Email: yadav.45@iitj.ac.in Phone No.: +91 6283202615

Abstract

Nus-factor SuhB protein from *Pseudomonas aeruginosa* (PaSuhB) is a pleiotropic regulator of multiple genes associated with the virulence of the pathogen. Herein, we have solved the crystal structures of PaSuhB in its apo and substrate-bound (D-myo-Inositol-1-phosphate) forms. Moreover, 3D pharmacophore modelling of the PaSuhB-bound substrate identified gallic acid as a potential inhibitor of the protein. *In-vitro* enzyme-kinetics studies indicate gallic acid is a non-competitive inhibitor of PaSuhB.



The high-resolution crystal structure of gallic acid-PaSuhB complex uncovers the existence of a novel allosteric ligand binding site of the protein. The novel allosteric ligand binding site was identified at the

dimerisation interface of the protein, in proximity of the $\alpha 4$ helix. Detailed structural analysis reveals that gallic acid, bound at the allosteric site of PaSuhB, induces structural distortion of the $\alpha 4$ helix and thereby promotes an asymmetric tetramer formation of dimeric PaSuhB. 'In-solution' dynamic light scattering experiments further fortify the gallic acid-mediated tetramerisation of PaSuhB. The asymmetric tetramer formation of PaSuhB shields the highly conserved NusA-AR2 domain binding site of the protein and thereby may impact its extragenic suppressor activity. *In vitro*, gallic acid at non-lethal concentrations induced the 'cold sensitive' growth phenomenon in *P. aeruginosa* and *E. coli*, a characteristic feature of *suhB* as well as *nus* deletion mutants. Moreover, gallic acid also inhibited the swimming motility of the bacteria. Altogether, our results demonstrated a novel allosteric inhibitor binding site of the pleiotropic virulence regulator SuhB from *Pseudomonas aeruginosa*, which can be exploited further to design specific inhibitors against this dreaded pathogen.

Keywords: *P. aeruginosa*-virulence-regulator; SuhB-Nus-factor; Crystal structure; Gallic acid; Allosteric site inhibitor.

Neutron macromolecular crystallography at the European Spallation Source

Esko Oksanen

Affiliation

European Spallation Source ESS ERIC; Lund University

Abstract

The European Spallation Source (ESS) is a European project to build and operate the world's most powerful neutron source in Lund, Sweden. The unique long-pulse technology and high-brilliance moderators make the ESS well suited for life science techniques such as macromolecular crystallography. The ESS is scheduled to produce first neutrons in January 2027 and open a user programme in 2028. The ESS instrument suite consists of 15 instruments and a test beam line. The NMX Macromolecular Diffractometer is the only instrument dedicated to life science. NMX is a time-of-flight quasi-Laue diffractometer optimised to make use of the bright long-pulse source.

The 156 m length allows the use of the full pulse with an acceptable wavelength resolution of 2-4% and the chopper system allows the wavelength range to be tuned to maximise signal-to-background. The robotic endstation enables multi-axis goniometry together with variable detector distance, which removes previous limitations on unit cell axes length. The user support in protein deuteration, crystal growth optimisation and data analysis ensures an optimal scientific throughput. NMX is scheduled to be commissioned among the first instruments in 2026.

The Cardiac Ankyrin-Repeated Protein CARP modulates force output in the muscle sarcomere

Nino Tabagari¹, Isabel Martin¹, Belinda Bullard², Jennifer Fleming¹, Olga Mayans¹; ¹Department of Biology, University of Konstanz, Germany; ²Department of Biology, University of York, United Kingdom

Cells respond to stressors by triggering adaptive responses that allow for an increased resistance to damage. Muscles, in particular, are exposed to high mechanical load that can lead to injuries. Consequently, muscles have a protection mechanism to preserve their integrity and contractile performance upon mechanical overload. The damage protection mechanism is provided by the protein CARP from the muscle ankyrin repeat family. Upon up-regulation, CARP targets the sarcomere, where it binds to the elastic titin protein at its N2A locus, cross-linking titin and actin and, thereby, inducing a stiffening of the sarcomere. Abnormally prolonged stiffening of the muscle is pathological and lead to disease. A closely related CARP homologue is MARP, which is constitutively present in muscle but that is not known to induce sarcomere stiffness. We investigate the structural and functional differences of CARP and MARP and their binding of the titin N2A region.

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QUANTUM-CHEMICAL STUDY OF CHONDROITIN SULPHATE AND HEPARIN.

Michal Hricovíni and Miloš Hricovíni

Institute of Chemistry, Slovak Academy of Sciences, 845 38 Bratislava, Slovakia, chemmike@savba.sk

Glycosaminoglycans (GAGs) are structurally diverse, linear, negatively charged polysaccharides that represent key regulatory and structural components of the extracellular matrix and cell surfaces. GAGs have been demonstrated to modulate tissue architecture, hydration, biomechanical properties and essential cellular processes, including adhesion, migration, proliferation, differentiation, and signal transduction [1]. Among these, heparan sulphate (HS) and chondroitin sulphate (CS) are of particular importance due to their distinct structural features and critical roles in both physiological and pathological contexts. HS facilitates the selective binding of growth factors, thereby modulating angiogenesis, developmental signalling, immune responses, and cell–matrix communication [2]. It is evident that CS plays a pivotal role in maintaining structural integrity and elasticity. Furthermore, CS functions as an agent in joint-related disorders, including osteoarthritis [3,4].

The three-dimensional structures of heparan sulphate and chondroitin sulphate tetrasaccharides are revealed through the utilisation of experimental nuclear magnetic resonance (NMR) data in conjunction with density functional theory (DFT) calculations [5]. The molecular structure of the studied tetrasaccharides was determined using the ω B97M-V/def2-TZVPD approach. The solute and the solvent were treated as a single supermolecular system, with tetrasaccharides hydrated with 160 water molecules. The calculation of proton–proton and proton–carbon coupling constants has revealed a strong dependence of one-bond proton–carbon coupling constants ($^1J_{C-H}$) on the sulphation position. Substituent effects were also observed on neighbouring atoms and the geometry of glycosidic linkages resulting in variations in the nJ_{C-H} magnitudes. The findings demonstrate that sulphate positioning exerts a significant influence on electronic distribution, molecular geometry, and nJ_{C-H} coupling magnitudes. This highlights the critical role of hydrogen bonding and ionic interactions in shaping the three-dimensional architecture of sulphated GAGs.

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Structural studies of dynamin-like proteins assisting synthetic cell division

Phuong Nguyen, Arjen Jakobi

Author affiliation: Delft University of Technology, The Netherlands

Abstract:

The work focuses on structural and mechanistic studies of dynamin-like proteins (DLPs) that may be suitable for use as a minimal divisome for synthetic cell division. DLPs are known for their roles in membrane remodeling processes, including membrane fission. The project will initially focus on the bacterial DLP DynA from *Bacillus subtilis*, as well as a novel class of DLPs called MidX possessing mitochondrial/plastid targeting signals as potential divisome candidates. These are interesting candidates in the context of synthetic cell division due to their capability to promote membrane scission in reverse topology. Utilising cryoEM/ET as the primary tool, this project aims to provide structural insights of DLP-mediated membrane remodeling and explore the feasibility of synthetic cell division by DLP-based divisomes. It will establish the molecular structure of reverse topology DLPs from cryoEM single-particle analysis, reconstitute DLPs on model membranes for cryoET analysis of their assemblies, and attempt to map their conformational changes during membrane remodelling, assisted by molecular dynamics simulations.

Elucidating the structure-function landscape of TAOK2 and its role in antiviral immunity

Lisse Thierens^{1,2}, Simon Pichard³, Arnaud Poterszman³, Savvas N. Savvides^{1,2}

¹Department of Biochemistry and Microbiology, Ghent University, ²Unit for Structural Biology, VIB-Gent Center for Inflammation Research, ³Institut de Génétique et de Biologie Moléculaire et Cellulaire IGBMC), Center for Integrated Structural Biology (CBI), Centre National de la Recherche Scientifique (CNRS), Institut National de la Santé et de la Recherche Médicale (INSERM), Université de Strasbourg, Illkirch, France.

TAOK2 belongs to the MAP3K family and is the largest of three enigmatic proteins (TAOK1, TAOK2, TAOK3), combining kinase and protein scaffolding functions to serve a variety of signaling pathways. Recent studies highlighted the involvement of TAOK2 in antiviral immunity, regulation of microtubule dynamics and requirement for nuclear speckle integrity. While the role of TAOK proteins in physiology and disease is still emerging, progress is hampered by a paucity of structure-function insights.

Here, we present our effort to recombinantly produce TAOK2 using different expression systems. Due to challenges producing TAOK2 in bacterial and mammalian systems, we expanded our strategy to insect cell-based production. Access to the INSTRUCT Protein Production Platform in Strasbourg enabled implementation of a baculovirus-insect cell expression pipeline, allowing successful expression and purification of TAOK2.

Ongoing single-particle cryo-EM studies aim to provide structural insights into TAOK2's role in antiviral immunity. These findings will deepen the current understanding on how the N-terminal kinase domain and C-terminal scaffolding domain of TAOK2 mediate interactions and how the domain interplay leads to its role in physiology and disease. Additional biophysical studies will further elucidate the molecular basis of viral sensing; thereby, integrating our understanding of antiviral defense mechanisms and the specific role of TAOK2 in these processes.

Crystal structures of host proteins bound to human papillomavirus peptides

Sujin Jung^{1,2} and Bonsu Ku^{1*}

¹Orphan Disease Therapeutic Target Research Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon 34141, Republic of Korea

²Department of Biochemistry, Chungnam National University, Daejeon 34134, Republic of Korea

Human papillomaviruses (HPVs) are etiological agents of abnormal cellular proliferation, resulting in either malignant or benign lesions, such as cervical cancer and warts. The HPV genomes encode two structural (L1 and L2) and seven functional (E1, E2, E4–E7, and E8) proteins. HPV uses distinct proteins to hijack host regulatory machinery at different stages of infection. One such case involves L2, the minor capsid protein of HPVs, which was reported to recruit polo-like kinase 1 (Plk1) for the delivery of viral DNA to mitotic chromatin during infection. Another case is that the CR2 domain of HPV16 E7 binds to the μ 2 subunit of clathrin-adaptor protein 2 (AP2- μ 2). This interaction modulates AP2-mediated internalization of epidermal growth factor receptor (EGFR). To address these interactions at the molecular level, we characterized direct interactions between viral proteins (HPV L2 and E7) and their host partners (Plk1 and AP2- μ 2). We also determined the crystal structures of host-virus protein complexes containing HPV derived peptides. These structural and biochemical findings provide evidence that HPV utilizes viral motifs to target host regulatory proteins, leading to disruption of cell cycle control and signaling dysregulation.

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[Keywords]: Plk1 ; serine/threonine-protein kinase ; PBD ; polo box domain ; clathrin-adaptor protein 2; AP2; μ 2; human papillomavirus; HPV16; L2; minor capsid protein, E7; CR2; crystal structure

Integrated structural characterization of gold(I)-loaded human ferritin nanocarriers

Lucrezia Cosottini^{a,b} and Paola Turano^{a,b}

^aCenter of Magnetic Resonance (CERM), University of Florence, Via Luigi Sacconi, 6, Sesto Fiorentino (FI), 50019, ^bChemical Department, University of Florence, Via della Lastruccia 3-13, Sesto Fiorentino (FI), 50019, (IT)

Human heavy chain ferritin (HuHf) is a promising nanocarrier candidate for the targeted delivery of metal-based anticancer agents. In this study, wild-type HuHf and its engineered variants were successfully employed to develop HuHf-based bioconjugates by loading three gold(I) compounds: the FDA-approved drugs auranofin (AF) and aurothiomalate (AuTM), and the experimental drug candidate gold carbene Au(NHC) [1–3]. Here, we report an integrative strategy for the structural characterization of these ferritin-gold(I) systems. By combining ESI-MS data from wild-type and site-specific mutants with ¹⁹F NMR spectroscopy, MD modeling, and cryo-EM structural determination [3–5], we precisely identified the binding sites and coordination modes of these gold species. Our findings revealed unique features in the coordination chemistry of gold(I) to the protein cage.

Biological data of TfR1 receptor-mediated internalization of HuHf-gold(I) bioconjugates in A2780 ovarian cancer cells demonstrate the selective entrance and cytotoxic activity of selected compounds. A detailed understanding of these bioconjugates, combined with the intrinsic ability of HuHf to selectively target cancer cells, provides valuable insights into their biological activity and therapeutic potential. Notably, the strong affinity of gold(I) compounds for HuHf confers enhanced stability against competing biomolecules in the cellular environment or blood stream.

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**Visualizing Mitochondrial Cristae Formation during Life Cycle
Differentiation of *Trypanosoma brucei* by Cryo-Electron
Tomography**

Oskar Lipinski¹, Brian Panicucci¹, Jiří Nováček², Alexey Amunts³, Alena Zíková¹

¹Institute of Parasitology, Biology Centre, Czech Academy of Sciences, České Budějovice, Czech Republic

²CEITEC Masaryk University, Kamenice 753/5, 625 00, Brno, Czech Republic

³University of Münster, Münster, Germany

Trypanosoma brucei is a unicellular parasitic protozoan responsible for African sleeping sickness in humans. Its complex life cycle alternates between the mammalian bloodstream form (BSF) and the procyclic form (PCF) in the tsetse fly vector. During this transition, the parasite undergoes a dramatic metabolic switch: BSFs rely predominantly on glycolysis for ATP production, whereas PCFs activate oxidative phosphorylation (OXPHOS), necessitating extensive remodeling of the mitochondrial inner membrane and cristae formation.

In vitro differentiation from BSF to PCF enables detailed examination of mitochondrial biogenesis and cristae emergence. While cristae in PCFs have been visualized previously using conventional electron microscopy techniques such as serial block-face imaging or heavy metal staining with uranyl acetate, these approaches lack preservation of the native ultrastructure. Here, we present preliminary cryo-electron tomography (cryoET) data of FIB-milled PCF *T. brucei* cells, revealing well-developed, disc-shaped cristae in a near-native state. Segmentation of tomograms highlights the organization of the inner mitochondrial membrane, providing new insights into the structural basis of OXPHOS activation.

Beyond visualizing cristae architecture, we aim to employ subtomogram averaging to resolve the spatial distribution of individual mitochondrial complexes (complexes I–IV and ATP synthase) and their assembly into supercomplexes. Preliminary results indicate that subtomogram averaging has the potential to resolve the precise arrangement of these complexes within the disc-shaped cristae, providing a high-resolution view of the bioenergetic transformation from BSF to PCF.

Overall, this study establishes a platform for connecting mitochondrial ultrastructure with functional metabolic adaptation in *T. brucei*, providing a framework for understanding organelle remodeling during protozoan differentiation.

**Fragment-Screen: Fragment-based drug discovery through
structural biology, medicinal chemistry, and artificial intelligence**

Natalie Haley^{1*}, Claudia Alen Amaro^{1*}, John Dolan^{1*}, Eirini Xemantilotou^{1*}, Sarah Townend^{1*}, **Pauline Audergon^{1*}**, Harald Schwalbe¹

¹Instruct-ERIC, Oxford House, Parkway Court, John Smith Drive, Oxford, OX4 2JY, UK.

*pauline@instruct-eric.org

The Fragment-Screen project (<http://fragmentscreen.org/>) is developing innovative instrumentation, workflows and experimental and computational methodologies for fragment-based drug discovery (FBDD) enabling access to early phase structure-based drug discovery for all biological targets and for scientists both in industry and academia. The established workflows use structural biology insights and associated data to feed artificial intelligence (AI) methodology to guide medicinal chemistry in drug development. Fragment-Screen brings together scientists from four ESFRI Landmark research infrastructures (RIs): ESRF (the European synchrotron) and the distributed RIs EU-OPENSOURCE ERIC (medicinal chemistry), ELIXIR (data resources for life science) under the coordination of Instruct-ERIC (integrated structural biology) and seven industry partners in scientific instrumentation and computational and AI sectors including SMEs to remove crucial bottlenecks in early phase drug discovery.

**Off-target structural insights: ArnA and AcrB in bacterial
membrane-protein cryo-EM analysis**

Mehmet Caliseki 1,2, Ufuk Borucu 2, Sathish K N Yadav 2, Christiane Schaffitzel 2, Burak Veli Kabasakal 1,2

1 Turkish Accelerator and Radiation Laboratory, 06830 Ankara, Türkiye.

2 School of Biochemistry, University of Bristol, 1 Tankard's Close, Bristol BS8 1TD, United Kingdom.

Abstract

Membrane-protein quality control in *Escherichia coli* involves coordinated actions of the AAA+ protease FtsH, the insertase YidC and the regulatory complex HflKC. These systems maintain proteostasis by facilitating membrane-protein insertion, folding and degradation. To gain structural insights into a putative complex formed by FtsH and YidC, we performed single-particle cryogenic electron microscopy on detergent-solubilized membrane samples, from which FtsH and YidC were purified using Ni-NTA affinity and size-exclusion chromatography. Although SDS-PAGE analysis indicated high purity of these proteins, cryo-EM data sets unexpectedly yielded high-resolution structures of ArnA and AcrB at 4.0 and 2.9 Å resolution, respectively. ArnA is a bifunctional enzyme involved in lipid A modification and polymyxin resistance, while AcrB is a multidrug efflux transporter of the AcrAB-TolC system. ArnA and AcrB, known Ni-NTA purification contaminants, were also consistently detected by mass spectrometry in Strep-Tactin affinity-purified samples, validating their presence independently of affinity-tag selection. ArnA, which is typically cytoplasmic, was consistently found in membrane-isolated samples, indicating an association with membrane components. Only 2D class averages corresponding to the cytoplasmic AAA+ domain of FtsH were observed; neither side views of full-length FtsH nor densities corresponding to an intact FtsH-YidC complex could be identified, due to the conformational flexibility of the FtsH complex and its transient interaction with YidC, which limited particle alignment and stable classification in cryo-EM data sets. Two-dimensional class averages revealed additional particles resembling GroEL and cytochrome bo3 oxidase. These results underscore the utility of cryo-EM in uncovering off-target yet structurally well defined complexes, which may reflect physiologically relevant interactions or purification biases during membrane-protein overexpression.

Cryo-EM Infrastructure Access at NOVA FCT: A Case Study on Fibrillar Polymorphism

Ana Luísa Moreira de Carvalho¹², Fernando de Sá Ribeiro¹²³, Luís Maurício Trambaioli da Rocha e Lima³, Himanshu Sharma¹²

¹ UCIBIO – Applied Molecular Biosciences Unit, NOVA School of Science and Technology, Universidade Nova de Lisboa, Caparica, Portugal

² Associate Laboratory i4HB – Institute for Health and Bioeconomy, NOVA School of Science and Technology, Universidade Nova de Lisboa, Caparica, Portugal

³ Laboratory of Pharmaceutical Biotechnology (pbitech), Federal University of Rio de Janeiro, Brazil

Amyloid fibrils are protein aggregates associated with numerous human diseases and can adopt structurally distinct polymorphs (“strains” in vivo) from the same polypeptide sequence. This structural polymorphism is a key determinant of amyloid pathogenicity. Several clinically relevant insulin analogues can form amyloid fibrils, yet their high-resolution structures remain poorly characterized. Addressing these questions requires advanced cryo-electron microscopy (cryo-EM) infrastructure and specialized workflows, such as those provided by the NOVA-FCT’s CryoEM Hub.

Insulin variants were subjected to in vitro fibrillation protocols and characterized using complementary electron microscopy approaches. Fibril morphology was first assessed by negative-stain TEM, followed by cryoEM and helical reconstruction. The CryoEM Hub supports sample preparation, data acquisition, and image processing while facilitating access to national and European cryoEM facilities.

Amyloid fibrils were obtained for all tested variants. TEM revealed distinct fibrillar morphologies, indicating polymorphic assemblies. CryoEM analysis of one variant produced a 3D density map with a global resolution of 5.87 Å. Directional resolution reached 3.89 Å along the fibril helical axis, revealing protofilament organization and confirming the high structural order of the sample. These results demonstrate structurally distinct amyloid polymorphs among insulin analogues and highlight the capability of the CryoEM Hub at NOVA FCT to support structural studies of complex biomolecular assemblies.

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Troubleshooting cryo-EM sample preparation using PUXANO Grids

João Ramos¹, **Moisés Maestro-López¹**

1 PUXANO, Ottergemsesteenweg-Zuid 713; 9000 Gent, Belgium

PUXANO is a platform-based Contract Research Organization (CRO) specializing in cryo-electron microscopy (cryo-EM) and protein design. Our design-to-structure workflow integrates cutting-edge tools to overcome major challenges in structural biology, including:

- A tailored cryo-EM grid portfolio that resolves critical sample preparation issues—such as preferred particle orientation, complex disassembly, and low sample yields—using solutions like tilted grids and graphene-supported grids.
- Proprietary protein design algorithms for generating soluble analogues of membrane proteins and multi-epitope scaffolds.

Through real-world case studies, we demonstrate how our integrated technology platform drives efficiency and innovation in protein research.

Molecular determinants for recognition of serotonylated chromatin

L. Pulido-Cortés¹, H. Gieling², V. Thijssen³, M. Liu³, R. Yoshisada³, L. Romão Soares³, S. Nizamuddin¹, F. Friedrich⁴, H. Greschik¹, L. Peng¹, R. Vargas Honorato², M. Jung⁴, A.M.J.J. Bonvin², M.L. Binossek⁵, R.Schüle¹, S. Jongkees³, H. van Ingen^{2*}† and H.Th.M. Timmers^{1*}†.

¹German Cancer Consortium (DKTK), Medical Center-University of Freiburg, Germany.

²NMR Spectroscopy, Bijvoet Center for Biomolecular Research, Utrecht University, the Netherlands.

³Department of Chemistry and Pharmaceutical Sciences, Vrije Universiteit Amsterdam, the Netherlands.

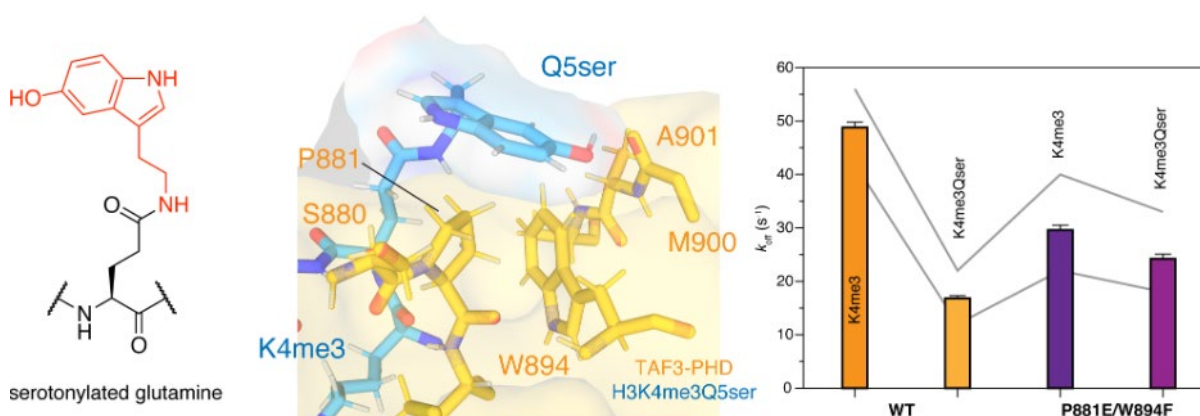
⁴Institute of Pharmaceutical Sciences, University of Freiburg, Germany.

⁵Institute of Molecular Medicine and Cell Research, Medical Center-University of Freiburg, Germany.

Post-translational modifications of histone tails constitute a key epigenetic mechanism controlling chromatin environment and gene transcription. Serotonylation of histone H3Q5 (H3Q5ser) is a recently discovered mark associated with active transcription of RNA polymerase II (pol II)-transcribed genes. The direct link between H3Q5ser and the pol II transcription machinery relies on the TFIID subunit TAF3. The presence of H3Q5ser enhances TAF3 binding to H3K4me3, but the molecular determinants underlying this interaction remained unclear.

Here, we resolve the binding mode of TAF3-PHD to H3K4me3Q5ser identifying a novel binding surface for H3Q5ser using solution NMR spectroscopy. This reveals how H3Q5ser recognizes a conserved surface of the TAF3-PHD via CH- π interactions in an edge-face conformation involving a proline residue stabilised by a tryptophan. This combination of proline and tryptophan is unique to the PHD finger of TAF3 and conserved amongst TAF3 orthologues. Combination of NMR and ITC data show that the additional interactions afforded by the serotonin group boost affinity ca. 3-fold by prolonging the life time of the complex.

Our findings establish a framework for the molecular recognition of serotonylated chromatin, laying the foundation for developing epigenetic inhibitors targeting serotonylation-dependent transcriptional regulation in neuronal development. In addition, this work highlights the ongoing need for experimental structural biology in the age of protein structure prediction to solve fundamental molecular mechanisms in biology.



Instruct Biennial Structural Biology Conference
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Figure. (left) structure of serotonylated glutamine; (middle) solution NMR based structure of a serotonylated histone tail peptide (H3K4me3Q5ser) bound to the TAF3-PHD finger; (right) complex dissociation rates based on NMR signal line shape analysis for wild-type (WT) and mutant TAF3-PHD bound to histone peptides with or without serotonylation.

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Deciphering the Structure and Mechanism of SaGpx: A Non-Canonical Glutathione Peroxidase from *Staphylococcus aureus***Sushobhan Maji¹**, Sudipta Bhattacharyya^{1*}¹Indian Institute of Technology Jodhpur, India

*Correspondence to: Sudipta Bhattacharyya, Department of Bioscience & Bioengineering, Indian Institute of Technology, Jodhpur 342037, Rajasthan, India. Email: sudipta@iitj.ac.in

Staphylococcus aureus encounters massive oxidative stress during infection. To counter this, the bacterium developed robust antioxidative defense mechanism. Glutathione peroxidases (Gpx) are well characterized antioxidative enzymes in eukaryotes; however, their bacterial counterparts remain poorly explored. *S. aureus* possesses two putative Gpx genes but lacks GSH biosynthetic machinery and glutathione reductase required for canonical Gpx function, suggesting alternate electron donor system(s) may be involved. This study aimed to elucidate structure-based biochemical characterization of one of the *S. aureus* glutathione peroxidases homologs (SaGpx, Uniprot Id: Q2FYZ0) and identify its plausible electron donor system. Herein, we cloned, purified and determined the high-resolution crystal structure of SaGpx (1.5 Å resolution) using X-ray diffraction crystallography. In vitro biochemical characterization of the highly conserved active site amino acid point mutants, as well as their structural disposition suggests their precise roles in the enzyme's catalysis. The crystal structure of SaGpx revealed that the enzyme adopts a canonical glutathione peroxidase fold with conserved catalytic tetrad composed of C36, Q70, W124 and N125. Also, SaGpx shows similarity with mammalian Gpx4, which was previously shown to exert phospholipid hydroperoxide peroxidase activity. Furthermore, biochemical assays suggest that SaGpx utilizes Staphylococcal thioredoxin1 as its cognate electron donor. The catalytic mechanism follows an atypical 2-cysteine peroxiredoxin-like pathway involving the formation of a sulfenic acid intermediate, followed by an intramolecular disulfide bond subsequently resolved by thioredoxin. This work provides the first structure-based biochemical characterization of a bacterial glutathione peroxidase homolog, establishing the novel structural insights of SaGpx as a noncanonical thioredoxin-dependent glutathione peroxidase.

**Structural and biophysical characterization of Themis2 complexes
in immune cell signalling**

Alba Sanz-Sanjuan^{1,2}, Jan Felix^{1,2,3}, Stephanie Olesco^{1,2}, Danielle Clancy^{1,2}, Savvas N. Savvides^{1,2}

1 Unit for Structural Biology, Department of Biochemistry and Microbiology, Ghent University, Ghent, Belgium.

2 Unit for Structural Biology, VIB-UGent Center for Inflammation Research, Ghent, Belgium.

3 BioImaging Core Ghent, VIB-UGent Center for Inflammation Research, Ghent, Belgium.

Themis2 is a cytosolic adaptor protein expressed in multiple immune cell types, such as NK cells, B cells, macrophages and epithelial cancer cells, yet its molecular function remains poorly understood. Themis2 regulates signalling through interactions with Grb2 and the phosphatase SHP-1, mediated by its tandem CABIT domains and C-terminal proline-rich sequence. We used an integrative structural biology approach to define how Themis2 engages these signalling partners. To enable structural analysis, an engineered Themis2-specific nanobody scaffold was used as a fiducial marker to facilitate data collection and processing. Cryo-EM analysis of the Themis2-ProMacrobody complex reveals extensive conformational heterogeneity, with interdomain flexibility between the CABIT domains. Binding to Grb2 and SHP-1 may stabilize Themis2, however biophysical analysis shows that Themis2 binds Grb2 with low affinity, in stark contrast to Themis, which forms a highly stable complex with Grb2 in T cells. To overcome this, we engineered a Themis2-Grb2 fusion protein to facilitate assembly of larger complexes including SHP-1. However, preliminary biophysical studies show difference between how both Themis and Themis2 interact with SHP-1. Together, these findings provide the first structural insights into Themis2 and establish a foundation to investigate Themis2-protein complexes in immune signalling.

An open and integrated ecosystem for live Cryo-EM/ET processing in Facilities

Mikel Iceta Tena

Affiliation

CNB-CSIC Biocomputing Unit + I2PC // SEMC-NYSBC

Abstract

Recent advances in cryo-electron microscopy have dramatically increased data throughput, creating challenges for facilities in data processing, project management, and stewardship. We present a system that streamlines the cryo-EM facility workflow, from acquisition to high-resolution three-dimensional structure delivery.

Customized streaming workflows in Scipion enable on-the-fly processing during microscope acquisition. Images are processed while data collection is ongoing, providing rapid feedback and often enabling near-final 3D reconstructions before sessions finish. The same infrastructure can also be deployed for cryo-electron tomography, supporting automated preprocessing, tilt-series handling, and early tomogram reconstruction during acquisition.

Processing pipelines are launched from EMHub, a laboratory information management system tailored for cryo-EM facilities. Operators can launch workflows directly from the acquisition context while EMHub records bookings, project history, facility usage, and administrative information such as reporting or billing. Metadata can be continuously communicated to the Instruct ARIA infrastructure using FandanGO, enabling transparent experiment tracking across the full lifecycle and supporting FAIR data principles that promote traceability, reproducibility, and open science.

Finally, datasets are delivered using iRODS as underlying data management technology, enabling scalable data-lake storage and efficient, ticket-based large data transfer. Together, this open ecosystem provides cryo-EM facilities with an adaptable all-in-one solution combining real-time processing, facility management, and FAIR data delivery, allowing users to leave with processed datasets and frequently high-resolution structures already available. This approach increases productivity for operators and users while adding value to facility services by providing automated, traceable, and interoperable structural biology workflow

High-resolution imaging of a trypanosome surface coat protein and its interaction with antibodies

Dr. Tanja Kuhm^{1,2}, Caterina Canetta¹, Dr. Simone Mattei², Dr. Erec Stebbins¹

1: German Cancer Research Center (DKFZ) Heidelberg, Germany

2: European Molecular Biology Laboratory (EMBL) Heidelberg, Germany

The African trypanosome *T. brucei*, is a unicellular pathogen responsible for sleeping sickness in humans and nagana in animals. It replicates extracellularly, where it faces constant exposure to the host's immune system. The pathogen evades immunity by serially varying their dense coat of Variant Surface Glycoproteins (VSGs), while immunodominant epitopes on the surface of a given VSG can dictate antibody responses, thereby enhancing antigenic variation by reducing cross-reactivity to different variants. VSG2 contains a surface-exposed calcium-binding pocket previously shown to be an immunodominant epitope, but the mechanistic basis of its immunodominance is unknown. Using single particle cryo-electron microscopy, we determined two structures of VSG2 in complex with two different monoclonal antibodies at an overall resolution around 2.5 Å. We found that both antibodies directly coordinate a calcium ion on VSG2. Chelation of the ion abolishes recognition, while a single loss-of-contact mutation of a central calcium-specific contact is able to eliminate binding. These data establish the ion itself as an essential component of the epitope, focusing diverse antibody responses onto the single, minimal structural feature of a single atom. Our results reveal a rare case of ion-dependent immunodominance on a pathogen surface, establishing that even simple cofactors can define epitopes and constrain immune recognition despite extensive antigenic variation in the protein sequence.

**Structural Characterization of Trypanosoma cruzi P5CS Filaments
by Cryo-Electron Microscopy: Insights in the Proline Metabolism
and Drug Discovery**

Maria Eduarda Souza Dias Lino^{1,2}, Leandro Farias Estrozi², Ariel Mariano Silber³, Otavio Henrique Thiemann¹

1- University of Sao Paulo, Institute of Physics

2 – Universite Grenoble Alpes, Institut de Biologie Structurale

3 - University of Sao Paulo, Institute of Biomedical Sciences

Proline metabolism is essential for trypanosomatids to adapt to diverse environments during their life cycle. In *Trypanosoma cruzi*, the enzyme Δ^1 -pyrroline-5-carboxylate synthase (TcP5CS) is crucial for proline biosynthesis and comprises two functional domains: glutamate kinase (TcGK) and glutamyl phosphate reductase (TcGPR). TcP5CS homologues have been shown to form filamentous assemblies, potentially linked to enzymatic regulation and function. Recent findings from our laboratory revealed that TcP5CS also forms filaments, displaying a structural organization distinct from its homologues representing a novel finding. This project aims to elucidate the three-dimensional structure of TcP5CS filaments and its domains using single-particle cryo-electron microscopy (cryo-EM). Cryo-EM grids will be analyzed under different biochemical conditions. The TcGK and TcGPR domains are also being crystallized for structural determination by X-ray crystallography, providing complementary atomic-resolution data. Biophysical techniques such as circular dichroism (CD), multi-angle light scattering (SEC-MALS), and small-angle X-ray scattering (SAXS) are being employed to better understand filament formation and enzyme architecture. It is expected to contribute to the structural and functional characterization of TcP5CS and supporting future drug discovery strategies targeting proline metabolism in *T. cruzi*, a key pathway for parasite survival.

**Structural and Biochemical Characterization of ALK: Bridging
ALKAL2 Binding and Kinase Activation**

Warre Dhondt^{1,2,3}, Steven de Munck^{1,2}, Jan Felix^{1,2,4}, Anice Remmery^{1,2}, Ahmad Reza Mehdipour³, Savvas Savvides^{1,2}

1. Unit for Structural Biology, VIB-UGent Center for Inflammation Research, Ghent, Belgium.
2. Unit for Structural Biology, Department of Biochemistry and Microbiology, Ghent University, Ghent, Belgium.
3. Center for Molecular Modeling, Ghent University, Ghent, Belgium.
4. BioImaging Core Ghent, VIB-UGent Center for Inflammation Research, Ghent, Belgium.

Anaplastic lymphoma kinase (ALK) is a receptor tyrosine kinase with critical roles in metabolism and neural development, but whose aberrant activation contributes to oncogenesis in several cancers. Current models of ALK activation by its cytokine ligand ALKAL2 are limited to the extracellular cytokine-binding domain. This leaves the contributions of other receptor domains to ALK's signaling potential poorly understood. To address this, we are studying a truncated ALK construct that spans from the extracellular cytokine-binding domain to the intracellular kinase domain. We demonstrate that this construct can be purified from HEK293S cell membranes in detergent while retaining its ligand-induced kinase activity. Biophysical characterisation of ALK and its interactions with ALKAL2 demonstrates an increased dimerisation potential of this transmembrane construct relative to the extracellular domain alone. We further present structural characterisations of this complex via negative-stain electron microscopy (NS-EM) and report preliminary cryo-EM grid screening results. This work advances our understanding of the mechanism of ALKAL2-induced ALK activation, and it represents an important step towards a cryo-EM structure of a full-length ALK-ALKAL2 complex that links extracellular ligand binding to intracellular kinase domain activation.

Novel ORFs in ssRNA bacteriophage metagenomes

Kaspars Tars

Affiliation

Latvian Biomedical Research and Study Center

Abstract

Phages belonging to family Leviviricetes typically have a short 3.5-4 kb ssRNA genome, enclosed in a T=3 capsid, built from 89 coat protein (CP) dimers and one or two copies of maturation or “A” protein (AP), responsible for attachment to cellular receptors – various kinds of bacterial pili. Genomes of all cultivated Leviviricetes phages have three core ORFs, encoding AP, CP and replicase subunit (RP), always in that order. Additionally, most ssRNA phage genomes have a separate ORF for lysis protein (LP), and some have also elongated variants of CP, called A1. Apart from the mentioned 5 proteins (AP, CP, RP, LP and A1) no other functional ORFs have been conclusively proven to exist in Leviviricetes genomes. However, in some metagenomic studies putative additional ORFs have been identified, which up to now have remained uncharacterized.

We looked for non-conserved open reading frames (ORFs) in Leviviricetes sequences from the IMG/VR virus metagenome database and used sequence- and structurebased clustering to organize them into similarity groups. ORFs encoding previously uncharacterized proteins were found throughout the ssRNA phage genomes although their prevalence in various phage lineages and at particular genome positions was markedly uneven. Most of the identified ORFs encode all- α proteins, a portion of which contain transmembrane segments that resemble a group of known ssRNA phage lysis proteins, while many others represent previously uncharacterized families of globular or semi-globular α -helical proteins. We additionally uncovered a major class of globular α/β proteins and experimentally determined the structure of a representative protein of this group which revealed that it exists as a dimer in solution. These results pave the way for further functional studies of novel ssRNA phage proteins for a better understanding of this diverse virus group.

Deposit your data in Molecular Biophysics Database

Jan Dohnálek¹, Terezie Prchalová¹, Jitka Plucarová¹, Tim Kadlec¹, Jan Stránský¹, Andrea S. Veiga², Juan Sabín², Miroslav Šimek³, Emil Dandanell Agerschou¹

¹ *Institute of Biotechnology of the Czech Academy of Sciences, v.v.i., Průmyslová 595, 252 50 Vestec, Czech Republic*

² *AFFINImeter, Software 4 Science Developments, Edificio Emprendia, Campus Vida, 15782 Santiago de Compostela, Spain*

³ *Czech Education and Scientific NETwork (CESNET), Generála Píky 430/26, 160 00 Prague 6, Czech Republic*

dohnalek@ibt.cas.cz

While FAIR deposition of data for structural biology techniques is covered to a large extent, molecular biophysics techniques lack systematic storage of original data. At the same several new biophysical techniques appear every year.

We contributed to systematic data management in molecular biophysics by development of a new database. The Molecular Biophysics Database (MBDB) stores raw data files together with metadata descriptions. The design of the database makes measurement results Findable, Accessible, Interoperable and Reusable.

The metadata for individual data sets consist of a general part and a method-specific part. The general part defines, in a unique way, descriptors for key parameters common for different experimental techniques, e.g. source organism, identity of individual molecules, including chemicals, with reference to external databases and unique identifiers for the most relevant types. The method-specific part is devoted to the metadata special for a particular technique (such as MST, BLI, etc.). This approach enables searching across different techniques and allows direct comparisons of results, e.g. interaction parameters, for the same molecular system measured by different techniques.

MBDB was launched in January 2025 (<https://mbdb-data.org/>) and is ready for deposition of raw measurement files with rich metadata describing experimental details for the techniques MST, BLI, SPR and ITC [1]. We invite researchers to deposit their raw data and make them publicly available, with each record receiving a DOI identifier. A demo of MBDB deposition is possible upon request. We invite experts in the named biophysical techniques to become expert reviewers.

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Analyzing the catalytic promiscuity of a non-specific endoxylanase from *Acetivibrio clariflavus*

P. Karampa¹, C. Pentari², E. Topakas² and **M. Dimarogona^{1*}**

¹Department of Chemical Engineering, University of Patras, Patras, Greece

²School of Chemical Engineering, National Technical University of Athens, Athens, Greece

*Corresponding author email: mdimarog@chemeng.upatras.gr

Xylan is the major constituent of hemicellulose, and the second most abundant polysaccharide of the plant cell wall after cellulose. Efficient utilization of xylan is essential for producing high value-added products, in the frame of the lignocellulosic biorefinery concept. Some members of the glycoside hydrolase family 30 (GH30) of CAZy database (<http://www.cazy.org/>) can degrade recalcitrant xylans, such as glucuronoxylan, by cleaving the β -1,4-glycosidic bonds between xylopyranose residues (1,2). This study focuses on a bacterial GH30 from *Acetivibrio clariflavus* (AcXyn30B; UniProt:G8M2Z1), the first biochemically characterized member of subfamily GH30_12. It is a non-specific xylanase acting on glucuronoxylan, arabinoxylan, and aryl glycosides of linear oligosaccharides (3). AcXyn30B is a modular enzyme, composed of a catalytic GH30_12 domain, a carbohydrate binding module 6 (CBM6), and a dockerin domain, sequentially placed from the N- to the C-terminal of the protein chain. Here, we present the crystal structure of the catalytic domain (AcXyn30B_CD) in apo and product-bound state, the former being the first crystal structure of a GH30_12 enzyme, determined at 1.14 Å resolution (PDB ID: 9QY5). Biochemical analysis showed that the main hydrolysis products of AcXyn30B_CD applied on xylopentaose (X5) are xylotriose (X3) and xylobiose (X2), while xylotetraose (X4) is slowly hydrolyzed to X3 and X1, suggesting preferable X5 binding on -3 to +2 subsites. This was further corroborated by the crystal structure of AcXyn30B_CD in complex with X3 at a resolution of 1.55 Å (PDB ID: 9SLE; Fig. 1). X3 was found bound at the -4, -3 and -2 subsites, while electron density probably originating from compounds of the crystallization solution was also observed at subsites -1 and +2. Analysis of the refined structures will provide insight into the catalytic mechanism of AcXyn30B and the structural determinants underlying its promiscuity, which is an asset for biocatalysts of industrial interest.

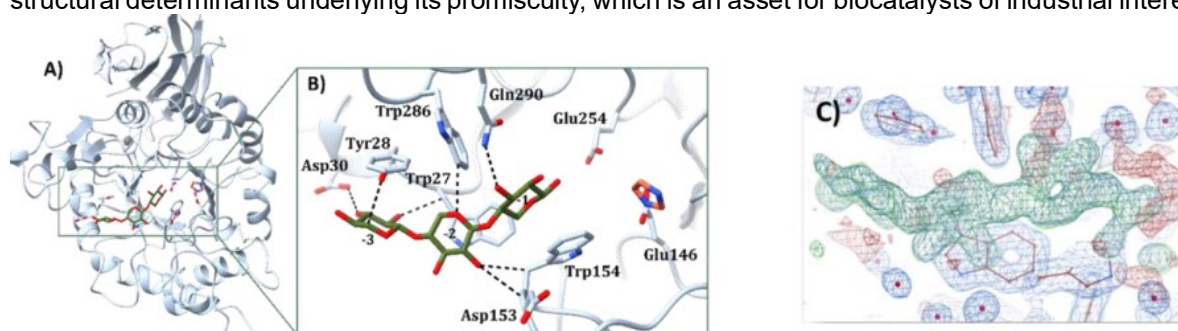


Figure 1. A. Crystal structure of AcXyn30B_CD in complex with X3. B) Close-up view of the substrate binding region C) Electron density map focused on the X3 binding site before incorporating it in the final model, $2F_o - F_c$ map in blue color (1σ) and $F_o - F_c$ differential map in green color (3σ).

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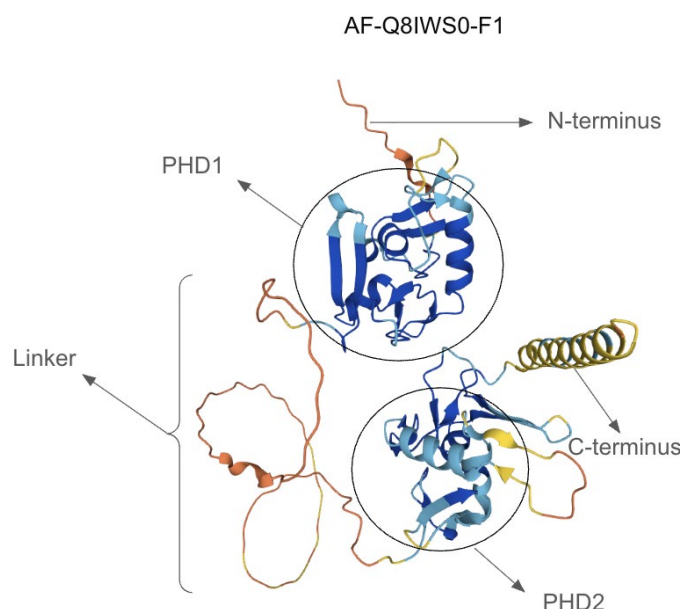
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Targeting undruggable intrinsically disordered regions in childhood malignant neoplasms

Author: Jacopo Miotto

Intrinsically disordered proteins and regions acquire this characteristic by exhibiting a dynamic behavior, a generally more extended structure, and a lack of a fixed three-dimensional structure. Most IDPs are deficient in having a stable drug-binding pocket. This makes them undruggable, therefore representing a challenge to researchers. Many of these proteins are oncosuppressors or oncogenes. However, regardless of their importance, they are poorly characterized, and their direct role in cancer is underestimated. By joining Pharos protein/cancer associations with MobiDB, it was found that for all major cancers, 25% of associated proteins are disordered. This allowed us to focus on IDPs and IDRs in childhood neoplasms. This type of cancer is enriched in these proteins with only a few protein associations. We aim to characterize and study the interactome, allosteric effects, and conformational changes of these proteins after being expressed and purified in bacteria and eukaryotic cells. This will be accomplished through cloning, protein purification, and eukaryotic cell transfection techniques, which will grant us the acquisition of the protein of interest that will be analyzed. As a first approach, we replicated preliminary experiments to clone, express, and characterize the whole protein of interest, PHF6 in our case, and its domains in *Escherichia Coli* BL21(DE3) and eukaryotic cells HEK293T. NMR, X-ray crystallography, and MS will be used to study the 3D structure, interactome, conformational changes, and allosteric effects of our protein. We hope that our project will contribute to filling the knowledge gaps regarding IDPs and IDRs characterization, and their relationships with cancer.



Functional and in silico Investigation of IRF4, cMyc, and Blimp1 Interactions in Multiple Myeloma**Christos Alamaniotis**

Affiliation

Department of Life Sciences, University of Sussex

Abstract

Interferon Regulatory Factor 4 (IRF4) is a master transcription factor essential for germinal centre B cell development and plasma cell differentiation. In Multiple Myeloma (MM), IRF4 overexpression creates a state of oncogene addiction critical for malignant cell survival. With over 180,000 annual global cases and a 62% five-year survival rate, MM remains an incurable malignancy defined by drug resistance and clonal heterogeneity, necessitating novel therapeutics targeting its molecular drivers. IRF4 orchestrates malignancy via aberrant activation of Interferon-Stimulated Response Element (ISRE) sequences (GAAA), yet the structural requirements for DNA binding and resulting systems-level dynamics remain poorly defined. In this study, we employed Fluorescence Polarization (FP) to quantify the binding affinity of the IRF4 DNA-binding domain across a set of endogenous DNA substrates representing validated genomic binding sites where IRF4 regulates key downstream transcriptional targets.

These biochemical parameters were integrated into a stochastic in silico model of the IRF4-cMyc-Blimp1 regulatory axis to predict the impact of targeted inhibition on the MM interactome. FP analysis identified a preferential binding architecture consisting of two tandem motifs separated by a 2-bp (CT) spacer, with highest affinity for GAAA-NN-GAGA configurations suggesting a specific sequence flexibility in its transcriptional recruitment. Our simulations revealed that while established MM states are resilient to single-node perturbations, cMyc inhibition is most efficacious during the pre-malignant transition, significantly delaying the shift to a myeloma-like state.

These findings suggest that cMyc serves as a critical gatekeeper during early oncogenesis, offering a potential window for early-intervention strategies. Future work will focus on validating these simulations in MM cell lines and in vitro assays to refine the use of inhibitors in plasma cell malignancies.

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

Adriana Erica Miele, Patrick England

Association of Resources for Biophysical Research in Europe – ARBRE, 6 avenue Gaston Berger,
69622 Villeurbanne, France. info@arbre-biophysics.eu

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

The aim of ARBRE, a full member of EBSA (European Biophysical Societies Association) is to promote the research field of molecular-scale biophysics and to bring together laboratories, core facilities and platforms, primarily in Europe, which study biological macromolecules and assemblies as a whole, at an intermediate level between atomic-resolution structural descriptions and cellular-level observations. ARBRE aims at building an optimal environment for the development of innovative integrative biophysical approaches, at the level of data acquisition, analysis and modelling.

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

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

We shall present our strategic plan and how ARBRE envisions its role among European research infrastructures and scientific societies.

For more info, please visit our website <https://www.arbre-biophysics.eu>

Linking the HTLV-1 pre-integration complex to the chromatin

Christine Hilcenko

Affiliation:

Imperial College London, Department of Infectious Disease, South Kensington Campus, Imperial College

Abstract

Human T-cell lymphotropic virus type 1 (HTLV-1) is a deltaretrovirus infecting an estimated 10-20 million individuals worldwide. Despite the clinical burden and the mechanistic parallels with HIV, no effective targeted therapeutics exist for HTLV-1 infection. A defining step in the HTLV-1 lifecycle is integration of a DNA copy of the viral genome into host chromatin, mediated by viral integrase (IN). Integration is not random; chromatin context of proviral insertion influences viral gene expression, clonal expansion, and oncogenic transformation. Our laboratory has uncovered a critical dependency of HTLV-1 integrase on the host phosphatase PP2A-B56. Using cryoEM, we solved the structure of the functional deltaretroviral intasome bound to the B56γ regulatory subunit of protein phosphatase 2A (PP2A), revealing a tetrameric IN:viral DNA synaptic complex engaging two PP2A-B56 molecules via a conserved short linear motif (SLiM). Mutational disruption of this interaction impairs viral infection. We have identified an interaction between PP2A-B56 and the cohesin complex component STAG2, a substrate of PP2A-B56. Preliminary data indicate that disruption of the integrase SLiM alters centromeric integration targeting, suggesting that PP2A-B56 and associated chromatin regulators contribute to integration site selection. These findings link viral integration, host phosphatase recruitment, and chromosomal architecture. Our goal is to structurally and functionally characterize the interaction and mechanism by which PP2A-B56 and its binding partners and substrates contribute to HTLV-1 infection and integration site selection. We are working to resolve higher-order complexes linking IN, PP2A-B56 and chromatin factors using an interdisciplinary approach that combines biochemistry, structural studies (cryoEM and NMR spectroscopy), flow-induced dispersion analysis and mass photometry. Here, we present the advantages of those complementary approaches with our preliminary

**Mechanistic investigation of CES1, nCEH1, ABCA1 and ABCG1
using Molecular Dynamics simulations****Georgios Kolypetris**Affiliation:
University of Helsinki**Abstract text**

Atherosclerosis is a leading cause of mortality worldwide and is closely linked to deregulation of cholesterol metabolism in macrophages. During disease progression, macrophages accumulate excess cholesterol and transform into foam cells due to an imbalance between cholesteryl ester hydrolysis and cholesterol efflux. Central components of this process include the cholesteryl ester hydrolases CES1 and nCEH1, as well as the cholesterol efflux transporters ABCA1 and ABCG1. In this project, we use molecular simulations and structural modeling to investigate the mechanisms governing cholesteryl ester hydrolysis and cholesterol transport. Our goal is to characterize catalytic and conformational dynamics in these proteins and identify mutations predicted to enhance cholesterol processing and efflux efficiency. We have established molecular dynamics simulation workflows for CES1–substrate complexes, enabling atomistic analysis of catalytic interactions. Further progress in mechanistic modeling will benefit from improved structural information for several key proteins, including nCEH1 and specific functional states of ABCA1 and ABCG1.

High-throughput Macromolecular Crystallography at Beamline P11

Spyros D. Chatziefthymiou, Alexander Grebentsov, Andrey Gruzinov, Olga Merkulova, Guillaume Pompidor, **Johanna Hakanpää**

Deutsches Elektronen-Synchrotron DESY, Photon Science
Notkestraße 85, 22607, Hamburg, Germany

We present a new service within Instruct Germany Hamburg Center in X-ray techniques. MX beamline P11 (1, 2) at PETRA III, DESY in Hamburg is dedicated to high-throughput macromolecular crystallography. It offers data collection as fast as 2 min per sample and the sample changer fits 23 unipucks simultaneously. The versatile focusing options, Eiger2 X 16M detector and remote operations with simple web-browser based solution make it ideal for various experiments including drug screening campaigns.

User interfaces at P11 are MXCuBE and ISPyB for data acquisition and laboratory information management. ISPyB also supports the sample shipments and tracking. We aim of establishing an option for unattended data collections in the near future. Beamline instrumentation upgrade will follow during 2026 with renewal of diffractometer and sample changer. This increases the sample changer dewar capacity to 37 unipucks, speeds up the sample mount cycle due to use of a double gripper, enable crystal reorientation and helical scans and improves the reliability of the instrument. Laboratories in the vicinity enable fragment-screening campaigns and DESY hosts e.g. OLTShifter for facilitated soaking and sample mounting.

Serial synchrotron crystallography at P11 is implemented with the CFEL tape-drive setup, also capable of time-resolved experiments by the mix-and-diffuse method (3). Real-time autoprocessing of serial data with CrystFEL (4) has been developed within a long-term proposal.

In preparation for PETRA IV, P11 is now generically accessed by the academic users via rolling access. The industrial use of the beamline has grown significantly and we further develop the features important for industrial access but also benefitting for the academic users.

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Cryogenic electron microscopy at IOCB Prague

Pavel Brazda

Affiliation:
IOCB Prague

Abstract

We are the newly established Cryogenic Electron Microscopy (cryo-EM) laboratory 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 HighPerformance Computing (HPC) systems. We also provide expertise in atomic-model building for the structures we analyze.

Our proficiency extends beyond cryo-EM, encompassing techniques at ambient temperatures and 3D electron diffraction. We currently operate an in-house screening electron microscope and collaborate with CEITEC in Brno to access high-resolution instrumentation.

At IOCB, we are committed to equipping our new cryo-EM facility with the most advanced, state-of-the-art instrumentation. Our overarching goal is to establish a cutting-edge cryo-EM infrastructure that will meet the needs of IOCB users and potential external collaborators in their scientific endeavors.

High-resolution cryo-EM structures of VDAC2–BAX/BAK complexes to elucidate mitochondrial apoptosis mechanisms

A.Hubert¹, V. Ravishank¹, C. Markarian¹, V. Prima¹, L. Bergdoll^{1*}

¹Affiliation: UMR7255, Marseille, France;

Apoptosis is a tightly regulated form of programmed cell death that plays a fundamental role in development and tissue homeostasis. In the intrinsic apoptotic pathway, cellular stress signals trigger the activation of the pro-apoptotic proteins BAX and BAK. These proteins translocate to the mitochondrial outer membrane, where they oligomerize to form pores that enable the release of cytochrome c, initiating the caspase cascade and ultimately leading to cell death. However, the molecular mechanism governing the recruitment and insertion of BAX and BAK into the mitochondrial outer membrane remains poorly understood.

The Voltage-Dependent Anion Channel protein VDAC2, located in the mitochondrial outer membrane, has been proposed to play a key role in this process. Previous electrophysiological studies and co-purification experiments suggest a direct interaction between VDAC2 and BAX. In our study, VDAC2 is produced in nanodiscs, and the VDAC2–BAX complex is generated using a cell-free expression system. Following purification through the His-tag on VDAC2, immunoblot analysis confirms the presence of both VDAC2 and BAX. Cross-linking experiments further support the formation of a stable complex between these proteins. Preliminary cryo-electron microscopy analyses are currently underway to visualize this complex at high resolution.

Our objective is to characterize the structural basis of the VDAC2–BAX interaction in order to elucidate the molecular mechanism regulating mitochondrial membrane permeabilization during apoptosis. Understanding this interaction could provide new therapeutic opportunities to modulate apoptosis, either by inhibiting cell death in neurodegenerative diseases or by promoting apoptosis in cancer cells. Structural insights into the complex may also enable future structure-based drug design targeting this pathway.

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**Structure-Based Functional Characterization of the Surface
Proteins Mpn444 and Mpn436 in Mycoplasma**

Irem Keles^{1#}, Sina Manger^{1#}, Pauline Roth¹, Margot P. Scheffer¹, Achilleas S. Frangakis^{1*}

¹Buchmann Institute for Molecular Life Sciences and Institute for Biophysics, Goethe University, Frankfurt am Main, Germany

[#]These authors contributed equally: Irem Keles, Sina Manger

^{*}Correspondence: Achilleas S. Frangakis (achilleas.frangakis@biophysik.org)

Mycoplasma pneumoniae is a human pathogen causing atypical community-acquired pneumonia. It serves as a model for a minimal cell and is notable for its absent cell wall, minimal genome, and use of antigenic variation to evade the host immune response. In this study, we present the structures of two previously uncharacterized essential surface proteins, Mpn444 and Mpn436, determined at 3.74 Å and 3.65 Å resolution, respectively. Structural analysis reveals that both proteins contain a peptidyl-prolyl isomerase (PPIase) domain and a chaperone-like domain, providing key insights into their potential biological roles. Furthermore, both proteins are conserved across multiple *Mycoplasma* species. In vitro PPIase activity assays further demonstrate that Mpn444 and Mpn436 exhibit foldase activity, suggesting that they play a role in extracellular protein folding in *Mycoplasma* species. Together, our findings highlight how structural determination enables the functional characterization of previously unknown proteins and reveal a potential extracellular folding machinery in *M. pneumoniae*, identifying Mpn444 and Mpn436 as promising targets for therapeutic intervention.

Development of nanobodies to neutralize short-chain α -neurotoxins from snakes

Mieke Munters¹, Marijke Brams¹, Mieke Nys¹, Chris Ulens¹

¹Laboratory of Structural Neurobiology, Department of Cellular and Molecular Medicine, Faculty of Medicine, KU Leuven, Leuven, Belgium

Purpose

Snakebite envenomation remains a major neglected tropical disease, with short-chain α -neurotoxins causing rapid neuromuscular paralysis through inhibition of muscle-type nicotinic acetylcholine receptors (nAChRs). There is a critical need for safe, affordable, and broadly effective antivenoms that efficiently neutralize these small, highly toxic components. This project aims to develop and characterize nanobodies derived from llamas immunized with recombinant short-chain α -neurotoxin ScNtx as next-generation therapeutics against a wide variety of short-chain α -neurotoxins.

Methods

Llamas were immunized with ScNtx, an immunogen designed based on the eleven most toxic short-chain α -neurotoxins from Elapid species. Nanobody libraries were expressed in *Escherichia coli* and subjected to biophysical characterization to determine binding affinities (K_d) for ScNtx. Functional neutralization was assessed in vitro using electrophysiological recordings of muscle-type nAChRs exposed to purified toxins and crude venoms. For structural insight, nanobody–ScNtx complexes were crystallized to resolve their three-dimensional structures.

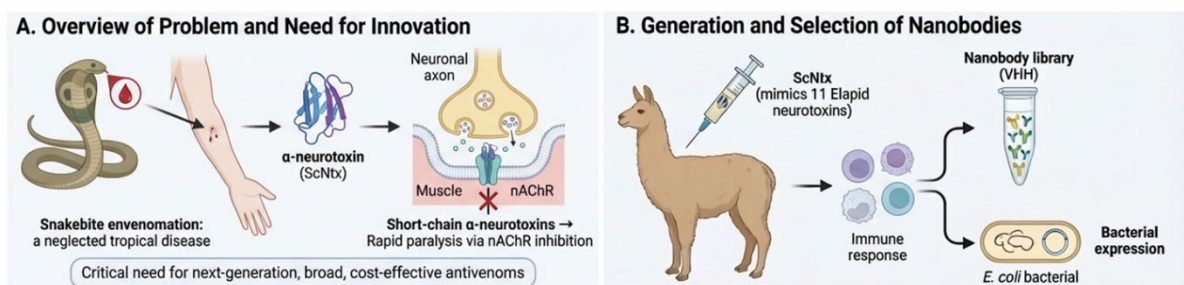
Results

ScNtx-specific nanobodies displayed high-affinity binding to ScNtx. Electrophysiological assays demonstrated that nanobodies prevented toxin-mediated inhibition of muscle-type nAChR currents, indicating effective in vitro neutralization for purified toxins and crude venoms. Structural studies of nanobody–ScNtx complexes resolve the binding mode that target key functional epitopes involved in receptor interaction.

Conclusion

These findings support nanobody-based inhibitors as promising, cost-effective therapeutic candidates for the neutralization of short-chain α -neurotoxins in Elapid snakebite envenomation. The combination of strong binding, broad functional neutralization, and structural information offers a rational basis for optimizing nanobody constructs and integrating them into next-generation recombinant antivenom formulations.

Llama-derived Nanobodies Efficiently Neutralize Short-chain α -neurotoxins from Elapid Snake Venoms



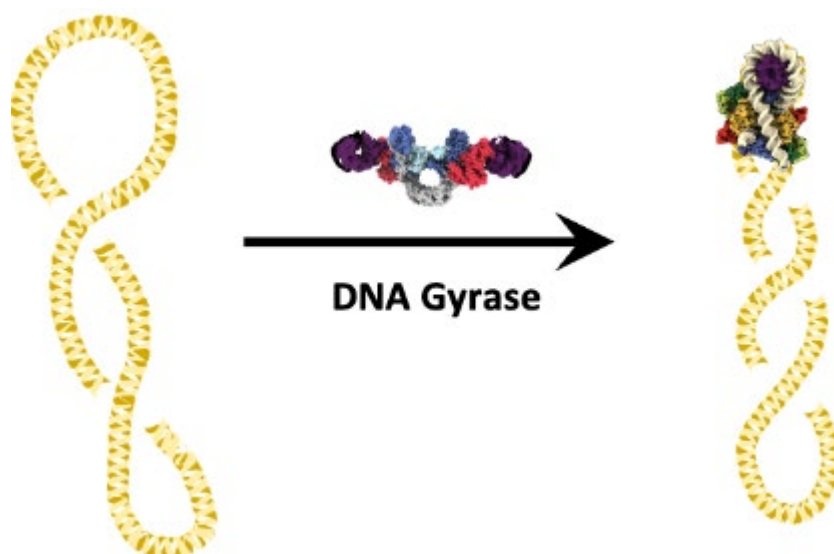
Structural Basis for the Chiral Preference of E. coli DNA Gyrase towards Positive Nodes

Naveen Kumar Murugasamy^{1*}, Luc Bonnefond^{1,2}, Valérie Lamour^{1,2}

1. IGBMC, UMR7104 CNRS, U964 Inserm, Université de Strasbourg, Illkirch, France ; 2. Hôpitaux Universitaires de Strasbourg, France; * naveen-kumar.murugasamy@igbmc.fr

DNA Topoisomerases have evolved to help maintain DNA's topological integrity in cellular processes such as replication and transcription. During the elongation phase of transcription, RNA polymerase (RNAP) generates positive supercoils downstream and negative supercoils upstream of the transcription bubble, which are relaxed by E. coli DNA gyrase (gyrase) and Topo I, respectively. In vitro, DNA gyrase can relax both negative (-SC) and positive supercoils (+SC). To date, gyrase structures have been resolved only in complex with linear DNA fragments, failing to capture the native topological state of physiological DNA. Due to this limitation, the twin-domain model of transcription has not been structurally explored.

To understand how Gyrase can recognise negatively and positively supercoiled DNA chirality during transcription. Cryo-Electron microscopy was used to analyse the structure of DNA gyrase reconstituted with +SC and -SC DNA using DNA minicircles (ideal templates to introduce and control the level of supercoiling). We obtained two different conformations of DNA gyrase: 1. the complex of DNA gyrase with +SC and -SC DNA, showing how the CTD domain binds differently to the DNA depending on the DNA crossover chirality and 2. A new open conformation of Gyrase without DNA, resembling a resting state gyrase before the DNA binding event of the catalytic cycle.



Molecular Insights into BoxA Modulation of CXCR4 Activity

M. Ghitti,[1,2] M. Morando,[2] A. Molinario,[3] AM Trotta,[4] S Scala,[4] M.E. Bianchi,[2,3] R. Mezzapelle,[2,3] and G. Musco[1]

[1] Biomolecular NMR Unit, IRCCS Ospedale San Raffaele, via Olgettina 60, 20132 Milan, Italy

[2] Università Vita Salute San Raffaele, via Olgettina 58, 20132 Milan, Italy

[3] Chromatin Dynamics Unit, IRCCS Ospedale San Raffaele, via Olgettina 60, 20132 Milan, Italy

[4] Microenvironment Molecular Target, IRCCS Pascale, via Semmola-80131, Naples, Italy

CXCR4 (GPCR) and its natural ligand CXCL12 regulate key processes such as immune cell trafficking, inflammation, and cancer evolution. Published data have shown that BoxA interferes with CXCL12-CXCR4 dependent cell-migration and favours CXCR4 internalization, leading to antitumor immunity through direct binding between CXCR4 and BoxA. Importantly, BoxA displays *in vivo* antitumor activity, supporting its potential as immunomodulatory agent. These observations prompted us to investigate the molecular determinants at the basis of BoxA-CXCR4 activity.

Based on the two-site two-step model, in which chemokines initially bind to the extracellular N-terminus of the GPCR and then move into the transmembrane pocket, we hypothesized that CXCR4 tail might also recognize BoxA. Therefore, we investigated by NMR the interaction of BoxA with CXCR41–38. We probed the interaction in native environment using membrane containing CXCR4. We observed that BoxA binds both CXCR41–38 and membrane embedded CXCR4, although with low affinity compared to CXCL12. Consistently, radioligand displacement assays and competition with an Anti-CXCR4 mAb indicate that BoxA weakly competes with CXCL12 binding to CXCR4 at μM concentrations. As a matter of fact, NMR and MST revealed that BoxA interacts not only with the receptor, but also with the chemokine in the μM range. Taken together, our data suggest that BoxA does not act as a classical orthosteric antagonist but rather as a multivalent modulator of the CXCL12–CXCR4 axis. In this model BoxA may alter the presentation of CXCL12 to CXCR4, thereby reshaping downstream signaling outputs. Such a mechanism may reconcile the modest affinities measured in biophysical assays with the significant functional effects observed in cell-based experiments.

These findings highlight how integrative structural biology can uncover unconventional mechanisms of receptor modulation and guide the development of new molecules targeting chemokine signalling pathways.

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MORPH: Structurally Aware Visualization and Models of Human Missense Variants in the Genomics Era

Lucas M.P. Chataigner¹, Hugues Aschard¹, Hanna Julienne¹

¹ Statistical Genetics Unit, Department of Computational Biology, Institut Pasteur, Université Paris Cité, 25–28 Rue du Dr Roux, 75015 Paris, France

Characterizing missense variants consequences at scale remains elusive, limiting prioritization in the clinic. Structure predictions could contribute, yet their applicability to variant interpretation remains to be fully evaluated.

Cytochrome P450 (CYP) enzymes metabolize most medications and have extensively documented pharmacogenetics, appearing thus as a relevant regime for addressing structure prediction for missense modelling. In particular, 97.1% (6,834/7,042) of observed missense variants across major CYPs remain to be studied, indicating an actionable area for variant prioritization. To understand CYP variations, we built: (i) a method to visually map missense variations on structure predictions, (ii) a pipeline exploring how predictions can inform variant prioritization.

Existing variant visualization methods predict properties or model substitutions but lack population frequency or ancestry information. Our method, MORPH (Missense On Reference Protein Human), enables mapping missense variants from 807,162 gnomAD v4 individuals onto structure predictions. It provides (i) a view of alternate allele and ancestry specific frequencies along sequences, and (ii) displays observed alternate sidechains. To further evaluate variant predictions for prioritization, we built a pipeline from open-access tools deriving descriptors reflecting variant related changes in residue interaction networks. Embedding variants in a reduced structural space, we then associate uncharacterized variants with clinically annotated ones with similar structural properties.

Our visuals reflect that reference sequences are subpart of the genetic landscape and enable analyses representative of diverse genetic ancestries. MORPH extends this to 19,060 UniProt references, supporting variant interpretation across clinical domains. While the scalable strategy for variant interpretation we propose highlights the potential of structure-based representations to advance personalized medicine.

Protein expression tags as scaffolds for peptide-based drug design**NABEEL AHMAD¹**, Virender Kumar², Vijay k Goel², Pradeep Sharma¹, Sujata Sharma¹, Tej P Singh¹¹ All India Institute of Medical Sciences, New Delhi,² Jawaharlal Nehru University, New Delhi

Protein expression tags are widely used to improve recombinant protein solubility and purification, yet they are generally assumed to be functionally inert. Structural analyses of phosphopantetheine adenylyltransferase (PPAT) from *Enterobacter* spp., however, reveal that N-terminal tag fragments can intrude into catalytic pockets and mimic natural substrate interactions with unexpectedly high affinity. In multiple PPAT crystal structures, 14 to 17-residue segments from the expression tag extend deep into the 4'-phosphopantetheine (PNS)-binding site. At the same time, small molecules such as citric acid and phosphonoacetic acid occupy the ATP-binding pocket, together fully blocking the enzyme's substrate cleft. Synthetic peptides corresponding to the tag fragments bind PPAT with micro- to nano-molar affinity, which is stronger than the native substrates, thereby demonstrating that expression-tag sequences can form stable, inhibitor-like interactions with essential metabolic enzymes. These findings reveal expression tags as an unrecognized source of structurally preorganized peptide scaffolds and highlight a novel strategy for peptide-based drug design that leverages tag-derived motifs, as directly revealed by crystallographic evidence.

Protein Production Facility – DNA & Proteins for Your Experiments

Miroslava Alblova, Veronika Klapstova, Tereza Kolarcikova, Agnieszka Szmitkowska, Lenka Vidrnova, Jan Dohnalek, Maros Huliciak

Centre of Molecular Structure, Institute of Biotechnology, Czech Academy of Sciences, Prumyslova 595, 252 50 Vestec, Czech Republic

miroslava.alblova@ibt.cas.cz

The Protein Production core facility at the Centre of Molecular Structure (CMS) of the Czech Infrastructure for Integrative Structural Biology (CIISB), part of Instruct-ERIC centre, offers comprehensive services covering every step of protein production from DNA to the purified protein. These include gene cloning into expression vectors, site-directed mutagenesis, and expression, followed by protein purification.

Our cloning services include both traditional cloning using restriction enzymes and restriction free (RF) methodologies. Furthermore, we perform small-scale expression and solubility tests using various *Escherichia coli* strains under different conditions. As an alternative to prokaryotic expression, we can provide protein production in human embryonic kidney cells (HEK293T, Expi293, and Expi293F GnTI-) or in baculoviral expression systems using Sf9 and High5 insect cells. Finally, we offer large-scale production and purification of target proteins.

For protein purification, we employ a range of steps, such as Strep-Tactin XT and immobilized metal chelate affinity chromatography (IMAC), both on FPLC or in gravity flow setups. We also provide ion-exchange chromatography and size exclusion chromatography using Superdex 75 and Superdex 200 columns (10/300 Increase or HiLoad 16/600). Customers may request adaptations of standardized protocols or provide us with established protocols. All services can be ordered individually or as a single end-to-end package.

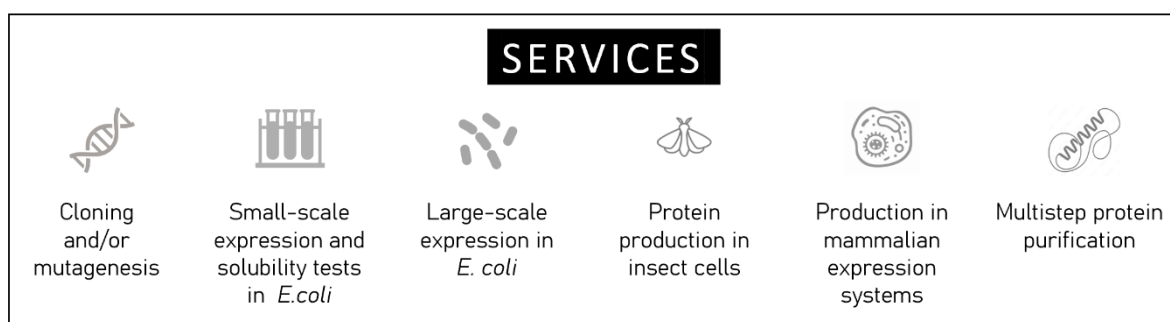


Fig. 1: Services of the Protein Production core facility in CMS.

The Protein Production core facility is a part of CMS operated by the Institute of Biotechnology, Czech Academy of Sciences. The Centre of Molecular Structure is supported by: Czech Infrastructure for Integrative Structural Biology (CIISB), Instruct-CZ Centre of Instruct-ERIC EU consortium, funded by MEYS CR infrastructure project LM2023042) and OP JAK project „Innovation of Czech Infrastructure for Integrative Structural Biology“ (No. CZ.02.01.01/00/23_015/0008175).

**European XFEL in Instruct-Germany: Serial Crystallography
Sample Preparation/Delivery/Characterization**

Christina Schmidt, Katerina Dörner, Robin Schubert, Joachim Schulz and Huijong Han

Sample Environment and Characterization (SEC) group, European XFEL, Holzkoppel4, 22869 Schenefeld

Serial crystallography (SX) has been a revolutionary technique in structural biology for more than a decade, providing insights into the structures and dynamics of biomolecules at room temperature. Using intense ultra-short X-ray pulses, SX make the collection of diffraction data from micron-sized crystals possible, avoiding radiation damage and allowing for the capture of transient states and intermediates in biological reactions.

The success of SX experiments relies heavily on the quality and delivery of the sample. However, preparing homogeneous and reproducible microcrystals in large enough amounts is yet a fairly unestablished technique.

We present our new Instruct-Hamburg services, **Serial Crystallography Sample Preparation, Characterization and Sample Delivery testing** at the EuXFEL.

We provide expert support for optimal sample preparation and characterization covering microcrystal growth and optimization, concentrating on the production of stable and uniform microcrystals suitable for serial data collection. Using our 'Sample Delivery Test for Serial Crystallography' service, users can test different delivery methods offered at the EuXFEL to find suitable delivery parameters. Moreover, experts can provide guidance in beamtime proposal writing.

The services aim to a detailed understanding of sample quality and properties, to improve data collection and data quality.

Inhibition of NLRP3 by a CNS-penetrating indazole scaffold

Torp, Jane (1); Ferber, Dominic (1); Buthmann, Hannes (1); Hagelueken, Gregor (1); Hartman, George (2); Py, Bénédicte F. (3); Wilhelmsen, Kevin (2); Geyer, Matthias (1)

1: Institute of Structural Biology, University of Bonn, Venusberg Campus 1, 53127 Bonn, Germany; 2: BioAge Labs, 5885 Hollis St., Suite 370, Emeryville, CA 94608, USA; 3: CIRI, Centre International de Recherche en Infectiologie, Univ Lyon, Inserm, U1111, Université Claude Bernard Lyon 1, CNRS, UMR5308, ENS de Lyon, Lyon, France

Low-grade inflammation is a hallmark of ageing and a key cause of age-related impairments and diseases. The NOD-like receptor NLRP3 senses a variety of danger signals and environmental insults, resulting in pro-inflammatory response, inflammasome formation and pyroptosis. Its aberrant activation has been linked to many acute and chronic diseases ranging from atherosclerosis to Alzheimer's disease and cancer, making NLRP3 an attractive therapeutic target. Here we report the discovery, characterization, and structure of an indazole-based NLRP3 antagonist, BAL-1516, which potently inhibits inflammasome formation in monocytes and microglia. The cryo-electron microscopy structure of BAL-1516 bound to NLRP3 reveals a previously undescribed compound binding site at a surface groove of the nucleotide-binding domain with contacts to the FISNA and WHD subdomains. The characteristic feature of BAL compound binding is the formation of three hydrogen bonds to the peripheral β -strand of the triple-ATPase; two from the indazole's nitrogen atoms and a third from the compounds' linker region. Additional phenyl and thiazole moieties render the compound hydrophobic, allowing excellent blood-brain barrier penetration. The compound binding site is highly specific for NOD-like receptors, and the optimized compound BAL-1516 is able to directly bind mouse NLRP3 despite two conservative residue changes in the binding interface. The BAL compounds represent a first-in-class family of NLRP3 inhibitors, providing a broad design space, including covalent and degradative properties, for the development of NLRP3-directed therapeutics.

**Selective inhibition of the *M. tuberculosis* lipase Rv0183 by
European-wide integrated structural biology**

L. Riegler-Berketa,^{*,1}, L. Gödla,¹, N. Polidoria,^b, P. Aschauer ^a, C. Griningera, G. Prosserc,^d, J. Lichteneggera, T. Sagmeistera, L. Pariggera,^e, C. C. Grubera,^e, N. Reilingc,^d, **M. Oberera,e,****

^aInstitute of Molecular Biosciences, University of Graz, Graz, Austria; ^bUniversità degli Studi di Torino, Italy;

^cMicrobial Interface Biology, Research Center Borstel, Leibniz Lung Center, Borstel, Germany, ^dGerman Center for Infection Research (DZIF), Partner Site Hamburg-Lübeck-Borstel-Riems, Borstel, Germany; ^dInnophore GmbH, Graz 8010, Austria; ^eBioTechMed-Graz, University of Graz, Graz 8010, Austria

Tuberculosis remains a major global health challenge and demands new therapeutic strategies, including approaches targeting lipid metabolism in *Mycobacterium tuberculosis* (Mtb). We focused on the monoacylglycerol lipase Rv0183, an α/β -hydrolase-fold enzyme involved in mycobacterial lipid processing as target for structure-guided inhibitor development. Using the European Lead Factory (ELF) as a collaborative European drug-discovery platform, we screened approximately 470,000 compounds and identified a hydroxypyrrolidine-based chemotype with low-micromolar inhibitory activity against Rv0183. Three lead compounds, ELF1, ELF5 and ELF8, were prioritized for detailed analysis.

High-resolution crystal structures of Rv0183 in complex with these inhibitors revealed a shared non-covalent binding mode centered on the 3-hydroxypyrrolidine-1-yl-methanone moiety near the catalytic site, while distinct heterocyclic substituents explored the substrate-binding pocket. Molecular docking, comparative structural analysis and AI-based cavity analysis supported stable binding, highlighted opportunities for future optimization, and indicated a basis for species-selective inhibition relative to human monoacylglycerol lipase.

In whole-cell assays under monoolein-dependent growth conditions, these compounds inhibited Mtb H37Rv growth, linking biochemical inhibition with a bacterial phenotype while also underlining the need for further optimization of potency, permeability and target validation.

This study highlights how European-wide integrated structural biology can connect screening, crystallography, computation and microbiology to accelerate early-stage tuberculosis drug discovery. For Austria, participation in the INSTRUCT framework would offer valuable opportunities to further develop such projects, for example through fragment screening, and to strengthen collaborative structural biology approaches against infectious disease.

Instruct-ERIC Biennial Structural Biology Conference

Giulio Tassini^{1,3}, Xhenti Ferhati¹, Steve J. Matthews⁴, Danilo Gomes Moriel¹, Anna Maria Colucci¹, Ilaria Ferlenghi², Antonio Rosato³ and Francesca Necchi¹

1: GSK Vaccines Institute for Global Health (GVGH), Siena, Italy. 2: GSK, Siena, Italy. 3: Università degli studi di Firenze, Florence, Italy. 4: Imperial College London, London, UK.

Characterization of the antibody-antigen binding aimed to develop an in vitro potency assay for a Group A Streptococcus vaccine. Growing ethical concerns about animal testing and the widespread adoption of the 3Rs (Replacement, Reduction, Refinement) have increased the need for validated in vitro assays that reliably demonstrate product potency and quality in pharmaceutical research. In this context, antibody-based immunoassays offer the possibility of correlating an antigen's in vitro potency with its in vivo immunogenicity. Developing a fit-for-purpose in vitro potency assay (IVRP) therefore requires detailed characterization of antibody binding sites and their interactions with vaccine antigens under reference and subpotent conditions.

This project combines development of an IVRP for a Streptococcus A vaccine with the structural characterization and epitope mapping of antibody–antigen interactions. Specific mouse monoclonal antibodies (mAbs) were preselected to identify and quantify the three recombinant proteins in our candidate vaccine (SLO, SpyCEP, and SpyAD) directly in the final formulation. Antigens and corresponding antibodies were expressed, and multiple structural techniques were applied to characterize complexes and define epitopes, including HDX–MS, X-ray crystallography, and cryo-EM. Fab fragments of each selected antibody were produced to facilitate the performance of such structural methods.

Furthermore, binding affinity and stability assessments were conducted using competitive ELISA (cELISA) and bio-layer interferometry (BLI) on antigens at reference conditions and after thermal stress to evaluate mAb suitability for monitoring vaccine stability. Antigen-specific functional assays were also performed to measure the inhibitory potential of the mAbs.

Together, the structural, binding affinity, and functional data will support the development and refinement of the IVRP, validating its use as a reliable tool to determine Streptococcus A vaccine potency.

Cryo-EM in Clinical Research: Insights into Lipid Acquisition by *Mycoplasma pneumoniae*

Pablo Guerra, David Vizarraga, Marina Marco, Noemi Rotllan, Jesús Marín, David Santos, Mercedes Camacho, Begoña Soto, Lorena Velasco-Reniu, Félix Pareja, María Collantes, Wanlu Wu, Irene Rodríguez-Arce, Luis Serrano, Jaume Piñol, Ignacio Fita & Joan Carles Escolà-Gil

- 1 Institut de Biologia Molecular de Barcelona (IBMB-CSIC), Parc Científic de Barcelona, Barcelona, Spain.
- 2 Centre for Genomic Regulation (CRG), The Barcelona Institute of Science and Technology, Barcelona, Spain.
- 3 Institut de Biotecnologia i Biomedicina and Departament de Bioquímica i Biologia Molecular, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Spain.
- 4 Institut de Recerca Sant Pau (IR SANT PAU), Barcelona, Spain.
- 5 CIBER de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), Madrid, Spain.
- 6 CIBER de Enfermedades Cardiovascular (CIBERCV), Madrid, Spain.
- 7 Servei d'Angiologia i Cirurgia Vascular i Endovascular, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain.
- 9 Unidad de Radiofarmacia, Servicio Medicina Nuclear, Clínica Universidad de Navarra, Pamplona, Spain.
- 10 Unidad de Imagen Molecular Traslacional (UnIMTRA), Servicio Medicina Nuclear, Clínica Universidad de Navarra, Pamplona, Spain.
- 11 Institut de Investigación Sanitaria de Navarra (IdiSNA), Pamplona, Spain.
- 12 ICREA (Institut Català d'Investigació i Estudis Avançats), Barcelona, Spain.
- 13 Institut de Biotecnologia i Biomedicina and Departament de Bioquímica i Biologia Molecular, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Spain. Jaume.Pinyol@uab.cat
- 14 Institut de Biologia Molecular de Barcelona (IBMB-CSIC), Parc Científic de Barcelona, Barcelona, Spain. ifrcr@ibmb.csic.es
- 15 Institut de Recerca Sant Pau (IR SANT PAU), Barcelona, Spain. jescola@santpau.cat
- 16 CIBER de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), Madrid, Spain. jescola@santpau.cat

Cryo-electron microscopy (cryoEM) is increasingly bridging structural biology and clinical research by enabling high-resolution characterization of molecular mechanisms involved in disease. Here, we highlight how cryo-EM approaches can contribute to understanding host–pathogen interactions with potential translational implications.

Mycoplasma pneumoniae (MPN), a major cause of atypical pneumonia, lacks the ability to synthesize essential lipids and therefore relies on host-derived lipids for survival. We identify the surface protein P116 as a key mediator of cholesterol acquisition from host lipoproteins, including LDL and HDL, as well as from mammalian cells. Using cryo-electron microscopy, we resolved the structure of the P116–monoclonal antibody complex, revealing the antibody epitope within the C-terminal domain of P116 and providing structural insight into the mechanism by which antibody binding blocks cholesterol uptake. This structural information was critical for interpreting functional assays showing that targeting this domain inhibits cholesterol acquisition and significantly reduces bacterial growth in vitro. These results reveal a mechanism by which MPN extracts essential lipids from host sources and suggest a potential tropism for lipid-rich pathological environments.

Cryo-EM provides a powerful framework to resolve the structural basis of this lipid acquisition system, opening new opportunities for therapeutic targeting and for the development of MPN-based strategies to selectively localize to atherosclerotic lesions or fatty liver.

Characterization of *Candida auris* cell wall architecture under caspofungin stress using solid state**Alvis Zvirgzdins**

Affiliation

Latvian Institute of Organic Synthesis

Candida auris (C.auris) is a human pathogenic fungus that causes severe hospital-acquired infections with high mortality rates and is difficult to identify and eradicate. At the moment echinocandins are the most commonly used antifungals against C.auris infections. It was shown that echinocandins can alternate the polysaccharide composition of C.auris fungal cell wall. However, the detailed analysis of cell wall composition at atomic level remains unknown. Here, we combined ¹³C, ¹⁵N and ³¹P detected solid-state nuclear magnetic resonance (NMR) spectroscopy (ssNMR) at magic angle spinning (MAS) with transmission electron microscopy (TEM) to structurally compare cell wall architecture between intact wild type and hog1Δ C.auris strains exposed to treatment of caspofungin. The TEM analysis can reveal overall cellular morphology at the 100-200 nm resolution, while MAS ssNMR can probe polysaccharides with different mobility, within cell walls at the atomic level.

The C.auris wild type and hog1Δ mutant were ¹³C,¹⁵N enriched solid media in presence and absence of caspofungin. All prepared samples were analysed with ssNMR spectroscopy and TEM. The multidimensional ssNMR spectra revealed structural changes in polysaccharide composition at different cellular compartments. This indicated that caspofungin treatment for wild type or hog1Δ mutant of is associated with reorganization of polysaccharide cell wall architecture rather than differences in produced cell wall mass. While the TEM analysis revealed that caspofungin treatment induced a pronounced increase in total cell wall thickness in the wild-type strain, whereas the hog1Δ strain retained an overall thickness.

In conclusion, our results demonstrated that a combination of TEM with ssNMR can help structurally dissect drug-induced cell wall perturbations at different resolution levels way.

Structure-Guided Optimisation of CRBN-recruiting TEAD PROTACs

Authors: **Natalia Karolak¹**, Angus D. Cowan^{1,2}, Aileen B. Frost^{1,2}, Gerhard Fischer³, Stefan Djukic¹, Sohini Chakraborti^{1,2}, Yvonne Westermaier³, Andre Wijaya^{1,2}, Sarath Ramachandran^{1,2}, Darren Darren^{1,2}, Luke M. Simpson^{1,2}, Ilaria Puoti^{1,2}, Elisha H. McCrory¹, Jeff Y. F. Wong^{1,2}, Lisa Crozier¹, Elizabeth Hogg¹, Conner Craigon^{1,2}, Johannes Wachter³, Ceren Bilgili³, Bharat Gadakh^{1,2}, Mokhitli Morake¹, Gemma Little¹, William Farnaby², David Schönbauer³, Nicola Chessum³, Nader Boshta³, Federico Mauri³, Alessio Ciulli^{1,2}, Kirsten McAulay^{1,2}, Harald Engelhardt³

Affiliations: 1Centre for Targeted Protein Degradation, School of Life Sciences, 1 James Lindsay Place, University of Dundee, DD1 5JJ, Dundee, Scotland, U.K.

2Division of Biological Chemistry and Drug Discovery, School of Life Sciences, James Black Centre, University of Dundee; DD1 5EH Dundee, Scotland, U.K.

3Boehringer Ingelheim RCV GmbH & Co KG, 1221 Vienna, Austria

TEA domain transcription factors – TEAD1-4, are key mediators of the Hippo pathway, which controls genes involved in cell proliferation and survival, and are frequently mutated in cancer. One of the strategies to inhibit TEAD activity are TEAD-targeting PROTeolysis Targeting Chimeras (PROTACs). They consist of target binder chemically linked to a ligand recruiting an E3 ligase, which by generation of ternary complex induce selective degradation by hijacking the ubiquitin-proteasome system.

During a campaign to develop TEAD-degrading PROTACs, we obtained a ternary cryo-EM structure of TEAD1 and CRBN:DDB1ΔBPB mediated by BI2133. In the ternary complex, TEAD1 was highly flexible relative to CRBN:DDB1ΔBPB, suggesting formation of only transient protein-protein interactions (PPI). We reasoned this could be due to a lack of conformational freedom between the two proteins caused by the rigid linker. By allowing greater flexibility between TEAD1 and CRBN with BI5745 we obtained a structure where most particles adopted a single rigid conformation with an extensive PPI between the LON domain of CBRN and the YAP binding region of TEAD. As the TEAD YAP interface region has at least 72% identity among TEADs, a similar ternary complex could likely be formed with all paralogues. Using the recently developed CRBNmidi, we were able to solve a higher resolution Xray ternary complex structure of TEAD3-BI5745-CRBNmidi, which showed the same ternary complex architecture as observed by cryo-EM with TEAD1. Using the ternary structures, we developed a PROTAC docking protocol for prioritization of the designs, yielding PROTACs with rigid linkers that stabilized the favorable PPIs. Optimization of the linker and binders resulted in BI4643 and BI5302 which showed improved degradation of all TEAD paralogues.

Together, this study illustrates how structural elucidation of PROTAC ternary complexes guides linker re-design to optimize intra-complex interactions and ultimately improve degradation.

Structural and Functional Characterization of the Amino Acid Transporter

LAT3: Toward Mechanistic Insight into Cancer Metabolism

AUTORS

Patricia Gómez-Gejo^{1,2}, Estefania Rusca¹, Joana Fort i Baixeras^{1,2,3*}, Manuel Palacín^{1,2,3*}

¹ Institute for Research in Biomedicine (IRB Barcelona), The Barcelona Institute of Science and Technology (BIST)

² Department of Biochemistry and Molecular Biomedicine, University of Barcelona

³ CIBERER

*Corresponding author: manuel.palacin@irbbarcelona.org and joana.fort@irbbarcelona.org

ABSTRACT

The human transporter SLC43A1 (LAT3) is a sodium-independent uniporter that mediates the facilitated diffusion of branched-chain amino acids (BCAAs) and phenylalanine. The transporter is overexpressed in several cancers, including prostate cancer, where it has been linked to mTORC1 signaling through leucine availability. However, as a facilitated diffusion system, LAT3 is predicted to operate bidirectionally, raising unresolved questions regarding its precise role in tumor amino acid homeostasis and mTOR activation. Despite its biological relevance, LAT3 remains poorly characterized at the molecular level, with no experimental structure currently available, limiting our understanding of its transport mechanism and hindering the development of specific molecular tools to study its function.

To address these questions, we are combining structural and functional approaches to define the molecular basis of LAT3 transport. Human LAT3 was heterologously expressed in HEK293 cells and purified to homogeneity. Single-particle cryo-electron microscopy (Cryo-EM) analysis yielded a 3D reconstruction at 4.7 Å resolution, revealing a monomeric transporter architecture in agreement with structural predictions. Current efforts focus on optimizing grid preparation and data processing to achieve sub-3Å resolution to resolve the substrate-binding pocket and transmembrane side-chain interactions.

In parallel, we successfully reconstituted purified LAT3 into proteoliposomes to enable mechanistic transport assays under defined lipid composition and gradient conditions. While successful reconstitution has been confirmed biochemically, functional assays highlight challenges related to background signal, protein orientation, and/or lipid-dependent activity, underscoring the importance of membrane environment in LAT3 function.

Together, this work aims to establish a structural framework for LAT3 transport and clarify its contribution to cancer cell amino acid regulation. These insights will support the evaluation of LAT3 as a potential therapeutic target in prostate cancer.

Deriving the molecular detail of lamin filaments through a hybrid approach

Sergei V. Strelkov

Affiliation : KU Leuven

Knowledge of atomic structure is indispensable towards the understanding of both the normal function of lamin filaments and the mechanism of laminopathies. We established the 2.2Å resolution crystal structure of a truncated lamin A variant forming the A11 tetramer. Given the previously determined structures of coil2 fragments, the new data reveal that the lamin rod is α -helical in its entirety. Furthermore, we use chemical cross-linking to probe the arrangement of lamin dimers in the filament.

The crystallographic and cross-linking data are supplemented with 3D structure prediction and modelling. As a result, we derive an atomic model of the tetrameric lamin filament which is in line with the previously reported 49nm periodicity.

**INTEGRATING THE EUROPEAN RESEARCH ECOSYSTEM FOR
MICRO- AND NANO-PLASTICS: LESSONS AND FRAMEWORKS
FROM THE FHERITALE INITIATIVE**

Antonio Rosato^{1,2}, Francesca Morelli¹

¹Consorzio Interuniversitario di Risonanze Magnetiche di Metalloproteine, Via Luigi Sacconi 6, 50019, Sesto Fiorentino, Italy

²Dipartimento di Chimica, Università di Firenze, Via della Lastruccia 3, 50019, Sesto Fiorentino, Italy

fmorelli@cerm.unifi.it

The pervasive presence of micro- and nanoplastics (MNPs), bioplastics, metal-based particles, and plastic additives in the environment and food systems poses a growing threat to human, animal, and ecosystem health. These particles can cross biological barriers and accumulate in organisms, yet current analytical, regulatory, and technological frameworks remain insufficient to fully assess their risks. Addressing this complex challenge requires a coordinated, interdisciplinary "One Health" approach. The One Health approach is a strategy that integrates human, animal, plant, and environmental health to achieve optimal outcomes for all by recognizing their interconnectedness.

The Horizon Europe project FHERITALE (www.fheritale.eu) aims to strengthen the European research landscape by enhancing access to integrated services across research infrastructures (RIs). The project focuses on four priority areas (<https://fheritale.eu/key-selected-priorities>) —MNPs, bioplastics, metals/metal oxides, and plastic additives—by mapping existing capabilities, identifying technological gaps, and fostering collaboration across scientific domains.

A comprehensive landscape analysis revealed critical gaps in current methodologies. These include the need for more sensitive detection techniques, validated exposure models, scalable biodegradation systems, harmonized protocols, and integrated digital infrastructures. For instance, Magnetic Resonance technologies remain underutilized despite their potential to enhance analytical precision.

FHERITALE's findings offer a roadmap for targeted innovation and highlight opportunities for industrial collaboration. By aligning technological development with regulatory and scientific needs, industry stakeholders can play a pivotal role in advancing safe polymer design, environmental monitoring, and risk mitigation strategies. The project invites engagement from industrial partners to co-develop scalable solutions and contribute to a sustainable, health-conscious future.

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Instruct Biennial Structural Biology Conference
Brussels, Belgium 27 – 29th May 2026**Substrate recognition by human separase****Authors:**

Jun Yu^{1†}, Sophia Schmidt^{1†}, Margherita Botto¹, Kitaik Lee², Chloe M. Ghent³, Jonah M. Goodfried³, Andrew Howe⁴, Francis J. O'Reilly², David O. Morgan³ and Andreas Boland^{1*}

Affiliations:

¹Department of Molecular and Cellular Biology, University of Geneva, Geneva, Switzerland

²Center for Structural Biology, Center for Cancer Research, National Cancer Institute (NCI), Frederick, MD 21702-1201, USA.

³Department of Physiology, University of California, San Francisco, San Francisco, CA 94143, USA.

⁴Bioimaging Center, University of Geneva, Geneva, Switzerland

†These authors contributed equally.

Abstract

The protein complex cohesin encircles the sister chromatids in early mitosis. At anaphase onset, sister separation is triggered by cleavage of the cohesin subunit SCC1/RAD21 by the cysteine protease separase. SCC1 contains two cleavage sites, where cleavage is stimulated by SCC1 phosphorylation. The molecular mechanisms of substrate recognition and cleavage are only partly understood. Here, we determined a series of cryo-EM structures of human separase in apo- or substrate-bound forms that, together with biochemical analysis, provide novel insights into the regulation of separase cleavage activity. We verify the first SCC1 cleavage site and reassign the second site. We show that multiple substrates, including separase autocleavage sites and the two SCC1 cleavage sites, interact with several docking sites in separase, including five phosphate-binding sites. We also describe the structural basis of the interaction between the cohesin subunit SA1/A2 and separase, which promotes cleavage at the second site in SCC1. Finally, using cross-linking mass spectrometry and cryoEM, we propose a model of how cohesin is targeted by human separase. Our work provides an extensive functional and structural framework that explains one of the most fundamental events in cell division.

In vivo fragment screening: A new high-throughput method

Mariana Louro¹, Vanessa Frazão¹, Carmen Casuso Souto², Tiago Rodrigues², Rui Moreira²,
Marta Sousa Silva¹, Carlos Cordeiro¹

¹ FT-ICR and Structural Mass Spectrometry Laboratory, Departamento de Química e Bioquímica,
Faculdade de Ciências, Universidade de Lisboa

² Research Institute for Medicines (iMed.Ulisboa), Faculdade de Farmácia, Universidade de Lisboa,
Portugal

E-mail: mplouro@fc.ul.pt

Fragment screening relies on purified macromolecular targets, often proteins, screened against fragment libraries under in vitro conditions. However, ligand binding and hit identification are strongly influenced by experimental parameters such as protein concentration, ligand excess, buffer composition and presence of organic additives for fragment solubilization. Approaches that combine biochemical screening with validation in vivo are therefore essential to prioritize biologically relevant hits at an early stage of drug discovery.

Here, we present a strategy to identify and validate inhibitors of the SARS-CoV-2 main protease (Mpro) using complementary cellular and native mass spectrometry approaches. Heterologous expression of Mpro in *Saccharomyces cerevisiae* severely impairs cellular growth due to the intrinsic toxicity of the protease, predicted to cleave at least 134 potential substrates in the yeast proteome. This phenotype provides a functional readout for inhibitor discovery: compounds that suppress Mpro activity restore normal yeast growth. Using growth-curve analysis as a high-throughput platform, fragment libraries were screened for compounds that restore yeast viability. Positive hits were subsequently validated by native mass spectrometry directly from crude cell extracts.

By combining functional cellular screening with native MS-based binding validation, this workflow bridges the gap between in vitro fragment discovery and biological relevance. Moreover, it enables an efficient prioritization of Mpro inhibitors and illustrates the potential of integrating cellular models with native mass spectrometry in antiviral drug discovery.

Fragment Screening of the SARS-CoV-2 Main Protease by Native Mass Spectrometry

Vanessa Frazão¹, Mariana Louro¹, Carmen Casuso Souto², Tiago Rodrigues², Rui Moreira², Marta Sousa Silva¹ and Carlos Cordeiro¹

¹FT-ICR and Structural Mass Spectrometry Laboratory, Faculdade de Ciências, Universidade de Lisboa, Portugal ² Research Institute for Medicines (iMed.Ulisboa), Faculty of Pharmacy, Universidade de Lisboa, Portugal

Email: vafracao@fc.ul.pt

Fragment-based drug discovery relies on the identification of weakly binding molecules that can be subsequently optimized into high-affinity ligands. Native mass spectrometry (MS) has emerged as a powerful tool for fragment screening because it enables rapid and direct detection of protein–fragment complexes while preserving non-covalent interactions in solution. In addition to identifying binding events, native MS provides quantitative information on stoichiometry, binding fractions, and dissociation constants.

Here, we explore the potential of native MS to probe the structural consequences of fragment binding through solvent-accessible surface area (SASA) analysis. By quantifying changes in protein charge state distribution upon fragment association, we can evaluate how ligand binding alters the exposed surface of the protein and infer structural rearrangements induced by fragment interaction.

This approach enables the investigation of ligand-induced structural through native MS, without requiring complementary structural techniques. The combination of fragment screening and SASA-based structural probing provides a rapid and information-rich strategy to characterize protein–fragment interactions and to guide fragment optimization at an early-stage in drug discovery pipelines.

In addition, native MS is a powerful tool when coupled to artificial intelligence (AI). Nowadays, AI is highly used, to predict protein-protein and protein-ligand interactions, however some models fail in the laboratory. Multimeric proteins are a clear example from one of the major challenges of AI, once ligand binding can interfere with complex stability causing complex dissociation, altering complex functional state, inhibiting complex formation or destabilizing complex interface. In that way, native MS structural data can be used to training some AI models to anticipate possible structural effects resulting from fragment interaction to multimeric proteins.

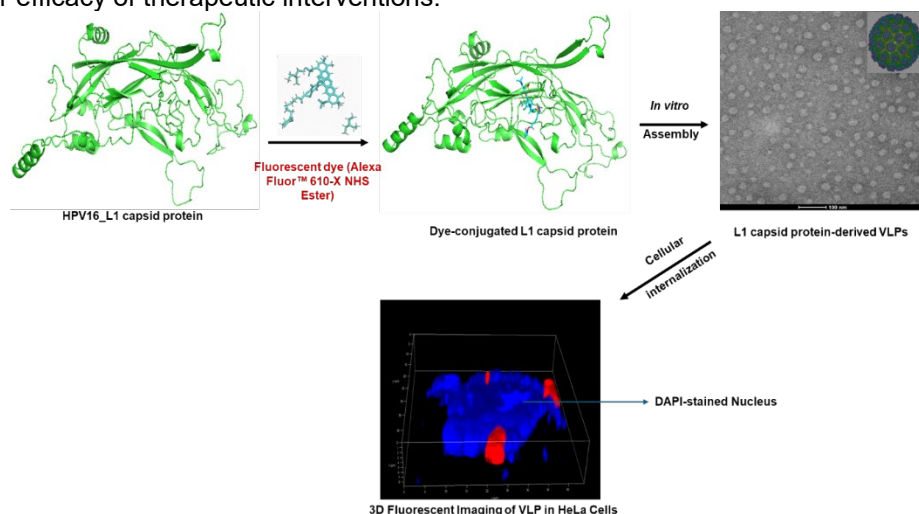
Bioengineered HPV16 L1 Capsid Protein-Based Fluorescent VLPs: Versatile Multifunctional Nano-Platform for Theranostic Applications

Sunil Kumar^{1 & 2}, Subhash Chandra Yadav^{2*}, Tulika Prasad^{1*}

¹Special Centre for Nanoscience and AIRF, Jawaharlal Nehru University, New Delhi, 110067, India.

²Electron Microscope Facility, Department of Anatomy, All India Institute of Medical Sciences, New Delhi, 110029, India.

Virus-like Particles (VLPs) derived from viral structural proteins, are self-assembled to mimic native virus morphology. VLPs emerged as robust platform for targeted theragnostic applications, owing to non-infectious, biocompatible and biodegradable nature. Herein, rationally designed VLPs with truncated N- and C-termini were bioengineered using Human Papillomavirus type 16 (HPV16) L1 capsid protein. Truncations in L1 protein enhanced soluble expression in *Escherichia coli* expression system. Purified capsid protein (55 kDa) was conjugated with fluorescent dye (Alexa Fluor 610-X-NHS ester) to generate structurally stable protein with preserved native folding. Truncated protein retained structural integrity even after conjugation and *in vitro* self-assembled into 50-60 nm VLPs. Fluorescent VLPs (red fluorescence) were primarily distributed in cytoplasm and frequently clustered in perinuclear region. Internalization of fluorescent VLPs demonstrated that conjugation did not compromise capsid stability/uptake and preserved their morphological and functional integrity in biological environment. Accumulation of VLPs in specific cellular compartments confirmed that these particles mimic native HPV internalization. Furthermore, 3D spatial analysis highlighted their ability to traverse cellular barriers to cellular compartments, a feature critical for drug delivery. Taken together, fluorescent VLPs offer promising potential as next-generation, multifunctional, trackable nanoplatform integrating drug delivery with simultaneous bioimaging for theranostic applications. Such fluorescent viral nano-platforms will facilitate precise drug delivery and real-time tracking within living systems, enabling observation and control over efficacy of therapeutic interventions.



Dynamic engagement of dual-role regulators by the Sin3 complex

Julien Olivet^{1,11}, **Neesha Rajesh Shewakramani**^{1,11}, Joseph Cesare^{2,11}, Florent Laval^{3,4,5,6,7,11}, Jules Nde², Jasper Van de Veire¹, Yasmine Brammerloo^{3,4,5}, Bastien Brebel^{3,4,8}, Olivia Debnath^{3,4}, Aaron D. Richardson^{3,4}, Hong Yue^{3,4}, Yang Wang^{3,4}, Kerstin Spirohn-Fitzgerald^{3,4}, Irma Lemmens^{9,10}, Tong Hao^{3,4}, Jan Tavernier^{9,10}, Michael A. Calderwood^{3,4}, Hyuk-Soo Seo^{3,4}, Frederick P. Roth^{3,7}, Sirano Dhe-Paganon^{3,4}, Jean-Claude Twizere^{3,5,6}, David E. Hill^{3,4}, Marc Vidal^{3,4}, Michael P. Washburn² & Kalyan Das¹

¹ Rega Institute for Medical Research, KU Leuven, Leuven, Belgium

² Department of Cancer Biology, University of Kansas Medical Center, Kansas City, KS, USA

³ Dana-Farber Cancer Institute, Boston, MA, USA

⁴ Blavatnik Institute, Harvard Medical School, Boston, MA, USA

⁵ University of Liège, Liège, Belgium

⁶ TERRA Teaching and Research Center, Gembloux Agro-Bio Tech, University of Liège, Belgium

⁷ Donnelly Centre for Cellular and Biomolecular Research, University of Toronto, ON, Canada

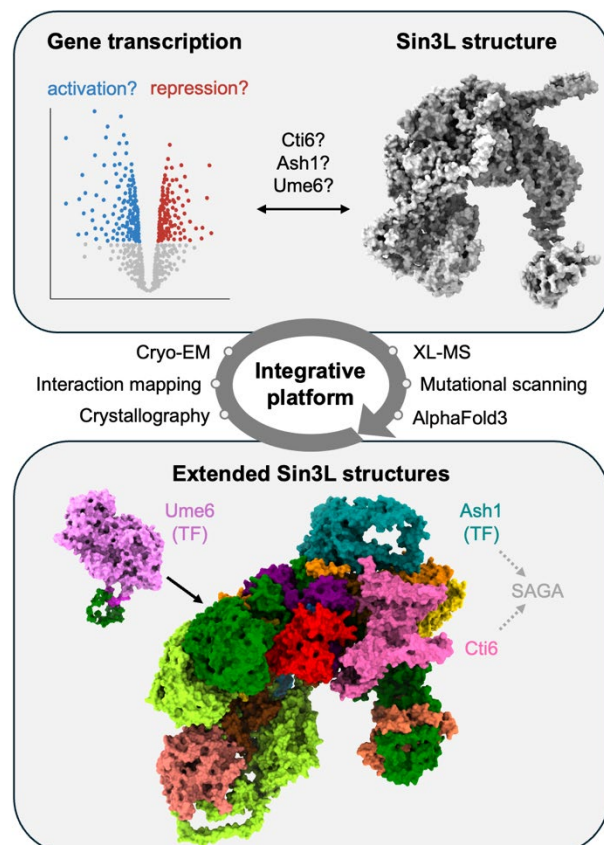
⁸ Institut Pasteur, Université Paris Cité, Centre National de la Recherche Scientifique, Paris, France

⁹ Center for Medical Biotechnology, Vlaams Instituut voor Biotechnologie, Ghent, Belgium

¹⁰ Department of Biomolecular Medicine, Ghent University, Ghent, Belgium

¹¹ These authors contributed equally.

Gene expression is governed by dynamic switches between repressive and activating transcriptional states. Among the molecules mediating these transitions, chromatin readers and transcription factors play pivotal roles. However, how they assemble with regulatory machineries to enable crosstalk between gene repression and activation remains unknown. Here, we apply an integrative structural dynamics approach – combining cryo-EM, crosslinking mass spectrometry, fragment-resolved protein interactome mapping and crystallography – to show how the dual-role chromatin reader Cti6 and transcription factors Ash1 and Ume6 engage the Sin3 deacetylase complex, a major regulatory hub in eukaryotes. We find that Cti6 competes with Ash1 to drive its dynamic recruitment to a shared peripheral module, while Ume6 engages the Sin3 scaffold through a defined, minimal interface. By positioning new flexible domains and subunits, we present a complete model of the core complex, extending structural coverage from 50% to 100%. Subunit interactions across core and peripheral modules are delineated by complementary fragment-resolved interactome mapping across $\sim 1.5 \times 10^6$ pairwise combinations. Using high-throughput mutational scanning, we further reveal 582 deleterious and 413 gain-of-function mutations



in the Sin3 scaffold, identifying evolutionarily conserved residues essential for anchoring transcription factors. Together, these results provide structural and functional insights into how dual-role regulators engage the central Sin3 complex, revealing subtle assembly principles that may facilitate crosstalk between gene repression and activation. Overall, this work establishes an integrative multidisciplinary framework to dissect the dynamics of macromolecular assemblies across biological system.

Bipartite binding of the N-terminal disordered domain of Src to ERK2 kinase

Alejandro Fernández^a, Marga Gairí^b, Maria Teresa González^b and Miquel Ponsa

^aBiomolecular NMR Laboratory, Department of Inorganic and Organic Chemistry, University of Barcelona

^bCentres Científics i Tecnològics de la Universitat de Barcelona

E-mail: alejandrofernandez@ub.edu

Src family kinases and extracellular signal-regulated kinases 1 and 2 (ERK1/2) are central components of signaling networks controlling proliferation, differentiation, migration, and survival. Dysregulation of these kinases is associated with numerous diseases, including cancer. Although their roles in signal transduction are well established, the molecular basis of their direct regulatory interactions remains incompletely understood.

Intrinsically disordered regions (IDRs) often mediate transient and regulatory protein-protein interactions in signaling pathways. The N-terminal region of Src contains an intrinsically disordered region whose functional contribution is still poorly characterized. Here, we investigated whether this region mediates interactions with ERK2.

Using nuclear magnetic resonance (NMR) spectroscopy, we characterized a bipartite interaction between the N-terminal disordered region of Src and ERK2 involving D- and F-motifs within the Src sequence. Biochemical assays show that ERK2 phosphorylates multiple residues in this disordered region, and that phosphorylation rates depend on the integrity of these motifs. Mutations disrupting the two motifs, as well as naturally occurring post-translational modifications, significantly alter ERK2-mediated phosphorylation kinetics.

In vitro immunoprecipitation from HEK293T cell lysates further demonstrates that the Src-ERK2 complex forms in a cellular context. Importantly, the abundance of the immunoprecipitated complex can be modulated using Src mutants affecting the D- and F-motifs, supporting the role of these elements in stabilizing the interaction in cells.

Together, our results identify the N-terminal intrinsically disordered region of Src as a docking platform for ERK2 and reveal a mechanism by which two short linear motifs within the disordered regions of Src together regulate kinase recognition and phosphorylation, highlighting a direct layer of crosstalk between Src and ERK signaling pathways.

**Production of single-chain dimer constructs of rat NKR-P1B
receptor and its ligands Clr-11 and RCTL**

Daniel Hoffman, Zuzana Urbanová, Jakub Jeníček, Jan Bláha, Ondřej Vaněk

Department of Biochemistry, Faculty of Science, Charles University, Hlavova 2030/8, 12840 Prague, Czech Republic

Natural killer cells are innate lymphoid cells that play a key role in immune surveillance by eliminating virus-infected and malignant cells. NK cell activation is regulated by the missing-self recognition mechanism that detects reduced expression of inhibitory ligands on target cells. In rats, the NK cell inhibitory receptor NKR-P1B recognizes the ligand Clr-11, which is expressed on healthy cells. Rat cytomegalovirus (RCMV) has evolved an immune evasion strategy targeting this pathway by encoding the RCMV C-type lectin-like (RCTL) protein, a viral ortholog of Clr-11. Infection of a healthy cell by RCMV leads to downregulation of Clr-11 expression and to simultaneous RCTL protein expression, thereby maintaining the inhibitory signal and preventing NK cell activation.

To mimic the dimeric states observed for these proteins in vivo and to overcome the problematic dimerization of Clr-11, single-chain dimeric constructs of NKR-P1B and Clr-11 were designed. In these constructs, two identical protomers containing a C-type lectin-like domain reside in tandem on a single polypeptide chain. NKR-P1B and Clr-11 single-chain dimers were expressed transiently in HEK293S GnTI⁻ cells to ensure uniform N-glycosylation suitable for crystallization in substantial yields; RCTL was produced using a stably transfected cell line. Proteins were purified using affinity and size-exclusion chromatography for downstream applications and analyzed by SDS-PAGE. Our aim is to study the structures of the prepared receptor and ligand constructs and their complexes, and to elucidate their interactions in solution and on the cell surface using biophysical methods. This research will lead to a better understanding of viral evasion of NK cells at the molecular level and to possible therapeutic applications.

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Molecular insights into DNA mismatch repair: Probing protein–protein and protein–ligand interactions**Janne Jänis**

Affiliation

University of Eastern Finland, Department of Chemistry and Sustainable Technology

Abstract text

DNA mismatch repair (MMR) is a conserved pathway that corrects replication errors that escape DNA polymerase proofreading, thereby maintaining genome stability. In *Escherichia coli*, this process is mediated by the coordinated action of three proteins, MutS, MutL, and MutH. MutL acts as a central regulatory hub, linking mismatch recognition to MutH-dependent strand incision through dynamic protein–protein interactions. Although nucleotide binding is known to modulate MutL activity, the binding affinities and stoichiometries of MutL–nucleotide interactions as well as their influence on MutL–MutH complex assembly remain elusive.

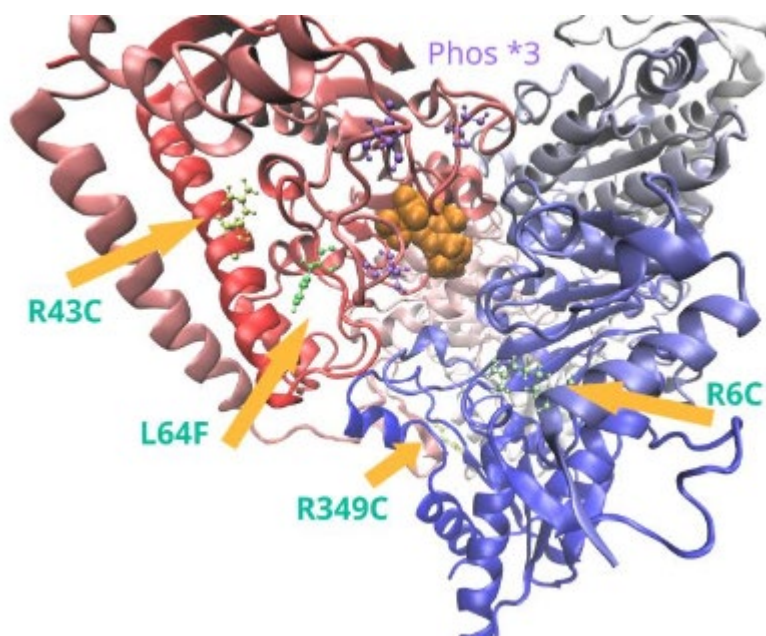
To address these questions, native high-resolution mass spectrometry was employed to quantify nucleotide binding to MutL and to resolve intact MutL–MutH complexes. All measurements were performed using a Thermo Q Exactive UHMR Orbitrap instrument, optimized for native MS. Ligand affinities for both free MutL and the MutL–MutH complex were determined through titration experiments. MutL was predominantly observed as a dimer and bound ATP and the nonhydrolyzable ATP analog AMP-PNP with high affinity, exhibiting the expected MutL₂Ligand₂ stoichiometry. In contrast, ADP binding was markedly weaker. MutL–MutH complex formation occurred both in the absence and presence of nucleotides. We also identified potential nucleotide-induced conformational changes and possible allosteric effects within the complex.

This work was supported by the RepState project funded by the European Union under HORIZON-MSCA-DN-2021 programme (Grant Agreement no. 101073485). The mass spectrometry facility is supported by Biocenter Kuopio, Biocenter Finland/FINStruct, Instruct-ERIC, and the Research Council of Finland.

Comparison of pathological amino acid substitutions of the human Pyruvate dehydrogenase E1 subunit

Eszter Kanóczki, Attila Ambrus, Oliver Ozohanics

Department of Biochemistry, Semmelweis University, 1085 Budapest, Hungary



The pyruvate dehydrogenase complex is a central metabolic hub that catalyzes the irreversible conversion of pyruvate to acetyl-CoA, thereby linking glycolysis to the tricarboxylic acid cycle and sustaining oxidative energy production in high-demand tissues such as the brain. The E1 component of human PDC is a thiamine-pyrophosphate (TPP)–dependent $\alpha_2\beta_2$ heterotetramer whose activity depends on precise subunit assembly, cofactor coordination, and inter-subunit communication. Pathogenic variants in the E1 α (PDHA1) and E1 β (PDHB) subunits are the most common cause of PDC deficiency, a severe mitochondrial disorder with wide clinical heterogeneity. This study examines four pathogenic variants—E1 β R6C, E1 α R43C, L64F, and R349C—that target conserved interface regions or structural pockets that are essential for proper folding and catalytic alignment, leading to reduced enzymatic activity despite variable effects on protein stability as revealed by the our molecular dynamics (MD) simulations and wet-lab validation.

Normal mode analysis (ANM) shows Loop B undergoing major fluctuations critical for catalysis (altered by R349C), while Loop A (a phosphorylation site) remains stable across variants. RMSF analysis reveals destabilization as follows: R43C elevates N-/C-terminal fluctuations in the α chain (similar to L64F); R6C loosens the α -chain region 295–315 (crucial for α - β interactions); and R349C destabilizes the entire protein. L64F stabilizes TPP binding (reduced flexibility), whereas R6C, R43C, and R349C disrupt channeling (increased TPP radius of gyration).

Predicted $\Delta\Delta G$ changes highlight destabilized E2 interactions for R43C and R6C. Wet-lab assays (circular dichroism, size-exclusion chromatography, enzyme kinetics) confirmed impaired folding and drastically reduced activity versus wild type.

Structural determinants of BC-loop dependent antibody escape variants in natural and patient-derived BK polyomavirus VP1 sequences

Cristina Úbeda Nicolau^{1,2}, Onno Akkermans¹, Steff De Graef¹, Senne Sienaert¹, Asanga Bandara³, Fernando H. Martins³, Erin E. Deans³, Stephen J. Ross³, Miriam Galindo Cerrada³, Vipul C. Chitalia^{5,6}, Nagraj Mani³, Stephen D. Weeks^{1,4}, Ali H. Munawar^{3,4}

1. Pledge Tx B.V., Leuven, Belgium
2. KU Leuven, Department of Chemistry, Faculty of Science, B-3001, Leuven, Belgium
3. Pledge Therapeutics LLC, Canton, MA, USA
4. Orthogon Therapeutics LLC (affiliate of Pledge Therapeutics), Canton, MA, USA
5. Boston Medical Center / Boston University Medical Center, Boston, MA, USA
6. Institute for Engineering & Science, Massachusetts Institute of Technology (MIT), Cambridge, MA, USA

BK polyomavirus (BKPyV) reactivation poses a threat to renal and hematopoietic transplant outcomes, with no approved antiviral therapies. Neutralizing monoclonal antibodies (mAbs) targeting the viral capsid protein VP1, such as 319C07, have recently advanced into clinical development, driven by promising *in vitro* potency. Since naturally elicited neutralizing mAbs arise following infection, we examined whether host immune pressure may have shaped the VP1 surface, and this has implications for the therapeutic application of biologics.

Using X-ray crystallography and cryo-EM we reveal that mAb 319C07, as well as two camelid derived single-domain antibodies ORT-016 and ORT-017, engage a shared epitope centered on the VP1 BC-loop which is involved target host cell receptor binding. Analysis of over 900 BKPyV VP1 sequences from public databases, combined with longitudinal VP1 sequence data from transplant recipients, highlighted widespread pre-existing diversity at the BC-loop contact sites. Serial passaging experiments further demonstrated the emergence of VP1 variants with reduced susceptibility to 319C07 and other mAbs, underscoring the evolutionary adaptability of BKPyV at this antigenic surface. Integrated BLI binding analyses and infection assays show that circulating BC-loop mutations, including single amino acid substitutions abrogate 319C07, ORT-016 and ORT-017 binding and neutralization. Notably, our data suggests that these mutations induce a conformational asymmetry within the VP1 pentamer, altering epitope stoichiometry as an additional mechanism of escaping the host immune response.

These findings underscore the remarkable evolutionary adaptability of the BKPyV antigenic surface, providing a mechanistic basis for the "translational gap" between *in vitro* potency and clinical efficacy of current neutralizing mAbs. Structural insights into viral target and its hypervariable regions are critical for developing the next generation of escape-resistant therapeutic approaches.

Preparation of an anti-CD20 nanobody-pHPMA conjugate and its selective binding to malignant B cells

Tereza Cibiková, Maxmilián Hejl, Jakub Jeníček, Jan Bláha, Ondřej Vaněk

Department of Biochemistry, Faculty of Science, Charles University, Hlavova 2030/8, 12840 Prague, Czech Republic

B cells play an essential role in the adaptive immune system; however, their malignant transformation can lead to various lymphomas and leukemias. A characteristic feature of these malignancies is the overexpression of CD20, which serves as a therapeutic target for mAbs such as rituximab. Although effective, antibody-based therapeutics are associated with systemic side effects and high production costs. In contrast, nanobodies are easier and less expensive to produce; however, their therapeutic use is limited by a short serum half-life. To address these limitations, we designed a macromolecular conjugate to increase the local concentration and binding avidity of anti-CD20 domains on malignant B cells.

The conjugate consists of an anti-CD20 nanobody covalently attached to a pHPMA carrier. The coupling was achieved using a two-step strategy: 1) sortase A mediated transpeptidation introduced an azide-functionalized linker; 2) click-chemistry coupling to a DBCO-modified polymer backbone. The resulting constructs were purified and characterized by SDS-PAGE, DLS, and AUC. Biophysical analyses confirm successful conjugation.

The biological activity of the fluorescently labeled conjugate was examined in both CD20(+) (MOLM-13, Raji) and CD20(–) (Karpas-299, K562) cancer cell lines. Confocal microscopy showed selective binding of the conjugate to CD20(+) cells, with minimal signal in the negative controls. Furthermore, exposure to the conjugate reduced the viability of MOLM-13 cells after 12 h, while no similar effect was detected in the CD20(–) Karpas-299 line.

Taken together, these results demonstrate the successful preparation of an anti-CD20 nanobody-pHPMA conjugate and support its selective binding to CD20(+) malignant B cells, representing a promising platform for further development of polymer-based therapeutics targeting B cell malignancies.

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Structure/function studies of two yeast Endonuclease III proteins

Diogo Agostinho 1,2, Célia M. Silveira 1, Sofia R. Pauleta 2, Smilja Todorovic 1, **Elin Moe**^{1,*}

1 ITQB NOVA, Universidade NOVA de Lisboa, Av. da República, 2780-157 Oeiras, Portugal;

2 UCIBIO, i4HB, DQ, NOVA FCT, Campus de Caparica, 2829-516 Caparica, Portugal.

* email : elinmoe@itqb.unl.pt

Keywords: Endonuclease III enzymes, FeS cluster proteins, vibrational spectroscopy, crystallography.

ABSTRACT

DNA glycosylases are enzymes that remove damaged bases from DNA by catalysing the hydrolysis of the glycosidic bond, between the base and the sugar phosphate backbone of DNA, at the onset of base excision repair (BER) [1, 2, 3]. Some of these highly conserved enzymes belong to the Helix-hairpin-Helix (HhH) superfamily of DNA glycosylases, and possess a FeS cluster, which raises a fundamental question about the role of the cluster in the BER [2, 3]. Here we address the still disputed role of these clusters by studying two yeast DNA glycosylases, one which carries a cluster (yNTH2) and one that does not (yNTH1). After the recombinant production of full-length yNTH2 and truncated yNTH2Δ39, we characterized their biophysical and biochemical properties by employing vibrational spectroscopy (resonance Raman and FTIR), electrochemistry and activity assays. We demonstrate that both forms are catalytically active, possess a typical cubane [4Fe4S]²⁺ cluster, and have comparable redox potentials ($E^0 = 0 \pm 14$ mV). We have now produced a chimeric enzyme containing a FeS cluster that we have characterised and crystallized. The result of these studies will allow us to understand the influence of the cluster on the structural and functional properties of FeS holding HhH family of DNA glycosylases.

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The conserved Fic enzyme SoFic targets switch-I of the bacterial elongation factor EF-Tu for AMPylation

Svenja Runge¹, Vivian Pogenberg¹, Alexander Baumgart¹, Aymelt Itzen^{1,2}

¹Institute of Biochemistry and Signal Transduction, University Medical Centre Hamburg-Eppendorf (UKE), 20246 Hamburg, Germany

²Centre of Structural Systems Biology (CSSB), Institute of Biochemistry and Signal Transduction, University Medical Centre Hamburg-Eppendorf (UKE), 20246 Hamburg, Germany

Fic enzymes are nucleotidyl transferases that post-translationally modify target proteins across all kingdoms of life. In a process termed AMPylation, Fic enzymes can use adenosine triphosphate (ATP) as a co-substrate to transfer adenosine monophosphate (AMP) onto their target proteins. Many Fic enzymes are also able to reverse this modification by deAMPylation. However, it remains incompletely understood how individual Fic enzymes switch between these antagonistic AMPylation and deAMPylation activities. Moreover, the physiological targets of many Fic enzymes are unknown and the complete range of consequences of AMPylation is only beginning to emerge.

We were able to identify the bacterial translation elongation factor EF-Tu as a novel AMPylation target of the conserved, but uncharacterized bacterial Fic enzyme SoFic. Using X-ray crystallography and biochemical assays, we were able to show that the AMPylation disrupts conserved structural features of EF-Tu, ultimately inhibiting translation and causing growth defects in bacterial cells. The AMPylation of EF-Tu may therefore be a novel instrument of bacterial growth regulation.

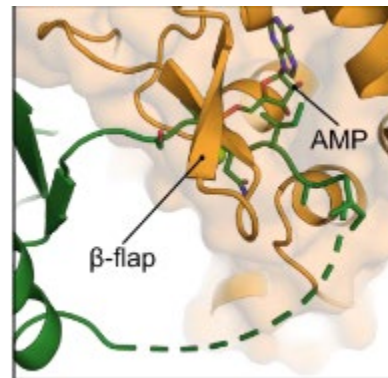
Furthermore, we resolved the enzyme-target interaction in the crystal structure of a non-covalently stabilized SoFic:EF-Tu complex, revealing a conserved target binding mode of Fic enzymes. Based on biochemical analyses, the functional switch between AMPylation and deAMPylation by SoFic appears to be tightly regulated by the dynamic monomer-dimer equilibrium of the enzyme. Therefore, this study does not only reveal a novel instrument of bacterial growth regulation, but also provides relevant insights into the function and regulation of the widespread, conserved family of Fic enzymes.



1: Crystal structure of a non-covalently stabilized SoFic:EF-TuAMP complex in a 2:2 stoichiometry.

(A) Each subunit of dimeric SoFic (light and dark orange) binds one subunit of AMPylated EF-Tu (light and dark green). The three domains of EF-Tu wrap around the catalytic Fic domain of SoFic. (B) The AMPylated switch-I region of EF-Tu is rearranged into the active center of SoFic.

(B)



Structural and functional insights into hydrogen sulfide homeostasis in bacterial pathogens**José Artur Alves de Brito**

Affiliation

EPCV - Universidade Lusófona & ITQB NOVA - Universidade NOVA de Lisboa

Abstract text

Hydrogen sulfide (H₂S) is an ancient molecule present in Earth's primordial atmosphere and organisms from all Domains of Life soon evolved to utilize it in their physiology. However, H₂S can have either beneficial or toxic effects, depending on the concentration. Therefore, tight regulation of intracellular H₂S and H₂S-derived more oxidized reactive sulfur species (RSS) is paramount for survival of all organisms. In bacterial pathogens, H₂S/RSS is regarded as an important component in microbial defence mechanisms against oxidative and antibiotic stress. For example, H₂S has been shown to revert intrinsic cephalosporin resistance in *Enterococcus faecalis*. The *cst* (copper-sensing operon repressor-like sulfurtransferase) operon in *S. aureus* encodes a nearly complete mitochondrial-like H₂S oxidation system (S₂- to thiosulfate, S₂O₃²⁻). In addition, a *cst*-like operon has also been described in the human opportunistic pathogen *E. faecalis*. Three enzymes encoded by these two operons include the *E. faecalis* coenzyme A persulfide reductase CoAPR, and the staphylococcal multidomain persulfide dioxygenase-sulfurtransferase fusion protein CstB and the sulfide:quinone oxidoreductase SQR, which collectively protect the organism against H₂S and RSS toxicity. In this work, we describe the X-ray crystallographic structures of wild-type and cysteine substitution variants of SaCstB and the CoA-bound crystal structure of EfCoAPR. These studies provide an enhanced understanding of the mechanisms of H₂S/RSS homeostasis encoded by the RSS-regulated *cst* operons in bacteria, providing new avenues to pursue alternative metabolic pathways to tackle antibiotic resistance in human pathogens. This work would not have been possible without Instruct-ERIC, namely through Access Proposals PID 37251, 38702 and Application APPID 3213

MicroED developments towards high-throughput and ease of use**Johan Unge**

Department of Chemistry, Umeå University, 901 87 Umeå, Sweden

The CryoEM technique MicroED, complimentary to both Single-particle analysis and X-ray crystallography, is being implemented since 2023 at Umeå Center for Electron Microscopy and is now an established technique in Umeå. Several targets have been successfully structurally determined using MicroED supporting local, national and international researchers. Small molecules structures can be determined directly from powder samples facilitating analysis of only partially crystallized samples. Initial protein crystals, directly from the crystallization plates without further refinement, may be suitable for MicroED structure determination, shortening the structure determination process substantially. MicroED can be considered an important tool in integrative structure biology projects.

At the same time as hardware and software have been updated at UCEM to support MicroED in both manual and automatic mode, new local developments of algorithms and software provide better data and a more robust workflow and ensures that the MicroED at Umeå is unique and enables structure determination of the most difficult samples. Current focus regards automation and high-throughput approaches to facilitate access to non-experts.

UCEM is open to all and collaborations on MicroED structure determination are welcome.

Structural studies of ryegrass mottle virus-encoded proteins.

Jēkabs Fridmanis^{1*}, Ina Balķe¹, Kaspars Tārs¹

¹ Latvian Biomedical Research and Study Center, Riga, Latvia.

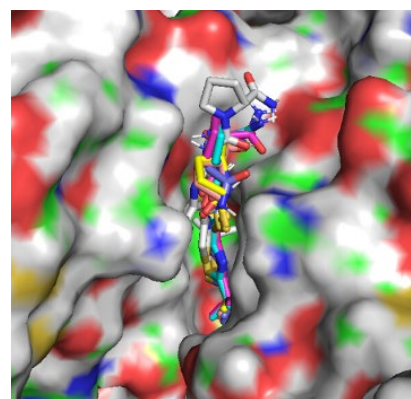
Plant-infecting viruses cause reduced crop yields in agriculture and therefore have a significant impact on the global economy. Despite this, many of these viruses remain poorly characterized, and the molecular mechanisms underlying plant virus infection are not well understood. The aim of this project is to conduct in-depth structural and functional analyses of proteins derived from ryegrass mottle virus (RGMoV), a member of the genus *Sobemovirus* known to infect cereal crops, including wheat, barley, and oat. Although progress has been made in the expression and purification of RGMoV proteins, the main advances achieved so far in this project have been the isolation of intact RGMoV virions from infected plants and their structural characterization using cryo-electron microscopy.

Computational drug screening Service at Instruct

*D.del Hoyo, B. Pueche, J. Algorta, J. Maestro, **C.O.S. Sorzano**
Biocomputing Unit, Natl. Center of Biotechnology, CSIC, Madrid, Spain*

Abstract

The increasing availability of high-resolution structures of biological macromolecules—obtained through techniques such as cryo-electron microscopy, X-ray crystallography, and integrative structural biology—creates new opportunities for structure-based drug discovery. However, transforming structural information into experimentally testable hypotheses about potential therapeutics requires specialized computational tools and expertise. To address this need, the Instruct Image Processing Center (I2PC) in Madrid provides a Computational Drug Screening Service as part of the Instruct-ERIC infrastructure.



This service enables researchers to perform structure-based virtual drug screening starting from a three-dimensional atomic model of a biological target. Virtual screening is a computational strategy used in drug discovery to evaluate large libraries of small molecules and identify those most likely to bind to a target protein, thus prioritizing compounds for experimental testing. The service integrates pocket detection, molecular docking, and ligand ranking within a unified computational environment implemented through the Scipion framework.

Users submit a structural model of the macromolecular target, obtained experimentally or through modeling. The workflow first identifies potential druggable pockets on the surface of the structure by analyzing geometric and physicochemical properties, including cavity shape, hydrophobicity, and electrostatics. Once candidate binding sites are detected, the system performs virtual screening of compound libraries against the selected pockets. Users may upload their own libraries of molecules (e.g., in SMILES format) or use curated collections, such as a reference set of approximately 1,500 FDA-approved compounds, for drug repurposing studies.

Each compound is computationally docked into the predicted binding sites and evaluated using scoring functions to estimate binding affinity. The service produces ranked lists of candidate ligands together with predicted binding poses and interaction maps. Results are provided through interactive visualization tools integrated in the Scipion environment, facilitating structural interpretation and further analysis.

The main goal of the service is to enable structural biologists to rapidly assess the druggability of newly determined macromolecular structures and generate hypotheses for ligand binding that can guide downstream experimental validation. Because the service is embedded within the broader Instruct ecosystem, it can be seamlessly combined with other infrastructure capabilities, including cryo-EM data processing, structural modeling, and functional analysis workflows.

IBS/ISBG Biophysics Facility – From Molecular Characterization to Interaction Analysis

Aline Le Roy¹, Jean-Baptiste Reiser¹, Philippe Mas² and **Caroline Mas²**

¹*Univ. Grenoble Alpes, CNRS, CEA, IBS, F-38000 Grenoble*

²*Univ. Grenoble Alpes, CNRS, CEA, EMBL, ISBG, F-38000 Grenoble*

The IBS/ISBG Biophysics Facility, located in Grenoble on the EPN campus, offers a broad spectrum of complementary biophysical techniques for the structural and functional characterization of biomolecules and their interactions. The facility enables the analysis of sample quality, size, oligomeric state, and homogeneity, as well as the quantification of biomolecular interactions in terms of affinity, kinetics, and thermodynamics. Techniques include dynamic light scattering (DLS), SEC-MALLS, mass photometry, microscale thermophoresis (MST), surface plasmon resonance (SPR), bio-layer interferometry (BLI), analytical ultracentrifugation (AUC), isothermal titration calorimetry (ITC), and circular dichroism (CD).

We provide access to our platforms and financial support for selected projects as a member of Instruct-ERIC and the FRISBI (French Infrastructure for Integrated Structural Biology) network. Beyond instrument access, our facility offers scientific expertise, experimental design assistance, and data analysis support. Examples of applications will be presented to illustrate how these techniques can address a wide range of biological questions.

Covalent Jo-In complexes for the assembly of artificial cellulosomes

Pauline Lizondo-Balderas[1], Thomas Enjalbert[1], Iker Pardo-Larrabeiti[2], Pierre Roblin[2], Cédric Montanier[2], Claire Dumon[2], Suzana Dos Reis[1], Samuel Tranier[1], Jean-Denis Pedelacq[1]

[1] Team « Structural Biophysics », Institute of Pharmacology and Structural Biology (IPBS), UMR5089, CNRS, Paul Sabatier University, Toulouse. [2] Team « Enzymatic Catalysis and Molecular Engineering », Toulouse Biotechnology Institute (TBI), UMR5504, CNRS, INSA, Toulouse

Lignocellulose is the primary structural component of plant cell wall, and Earth's most abundant renewable organic resource[1,2]. Certain anaerobic bacteria are able to efficiently degrade lignocellulose through cellulosomes, large extracellular multienzyme assemblies with exceptional enzymatic diversity and catalytic synergy[3,4]. These systems have inspired the work of designer cellulosomes (DC) for biomass deconstruction[5,6]. However, DC architectures rely on non-covalent interactions, whose intrinsic flexibility and potential for cross-reactivity can limit the precise control of the fused enzymes[7,8].

This project aims to explore a covalent assembly system, named Jo-In, derived from the *Streptococcus pneumoniae* RrgA adhesin. This system facilitates the spontaneous formation of an intramolecular isopeptide bond, providing a robust and irreversible linkage[9,10]. This system has been previously validated in-house for in vitro enzyme fusion applications[11]. The project focuses on the identification and characterization of orthogonal Jo-In pairs to enable the modular assembly of multi-component enzymes with enhanced structural stability and defined stoichiometry. To achieve this, we screened multiple Jo-In candidates and evaluated their cross interaction through covalent bonding assays. We further investigated their 3D structure using X-ray crystallography, revealing a large interface within the complex, stabilized by two conserved salt bridges across all studied pairs. Guided by these structural insights, we engineered rational mutations that directly impacted complex formation. Finally, we examined the shape and size of a newly designed Jo-In fusion pair using Small-Angle X-ray Scattering (SAXS) experiments, providing low-resolution envelopes consistent with the expected overall conformation.

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Microsecond time-resolved and conventional cryoEM reveal early intermediates to acid-induced capsid dissociation of foot-and-mouth disease virus

Delloso GKY^{1,2}, Hruby J³, Shah PNM¹, Porta C¹, Duyvesteyn HME¹, Kelly JT², Lorenz UJ³, Tuthill TJ², Fry EE¹, Stuart DI¹

¹University of Oxford, Oxford, United Kingdom

²The Pirbright Institute, Pirbright, United Kingdom

³École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland

ABSTRACT

Picornaviruses are +ssRNA viruses with 60 copies of each of 4 capsid proteins (VP1-4) icosahedrally arranged around the genome. Capsid intermediates typically mediate picornavirus entry into host cells (e.g. Enteroviruses), but for the acid-labile foot-and-mouth disease virus (FMDV), such intermediates have remained elusive because the capsid dissociates rapidly into pentamers at low pH. We are attempting to identify intermediates using two approaches.

Firstly, we have found that the relatively acid-tolerant FMDV A10₆₁ serotype can be incubated at pH 6.2 without capsid dissociation. Analysis of these pH 6.2 particles by cryoEM yielded a 2.6 Å map, which, relative to the pH 7 structure, reveals that the N-terminus of VP1 replaces the C-terminus of VP4 in an extended β-sheet spanning the pentamer interfaces. This association of VP1 to complete the extended β-sheet is structurally similar to that of the more acid-stable Enteroviruses, perhaps suggesting a conserved strategy for maintaining capsid integrity under slightly acidic conditions.

Secondly, we performed microsecond time-resolved cryo-EM on the highly acid-labile SAT2 serotype. SAT2 was prepared in the presence of a “caged proton” and plunge-frozen before exposure to a UV-A laser, which released the protons and reduced the pH on the vitrified grids. Portions of the grids were transiently melted *in situ* for 30 μs using a laser pulse and immediately revitrified, to trap any acid-induced structural intermediates that evolved over 30 μs. Single particle data were collected and analysed, revealing three distinct states: unexpanded particles similar to laser-unexposed control particles (at 2.6 Å resolution); intermediate particles with disordering of the C-terminus of VP4 within the extended β-sheet (at 2.8 Å resolution); and expanded particles with the altered extended β-sheet similar to the intermediate particles, along with a rearrangement at the twofold interface (at 3.2 Å resolution).

Overall, we have identified novel low-pH FMDV structures, potential intermediates in the uncoating and dissociation pathways, that could shed light on FMDV entry mechanisms and aid the rational design of more stable vaccines.

The Integrated Structural Biology Workflow at Human Technopole's National Facility for Structural Biology

Marko Nedeljković¹, Nataliya Danilenko¹, Sebastiano Pasqualato¹, Joanna Andrecka¹, Andrea Graziadei¹, Alessandro Scardua¹, Paolo Swuec¹

¹ Human Technopole, National Facility for Structural Biology, Viale Rita Levi-Montalcini 1, 20157 Milan, Italy

Email address: marko.nedeljkovic@fht.org

Founded in 2018 in Milan, Human Technopole (HT) is a life science institute dedicated to advancing the Italian scientific landscape. As part of this mission, HT's five National Facilities share cutting-edge resources and expertise with the broader scientific community. Among these, the National Facility for Structural Biology provides a rare, fully integrated approach to molecular research through five specialized units: Biomass, CryoElectron Microscopy (Cryo-EM), Biophysics, Structural Proteomics, and Single-Molecule analysis.

Equipped with state-of-the-art infrastructure, the facility supports both internal and external users. External researchers apply through year-round open calls by submitting proposals evaluated by an independent international committee. Successful applicants can choose between mail-in remote service, on-site access with personalized training, or fully independent instrument operation for experienced users.

Multiple projects have already demonstrated the power of this integrated pipeline. Typically, macromolecular complexes are expressed using the Biomass Unit's diverse expression hosts. These complexes are then biochemically and thermodynamically characterized by the Biophysics Unit before high-resolution structural visualization by the Cryo-EM Unit. Topology and structural restraints are validated using the cross-linking mass spectrometry (XL-MS) pipelines of the Structural Proteomics Unit. Finally, biological processes are tracked in real-time by combining fluorescence microscopy and optical tweezers in the Single-Molecule Unit.

By unifying these multidisciplinary workflows under one roof, the National Facility for Structural Biology lowers technical barriers and accelerates high-impact mechanistic studies for the Italian scientific community.

Establishing A Chitin Synthase Inhibition Reporter Assay Using SMALPs

James Irvine (1), Joe Gingell (2), John Mina (2), Shradha Singh (2), Xinghao Zhou (3), Tim Dafforn (1), Graeme Kettles (1)

(1) *University of Birmingham, UK*

(2) *Syngenta, UK*

(3) *Syngenta (SBC), China*

Chitin is an important fungal and insect cell wall component made of long straight chains of β -1,4 *N*-acetylglucosamine. It is produced and transported to the cell wall by transmembrane proteins called chitin synthases. These proteins are an attractive pesticide target due to chitin's vital role in the cell wall structure of pathogenic fungi and grazing insects as well as its absence from both plants and mammals. Despite this, chitin synthases are the target of few commercial pesticides, in part due to the lack of high-throughput assays to screen chemical libraries for this mode of action. This project aims to determine if heterologous expression of chitin synthase and subsequent extraction using styrene-maleic acid (SMA) can be used to create a high-throughput protein activity assay. Three chitin synthases from the economically important wheat pathogen *Zymoseptoria tritici* will be expressed in both *Pichia pastoris* (yeast) GS115 and *Spodoptera frugiperda* (insect) Sf9 cell lines to determine the best heterologous expression system. Following this the protein will be extracted using SMA to generate SMA Lipid Particles (SMALPs) that can be used to assay the chitin synthesis rate in the presence of chemicals from a library containing both known and putative inhibitors.

Murine NKR-P1C, a major activating receptor responsible for driving NK cell immune surveillance**Mgr. Nadezda Bragina**

Institute:

Faculty of Science, Chemistry dep., Charles University, Hlavova 8, 128 00 Prague 2, Czech Republic

Abstract

Natural Killer (NK) cells are a part of the vast repertoire of innate immune response agents in mammals. Amongst their primary functions is a process called immune surveillance. They use a variety of activating and inhibitory receptors on their surfaces to “scan” other cells in the organism to determine whether their target cells are healthy or infected, malignantly transformed, or otherwise negatively altered.

One such activating receptor is the murine Natural Killer Receptor – Protein 1C (mNKR-P1C). Despite being first described over 50 years ago, its structure and natural ligand are still unknown. A lack of knowledge about this activating receptor limits our understanding of its precise role in immune responses and regulation, which is essential, as mice serve as model organisms and their NK cell receptor repertoire differs from that of humans.

This project focuses on the recombinant production of mNKR-P1C in two types of human cell lines with distinct N-glycosylation patterns. This allows mNKR-P1C to be studied using several biophysical methods that could help paint a comprehensive picture of the receptor's structure and function. Initially, we expressed a construct encompassing the entire extracellular domain of mNKR-P1C in a HEK293S GnTI⁻ stable cell line, providing excellent yields; however, this construct never crystallized. Next, we prepared a truncated version of the extracellular domain of NKR-P1C lacking the flexible hinge region. The second construct was transiently produced in both HEK293T and HEK293S GnTI⁻ cell lines. Based on previous structural studies of homologous receptors, such as a human NKR-P1, we believe that truncating the hinge can enhance protein crystallizability. The oligomeric state, glycosylation, and disulfide bond patterns were assessed by analytical ultracentrifugation and mass spectrometry.

This work was supported by the Czech Science Foundation (25-18490S)

Exploring Different Conformations of the Hepatic ABCB11 Transporter Under Turnover Conditions

Hongtao Liu^{1,5,7}, **Ana Cuervo**^{2,7}, Dongchun Ni^{3,6,7}, Somnath Mukherjee⁴, Rose Bang-Sørensen¹, Rossitza N. Irobalieva¹, Kamil Nosol¹, Patricia Losana², David Herreros², Henning Stahlberg³, José M. Carrazo², Anthony Kossiakoff⁴, Julia Kowal¹ & Kaspar P. Locher¹

¹Institute of Molecular Biology and Biophysics, ETH Zürich, Zürich, Switzerland

²National Center of Biotechnology (CNB-CSIC), Madrid, Spain

³Laboratory of Biological Electron Microscopy, Institute of Physics, School of Basic Science, EPFL, and Department of Fundamental Microbiology, Faculty of Biology and Medicine, University of Lausanne, Lausanne, Switzerland

⁴Department of Biochemistry and Molecular Biology, The University of Chicago, Chicago, IL, USA

⁵Hangzhou Institute of Medicine, Chinese Academy of Sciences, Zhejiang Cancer Hospital, Hangzhou, Zhejiang, 310018, China

⁶International Cancer Center, Shenzhen University Medical School, Shenzhen University, Shenzhen, Guangdong, China

⁷These authors contributed equally: Hongtao Liu, Ana Cuervo, Dongchun Ni

email: acuervo@cnb.csic.es

The Bile Salt Export Pump (BSEP), also known as ABCB11, is an ATP-binding cassette transporter expressed in hepatocytes, responsible for exporting bile salts into bile canaliculi. This process is powered by ATP hydrolysis, enabling the active transport of bile salts against their concentration gradient. Disruptions in BSEP function, due to genetic mutations or drug interactions, are commonly linked to severe liver disorders such as cholestasis. Structurally, ABCB11 comprises two transmembrane domains (TMDs), each formed by six helices, and two nucleotide-binding domains (NBDs). During its transport cycle, ABCB11 adopts multiple conformational states [1,2,3]: the inward-facing (IF) state, which permits bile salt binding from the cytoplasmic side; and the outward-facing (OF) state, triggered by the binding of two ATP molecules, which enables substrate release into the bile canaliculus. ATP hydrolysis and substrate release then reset the transporter to the IF state.

In this study, we employed cryo-electron microscopy (cryo-EM) and single-particle image analysis to investigate ABCB11 embedded in nanodiscs in the presence of ATP and chenodeoxycholic acid (CDCA), a major bile salt under turn-over conditions. Our analysis revealed two distinct inward-facing (IF) conformations: IFwide, characterized by an autoinhibitory loop that hinders substrate access; and IFnarrow, where the NBDs are closely apposed with two ATP molecules bound, and no substrate present, suggesting a post-translocation state. These structural insights expand our understanding of ABCB11's dynamic mechanism and its critical role in bile salt transport.

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Title: Cryptic Pocket Dynamics and Druggability in the Coronavirus Spike Protein**Author list:**Firdaus Samsudin [1], **Peter J. Bond [1,2,*]*****Corresponding/presenting author:** peterjb@a-star.edu.sg**Affiliations:**

[1] Bioinformatics Institute (BII), Agency for Science, Technology and Research (A*STAR), 30 Biopolis Street, #07-01 Matrix, Singapore 138671, Republic of Singapore

[2] Department of Biological Sciences, National University of Singapore, Singapore 117543, Singapore

Abstract:

The coronavirus spike glycoprotein mediates viral entry and is a key target for pan-coronavirus therapeutics, yet conserved transient conformations and cryptic pockets remain difficult to exploit. Here, we combine integrative structural modelling and molecular simulations of a full-length, glycosylated spike embedded in a native membrane to identify conserved lipid-binding sites and functionally relevant and druggable cryptic pockets. Our simulations reveal multiple lipid interaction sites across the spike that modulate local structure and inter-domain coupling. These interactions, together with the dynamic opening of cryptic pockets, are supported by complementary biophysical measurements, consistent with underlying conformational heterogeneity. Co-solvent “flooding” simulations recapitulate known pockets and identify a previously uncharacterised pocket at the multimerisation (MM) loop proximal to a site of frequent mutation during variant evolution. This pocket exhibits reversible opening behaviour and is structurally coupled to regions governing S1 shedding. Ligand binding stabilises alternative MM loop conformations, revealing a structural mechanism by which this region modulates spike stability and influences the prefusion-to-postfusion transition. Virtual screening further identifies candidate compounds targeting this site, currently under evaluation using in vitro spike-mediated membrane fusion assays. Notably, lipid-binding interactions and cryptic pockets are conserved across SARS-CoV-2 variants, consistent with recent work showing preservation of lipid engagement through compensatory structural mechanisms. Collectively, these results provide a structural framework linking lipid interactions, conformational dynamics, and cryptic pocket formation in the spike glycoprotein, and establish a structural basis for the rational design of broad-spectrum coronavirus entry inhibitors

Structural studies of native multi-protein complexes essential for reproduction and development

Zeynep Ilgın Kılıç, Hubrecht Institute, Uppsalalaan 8, 3584 CT, Utrecht

Early embryonic development is driven by macromolecules stored in the egg. These maternal reserves are stockpiled in the developing egg cell – the oocyte – as it grows to be one of the largest cells in the body. Mammalian oocytes store proteins for embryonic development on abundant structures known as cytoplasmic lattices (CPLs), but the mechanisms by which they achieve this are unclear. Here, we use cryo-electron microscopy and artificial intelligence (AI)-based modeling to elucidate the molecular architecture and composition of native CPLs from mouse oocytes. We find that CPLs are formed by at least 13 different proteins assembling into a megadalton-scale complex, including maternal effect factors and proteins essential for early embryonic development. This represents an elegant molecular mechanism by which oocytes stockpile proteins through direct incorporation into highly stable supramolecular assemblies. Our structures solve the decades-long mystery of the CPLs and rationalize numerous functional and genetic studies on CPL-associated proteins, thereby providing a structural framework for understanding how disrupting these maternal factors leads to infertility and developmental defects.

Visualizing Natural Killer Receptors with DNA-PAINT

Ondřej Skořepa^{1,2}, Ondřej Vaněk¹, Ralf Jungmann², Barbora Kalousková³, Mario Brameshuber³, Ario de Marco⁴, Lucia Cikatricisová⁴, Jan Bláha⁵

¹ Department of Biochemistry, Faculty of Science, Charles University, Hlavova 2030, 12840 Prague, Czech Republic

² Max Planck Institute of Biochemistry, Am Klopferspitz 18, 82152 Martinsried, Germany

³ Institute of Applied Physics - Biophysics group, TU Wien, Getreidemarkt 9, 1060, Vienna, Austria.

⁴ Laboratory for Environmental and Life Sciences, University of Nova Gorica, Vipavska cesta 13, S-5000, Nova Gorica, Slovenia

⁵ EMBL, Hamburg Unit c/o DESY, Notkestrasse 85, 22607, Hamburg, Germany.

Natural killer (NK) cells are key components of the innate immune system, exerting cytotoxic effects on infected and transformed cells. Their activity is regulated by surface receptors that often engage in low-affinity interactions with cognate ligands a process thought to be amplified through receptor oligomerization and clustering. However, direct observation of these dynamic events at the membrane level remains a major challenge.

In this study, we use DNA-PAINT super-resolution microscopy to investigate the spatial organization, oligomeric state, and ligand-induced clustering of several human and murine NK cell receptors and their ligands, including NKp30:B7-H6, NKR-P1A:LLT1, NKR-P1B:Clr-11, and NKp80:AICL. Using recombinant receptor ectodomains in solution or anchored to supported lipid bilayers, DNA-PAINT reveals nanoscale receptor distributions on the surface of fixed cells.

Our data suggest that NKp30 oligomerization strictly dependent on N-glycosylation and the presence of the stalk domain when characterized as isolated protein persists on the cell membrane. However, the densely packed nature of such oligomers presents a significant challenge for resolving the precise architecture. Similarly, for the NKR-P1:LLT1 system (as well as the homologous NKR-P1B:Clr-11 and NKp80:AICL pairs), super-resolution imaging confirms the dimeric nature of both receptors and ligands, though clustering patterns consistent with structural models remain to be fully validated.

This approach establishes a powerful framework for resolving low-affinity, transient interactions at the single-molecule level. The findings deepen mechanistic understanding of NK cell activation and contribute the design of receptor-targeted immunotherapies.

This work was supported by the Czech Science Foundation (23-08490L, and 24-12553O).

Support Facilities for the NMX Instrument at the European Spallation Source: Crystal Growth and Protein Deuteration

Swat Aggarwala^{a,b}, Zoë Fishera^c, Justn Bergmanna, Aaron Finkea, Esko Oksanen^{a,b}

European Spallation Source, Lund, Sweden, ^bDepartment of Computational chemistry, Lund University, ^cDepartment of Biology, Lund University

The European Spallation Source (ESS), nearing completion in Lund, Sweden, is set to become the world's leading neutron source for research addressing key societal challenges in health, energy, and the environment. Life sciences and biophysics form core pillars of the ESS scientific program, supported by a suite of 15 state-of-the-art instruments employing methods such as Neutron Macromolecular Crystallography (NMX), Small-Angle Neutron Scattering (SANS), Neutron Reflectometry, and Neutron Spectroscopy.

The primary emphasis lies in structural biology and biophysics, exploring both the structure and dynamics of biomolecules and their assemblies. The long-pulse neutron source at ESS is particularly advantageous for structural biology techniques, including macromolecular crystallography and scattering experiments. Among the instruments, NMX will enable atomicscale studies of biomolecular structures, while SANS (LOKI) and reflectometry (FREIA) will allow investigations of biomolecular assemblies at the molecular level.

To facilitate high-impact neutron science, ESS hosts advanced scientific support facilities, including the DEuteration and MACromolecular Xtallization (DEMAX) platform. DEMAX provides researchers with deuterated inorganic and organic compounds, as well as access to crystallization laboratories for growing large single crystals suitable for neutron studies. In protein crystallography, deuteration labelling enhances weak signal-to-noise ratios and minimizes incoherent background from hydrogen, thereby enabling direct visualization of hydrogen atoms, hydrogen bonds, and water networks in enzymatic and macromolecular complexes. Access to DEMAX services is granted through a peer-reviewed proposal system and is free of charge to accepted users.

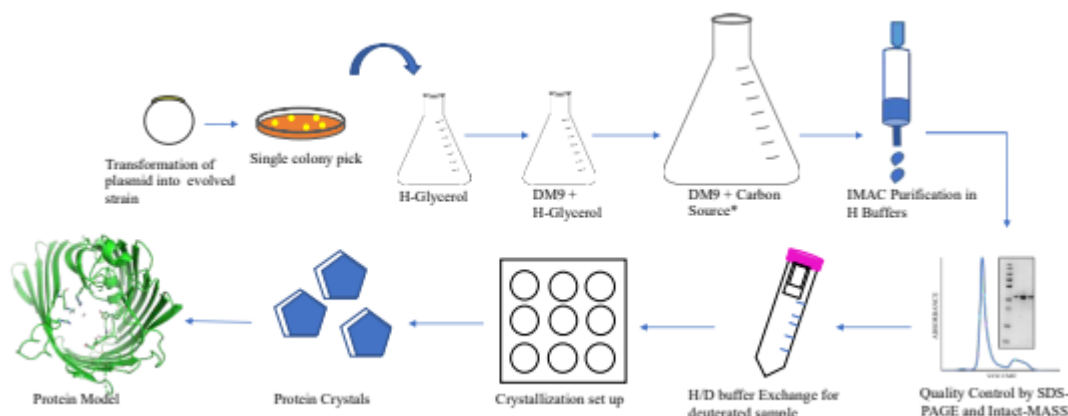


Figure- Workflow for production of perdeuterated protein (from gene to structure)

**Integrated Biological Cryo-Imaging workflows at the UK Harwell
Campus centre**

Yuriy Chaban^{1§}, Valentina Loconte¹, David Owen¹, James Gilchrist¹, Daniel Hatton¹, Emma Buzzard¹,
Ellis Bragginton¹, Peter Harrison¹.

¹Diamond Light Source Ltd., Diamond House, Didcot, OX11 0DE. Oxfordshire, UK.

§yuriy.chaban@diamond.ac.uk

Biological Cryo-Imaging (BCI) at Diamond Light Source synchrotron (Diamond) comprises the electron Bio-Imaging Centre (eBIC) and B24 beamline. eBIC offers high-throughput cryo-EM, including data collection for Single Particle Analysis (SPA), cryo-Electron Tomography (cryo-ET) and 3D Electron Diffraction (3DED). B24 is a 3D correlative cryo-imaging beamline offering cryo-Soft X-ray Tomography (cryoSXT), complemented by super-resolution fluorescence structured illumination microscopy (cryoSIM). Access to the BCI services is available through the Diamond user system and via the Instruct Harwell Campus centre proposals. Many techniques can be used in conjunction with others to form integrated workflows. Examples include combining Membrane Protein Lab services with subsequent data collection at eBIC. We also offer combined experiments between B24 and eBIC, where cells are characterised in their entirety by cryoSIM and/or cryoSXT, with detailed in-situ characterisation using ion beam lamellae milling and cryo-ET. Another combined workflow includes possibility of collecting micro-crystal 3DED data at eBIC or X-ray diffractions data using VMXm Diamond beamline, or both. 3DED on larger crystals is possible after lamellae milling. We offer a range of services for cryo-sample preparation. These include a micropatterning using PRIMO (by Alvéole) for controlling cell adhesion on grids; blot-free sample preparation using Chameleon (by SPT Labtech); rapid sample preparation using Speed Blot (by Cryopreparation); the waffle method, involving high-pressure freezing and subsequent lamellae milling (typically used for thick bacterial suspensions). We have an active commissioning call for cryo-lift-out to prepare multiple slices of the same object for cryo-ET. Correlative imaging workflows are possible using a custom built cryoSIM, Leica cryoCLEM solution, or Delmic Meteor in-chamber fluorescence device installed on our Aquilos focused ion beam/scanning electron microscope. Complementing our services are developed and customised pipelines for sample shipping, electronic tracking of specimens and experimental conditions, on-the-fly data processing, easy-to-use algorithms for combining data from different experiments, and automated data archiving. On-the-fly data processing pipelines for SPA and cryo-ET/ sub-tomogram averaging are fully compatible with CCP-EM Doppio and could be easily continued at home institute post data collection. For the Instruct-ERIC users, we now offer a new possibility to access our HP computing resources for data analysis within 40 days post data collection.

Investigating the role of VDAC2 in mitochondria- mediated apoptosis**Agathe HUBERT**

Institute : Centre National Recherche Scientifique (CNRS)- LISM – Laboratoire d'Ingénierie des Systèmes Macromoléculaires (LISM)

Abstract

Apoptosis is a tightly regulated form of programmed cell death that plays a fundamental role in development and tissue homeostasis. In the intrinsic apoptotic pathway, cellular stress signals trigger the activation of the pro-apoptotic proteins BAX and BAK. These proteins translocate to the mitochondrial outer membrane, where they oligomerize to form pores that enable the release of cytochrome c, initiating the caspase cascade and ultimately leading to cell death. However, the molecular mechanism governing the recruitment and insertion of BAX and BAK into the mitochondrial outer membrane remains poorly understood.

The Voltage-Dependent Anion Channel protein VDAC2, located in the mitochondrial outer membrane, has been proposed to play a key role in this process. Previous electrophysiological studies and co-purification experiments suggest a direct interaction between VDAC2 and BAX. In our study, VDAC2 is produced in nanodiscs, and the VDAC2–BAX complex is generated using a cell-free expression system. Following purification through the His-tag on VDAC2, immunoblot analysis confirms the presence of both VDAC2 and BAX. Cross-linking experiments further support the formation of a stable complex between these proteins. Preliminary cryoelectron microscopy analyses are currently underway to visualize this complex at high resolution.

Our objective is to characterize the structural basis of the VDAC2–BAX interaction in order to elucidate the molecular mechanism regulating mitochondrial membrane permeabilization during apoptosis. Understanding this interaction could provide new therapeutic opportunities to modulate apoptosis, either by inhibiting cell death in neurodegenerative diseases or by promoting apoptosis in cancer cells. Structural insights into the complex may also enable future structure-based drug design targeting this pathway.

Submission Statement

**From Biological Samples to High-Resolution Structures: The
Multiplatform Research Infrastructure of Instruct Centre Finland**

Hanna M. Oksanen 1*, Sarah J. Butcher 1, Minna M. Poranen 1, Katri Eskelin 1, Tommi Kajander 1, Salla Keskitalo 1, Markku Varjosalo 1, Lari Lehtiö 2, Tiila R. Kiema 2, Rajaram Venkatesan 2, Janne Jänis 3, Juha Rouvinen 3

1 University of Helsinki

2 University of Oulu

3 University of Eastern Finland

* presenting author

Instruct Centre Finland serves as a national hub integrating advanced structural biology technologies into a coherent, expert-supported research platform. Formed through collaboration among the Universities of Helsinki, Oulu, and Eastern Finland, the Centre provides coordinated access to high-end instrumentation and specialized expertise spanning biomolecular complex purification, cryo-electron microscopy, single-cell proteomics, macromolecular crystallisation, molecular biophysics, X-ray diffraction, and native mass spectrometry.

With its strong connection to the national FINStruct infrastructure (Structural Biology Finland), the Centre plays a pivotal role in Finland's research ecosystem through its advanced services, which accelerate innovation across both academic and industrial communities.

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**Core Facility for Crystallization and Diffraction,
Centre of Molecular Structure, IBT CAS, BIOCEV.**

Jiří Pavlíček, Jan Stránský, Kristýna Adámková, Ľubica Škultétyová, Jan Dohnálek, Maroš Huličiak

Institute of Biotechnology, Czech Academy of Sciences, Průmyslová 595, 25250 Vestec, Czech Republic

jiri.pavlicek@ibt.cas.cz

The Centre of Molecular Structure at the Institute of Biotechnology of the Czech Academy of Sciences (IBT CAS; BIOCEV, Vestec, Czech Republic) is an advanced complex of scientific core facilities dedicated to structural biology. Their services are delivered within the frameworks of the Czech Infrastructure for Integrative Structural Biology (CIISB) and the pan-European research infrastructure Instruct-ERIC.

The crystallization and diffraction facility is equipped with state-of-the-art instrumentation, including automated robotic platforms for high-throughput crystallization screening of proteins and nucleic acids in specialized crystallization hotels with comprehensive imaging capabilities. The facility also provides advanced services for biomacromolecular structure characterization using single-crystal X-ray diffraction, as well as small-angle X-ray scattering (SAXS) for the analysis of samples in solution. The high quality and intensity of the X-ray beams are ensured by the use of MetalJet sources (Excillum).

A particularly notable technological asset is the SONICC instrument (Formulatrix), integrated with the RI1000 crystallization hotel. This system combines second harmonic generation (SHG) and ultraviolet two-photon excited Fluorescence (UV-TPEF), enabling highly sensitive detection of micro- and nanocrystals.

Overall, the facility represents the forefront of contemporary technology, enabling sophisticated crystallization and diffraction experiments as well as detailed structural analyses of biomacromolecular complexes.

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Multi-technique buffer screening pipeline at Instruct-CZ

Jan Stránský

Affiliation: Institute of Biotechnology, CAS

Sample environment is intrinsic, but often overlooked part of the experiment. Especially buffer conditions are very important for studies in molecular biophysics and structural biology. Over the years, number of techniques was introduced, which enables high throughput testing of chemical environment, however, each focuses on different aspect of protein solution quality. Therefore, a pipeline was developed, which covers sample preparation, multiple screening techniques available in Centre of Molecular Structure (Instruct-CZ, Vestec) and software solution to present all the data to even unexperienced user in a digestible way.