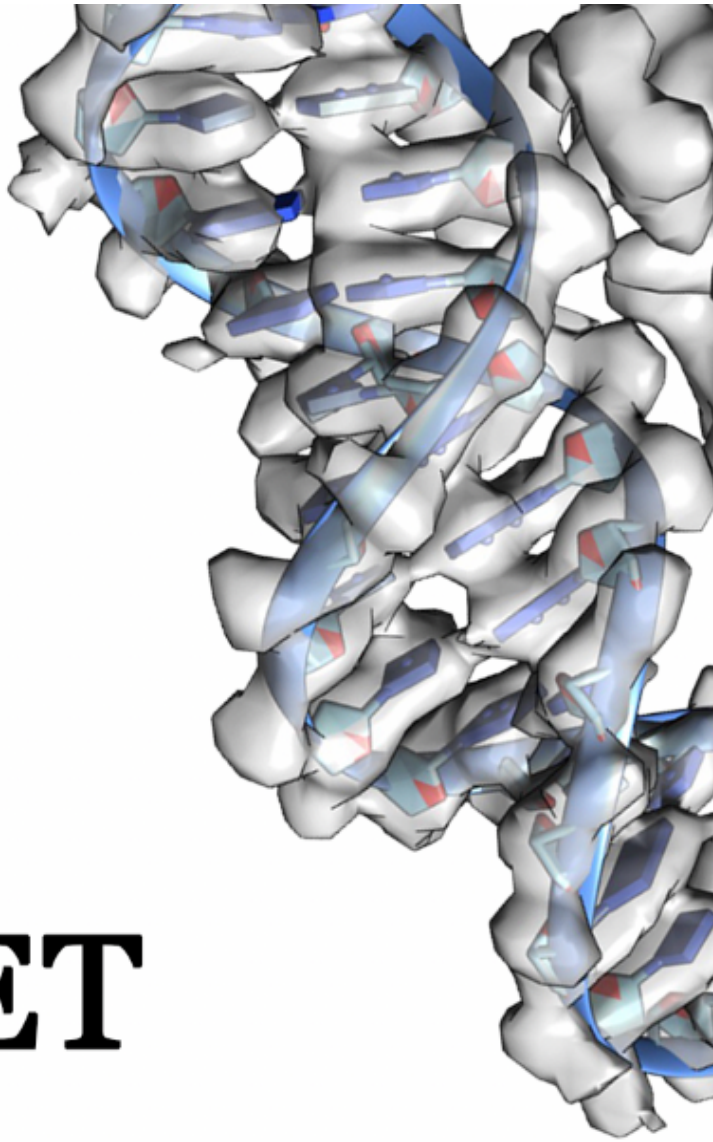
cryoNET

UMEÅ UNIVERSITY



cryoNET Symposium

1-2 October, 2025

Knut and Alice Wallenberg Foundation

Wenner-Gren Stiftelserna
Wenner-Gren Foundations

ThermoFisher SCIENTIFIC

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MIMS Molecular Infection Medicine Sweden
Nordic EMBL Partnership for Molecular Medicine

UCMR Umeå Centre for Microbial Research

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Stockholms universitet

Web-site: cryoNETsymposium

Image on the first page: Vinod Kumar Vogirala/Sara Sandin

Welcome to the 8th cryoNET Symposium

cryoNET is Nordic network in cryogenic electron microscopy, with a clear goal: to promote collaboration and knowledge sharing across borders, and to establish state-of-the-art cryo-EM as a strong pillar of the science research in the Nordic region.

At the core of the network are the cryo-EM facilities at the **University of Copenhagen**, **Aarhus University**, **Stockholm University**, and **Umeå University** – four institutions working closely to strengthen the regional foundation of cryo-EM. The network also continues to grow, reaching out to additional sites in **Norway** and **Finland.**

This initiative is generously supported by the **Novo Nordisk Foundation** and the **Knut and Alice Wallenberg Foundation**, whose commitment to advancing research has helped make this collaboration possible. This symposium is also supported by Wenner-Gren Foundation, Thermo Fisher Scientific, MiMS, UCMR, SciLifeLab, Aarhus University, University of Copenhagen, Umeå University, Stockholm University.

On behalf of the symposium organising committee:

Linda Sandblad

Lars-Anders Carlson

Max Renner

Sara Sandin

Tanvir Shaikh

Symposium program

DAY 1: Wednesday 1st October 2025

8:45 Registration & Poster Mounting

9:00 Welcome and opening remarks

Session 1

(Chair: Linda Sandblad)

9:15 **Extending the reach of single particle cryoEM**
Christopher Russo
MRC Laboratory of Molecular Biology, Cambridge, UK

9:45 **From in situ beginnings in Umeå to multiscale structural insights into an extremophile and a pathogen**
Irina Gutsche
Institut de Biologie Structurale, Grenoble, France

10:15 Coffee Break

10:45 **Unleashing the activity of PC flipping ATP10A - a driver on endocytosis**
Klara Theresa Scholtissek
Aarhus University, Denmark

11:15 **A top-down approach for studying Xenopus laevis MCM double hexamer formation by Cryo-EM**
Manil Kanade
University of Copenhagen, Denmark

11:35 **Cryo-EM data-driven modeling of biomolecular recognition**
Nandan Haloi
KTH Royal Institute of Technology, Stockholm, Sweden

12:00 – 13:00 Lunch

Session 2

(Chair: Lars-Anders Carlson)

13:00 **Cryo-ET of influenza A virus infected cells and on-lamella dual-axis tomography**
Petr Chlanda
Heidelberg University, Heidelberg, Germany

13:30 **Balancing user support, throughput and innovation in a multi-user EM facility**
Sonja Welsch
Max Plack Institute of Biophysics, Frankfurt, Germaany

14:00 **Small molecules restore mutant mitochondrial DNA polymerase activity**
Sebastian Valenzuela
University of Gothenburg, Sweden

14:30 Group photo, Coffee break

15:00 **Uncovering molecular determinants of antibody immunodominance using cryoEM**
Aleksandar Antanasijevic
École polytechnique fédérale de Lausanne, Lausanne, Switzerland

15:30 **Seeing the small move: dissecting flexibility in a hemoglobin-binding receptor from Staphylococcus aureus**
Valeria Buoli Comani
Department of Food and Drug, University of Parma, Italy

15:50 **The Structure and Function of the CCHF Virus Polymerase**
Jeremy Keown
University of Warwick, United Kingdom

16:10 – 17:30 Poster session & Refreshments

18:30 Symposium dinner at *P5 downtown Umeå on floor 5 in Väven* (Address: Storgatan 46A, Entré Väven Norra.)

DAY 2: Thursday 2nd October 2025

Session 3

(Chair: Sara Sandin)

9:15 **The molecular basis of lamin specific chromatin interactions**
Ohad Medalia
University of Zurich, Zurich, Switzerland

9:45 **New developments in Cryo-EM: Pushing boundaries for in situ structural determination and contextual imaging**
Itziar Serna Martín
Thermo Fisher Scientific, Netherlands

10:15 Coffee Break & Announcement of Best Poster

10:45 **Open and closed forms of assembled henipavirus nucleoprotein suggest structural basis of genome access**
Max Renner
Umeå University, Umeå, Sweden

11:15 **Decoding the nanoscale architecture of intracellular lipid flux**
Veijo Salo
European Laboratory of Molecular Biology, Heidelberg,Germany

11:45 **Simplified High-Pressure Freezing Workflow in Autogrids**
Miriam Weber
ETH Zürich, Zürich, Switzerland

12:05 – 13:00 Lunch

Session 4

(Chair: Tanvir Shaikh)

13:00 **A high-throughput workflow towards quantitative cryo electron tomography**
Sebastian Tacke
Max plank Institute of Molecular Physiology,Dortmund, Germany

13:30 **Caught in a moment: dynamics of proteins at the nanoscale**
Ilaria Testa
KTH Royal Institute of Technology, Stockholm, Sweden

14:00 Coffee break

14:15 **Towards elucidating the structure and operating principles of primase-helicase TWINKLE from Arabidopsis Thaliana**
Mira Dombi
University of Copenhagen, Denmark

14:45 **Continuous serial electron diffraction (c-SerialED) for studying protein-ligand interactions and enzymatic redox reactions**
Xiaodong Zou
Stockholm University, Sweden

15:15 Closing remarks

Abstract booklet

1. Symposium venue
2. Symposium dinner venue
3. Abstracts by speakers
4. Poster List
5. List of participants
6. Feedback

Symposium Venue

All presentations will be held in the lecture hall **Rotundan**, upstairs in the **Universum** building, Umeå University. Once you enter the building you need to go up one floor.

Coffee and lunch breaks

Coffee and refreshments will be served in **Brashörnan** a room adjacent to lecture room Rotundan.

Lunches will be served in the **Restaurant Lingon**, opposite to the lecture hall Rotundan (look for the cryoNET Symposium reservation signs).

MAP

UMEÅ UNIVERSITY

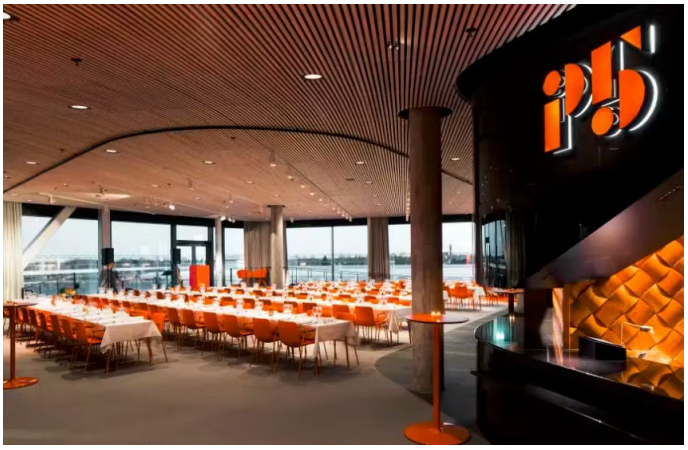
Symposium dinner venue

Everyone who signed up for the dinner on **1 October** at **18:30**, welcome to the **P5 Restaurant**. P5 is located on the 5th floor of Väven building, downtown Umeå. (Address: Storgatan 46A, Entré Väven Norra.)

Väven is approximately a 7-minute walk from Hotel Mimer and about 30-40 minutes from Universum. You can also catch a bus (numbers 5 or 8 from Universum to Renmarkstorget or Vasaplan; for ticket information, see LINK).

Map

Möten
Utbildningar
& Event



Abstracts by speakers

Christopher Russo, *MRC Laboratoy of Molecular Biology, United Kigndom*
Irina Gutsche, MICA, *Institut de Biologie Structurale, France*
Klara Theresa Scholtissek, *Aarhus University, Denmark*
Manil Kanade, *University of Copenhagen, Denmark*
Nandan Haloi, *KTH Royal Institute of Technology, Stockholm, Sweden*
Petr Chlanda, *Heidelberg University, Germany*
Sonja Welsch, *Max Plack Institute of Biophysics, Germany*
Sebatian Tacke, *Max Planck Institute of Molecular Physiology, Germany*
Aleksandar Atanasijevic,*École polytechnique fédérale de Lausanne,Switzerland*
Valeria Buoli Comani, *Department of Food and Drug, University of Parma,Italy*
Jeremy Keown, *University of Warwick, United Kingdom*
Ohad Medalia, *University of Zurich, Switzerland*
Itziar Serna Martin, *Thermo Fisher Scientific, Netherlands*
Max Renner, *Umeå University, Sweden*
Veijo Salo, *EMBL, Germany*
Miriam Weber, *ETH Zürich, Zürich, Switzerland*
Sebastian Valenzuela, *University of Gothenburg, Sweden*
Ilaria Testa, *KTH Royal Institute of Technology, Sweden*
Mira Dombi, *University of Copenhagen, Denmark*
Xiaodong Zou, *Stockholm University, Sweden*

Extending the reach of single-particle cryoEM

Christopher J. Russo

MRC Laboratory of Molecular Biology, Francis Crick Avenue, Cambridge, United Kingdom CB2 0QH.
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Ten years on from the "resolution revolution", molecular structure determination using electron cryomicroscopy (cryoEM) is poised to soon surpass X-ray crystallography as the most used method for experimentally determining new atomic structures [1]. But the technique has not reached the physical limits set by radiation damage and the signal-to-noise ratio in individual images of molecules and costs far too much per structure to be used on the breadth of biology that could potentially benefit from experimental structure determination. By examining the physical limits of imaging biological molecules with electrons and drawing on recent work on radiation damage to biological molecules at different temperatures [2,3] and energies [4,5], I will identify opportunities for making cryoEM cheaper. I will also examine the potential to extending the application of single-particle cryoEM to smaller, larger and more difficult structures, to resolve structures determined with high temporal precision, and identify structures in cryogenically preserved specimens taken directly from vitrified cells. This will help guide technology development to continue the exponential growth of structural biology in the coming decade.

References

[1] A. Patwardhan, R. Henderson, C.J. Russo, Extending the reach of single-particle cryoEM, *COSB* 92 (2025) 103005.
[2] K. Naydenova, A. Kamegawa, M. J. Peet, R. Henderson, Y. Fujiyoshi, C. J. Russo, On the reduction in the effects of radiation damage to two-dimensional crystals of organic and biological molecules at liquid-helium temperature, *Ultramicroscopy* 237 (2022) 113512.
[3] J. L. Dickerson, K. Naydenova, M. J. Peet, H. Wilson, B. Nandy, G. McMullan, R. Morrison, C. J. Russo. Reducing the effects of radiation damage in cryo-EM using liquid helium temperatures. *PNAS* 122 (2025) e2421538122.
[4] M.J. Peet R. Henderson C.J. Russo, The energy dependence of contrast and damage in electron cryomicroscopy of biological molecules, *Ultramicroscopy* 203 (2019) 125–131.
[5] G. McMullan *et al.* Structure determination by cryoEM at 100 keV. *PNAS* 120 (2023) e2312905120.

From in situ beginnings in Umeå to multiscale structural insights into an extremophile and a pathogen

Irina Gutsche

MICA group, Institut de Biologie Structurale, Grenoble, France

In this talk, I will focus on two projects that I brought with me to UCEM when I joined Umeå as a visiting researcher in 2021: the extraordinarily radiation-resistant bacterium *Deinococcus radiodurans* and the leading cause of bronchiolitis and pneumonia in babies and small children, Respiratory Syncytial Virus (RSV). I will show how cryo-electron tomography of *D. radiodurans* cryo-lamellae enabled us to uncover and characterise its unusual cell division mechanism by "sliding door" closure, while directly observing concurrent nucleoid replication and segregation into daughter cells. I will then present our recent progress in elucidating the mechanisms of action of the central divisome protein FtsZ and the key nucleoid-associated histone-like protein HU, highlighting the value of a synergistic multimodal imaging approach. In the second part of the talk, I will turn to RSV, summarising our current understanding of its nucleocapsids both *in vitro* and within cytoplasmic virus-induced biocondensates, and conclude by opening perspectives for an integrative dynamic structural analysis of RSV replication.

Unleashing the activity of PC flipping ATP10A - a driver on endocytosis.

K. Scholtissek[1], F. Pamula[1], P. Vangheluwe[2] and J. Lyons[1,3]

[1] *MBG Århus Universitet, Århus, Denmark*
[2] *KU Leuven, Leuven, Belgium*
[3] *iNano Århus Universitet, Århus, Denmark*

Lipid asymmetry of cellular membranes is crucial for cellular function and P4- ATPases or so called flippases (members of the P-type ATPase family) take part in the maintaining of it. Furthermore, endocytosis is triggered by flippases by inducing membrane curvature [1]. They actively transport lipids to the cytoplasmic leaflet via the Post-Albers cycle. All three members of the family of ATP10s have been associated with different diseases such as insulin resistance (ATP10A) [2]. Central to the function of lipid flippases is the recognition and transport of distinct lipid species. The substrate specificity of ATP10s is interesting due to their ability to transport select phospholipids and glucosphingolipids [3,4,5]. How this specificity and transport occurs is unknown. Some is known about the regulation and activation of flippases. Their N- and C-termini can bind the cytosolic domains in a manner of autoinhibition which can be released by phosphorylation events, binding to other proteins or regulatory lipid binding [6]. While the C-termini of ATP10s lack the described motif required to bind the cytosolic domains, literature suggests a role for autoinhibition/activation through a yet unknown mechanism [7]. The family of ATP10s is yet to be characterized structurally and biochemically. I have been able to solve two distinct conformational structures of ATP10A by cryoEM without addition of inhibitors or artificial lipids. Two different sample preparations show the majority of the particles to be stalled in an E2P like conformation with a lipid bound in the substrate binding pocket. This poses questions about potential autoinhibition of the flippase which differs greatly from known autoinhibitory mechanisms. The smaller subset shows an empty binding pocket and the transporter adopts an unknown conformation which could resemble the for now unsolved E2 lipid exit state. With this finding we could illuminate lipid exit beyond biochemical insights. Furthermore, purified ATP10A exhibits ATPase activity in a malachite green assay and is activated by phosphatidylinositol phosphate. These results taken together pose the basis to answer questions about autoregulation of ATP10A. Further structural investigations of ATP10A by cryoEM in parallel to MD simulations and biochemical characterization is ongoing.

References

[1] Takada, N., EMBO J. **2;37(9),** e97705 (2018)
[2] Norris, A. C., Scientific Reports **A14**, 343 (2024)
[3] Kita, N., J Lipid Res., **65(3)**, 100508 (2024)
[4] Martin, S., Acta Neuropathol., **139(6):** 1001-1024 (2020)
[5] Naito, T., J Biol Chem., **290(24):**15004-17 (2015)
[6] Dieudonne, T., Elife, **13:11:**e75272 (2022)
[7] Okamoto, S. MBoC, **31:19** (2020)

A top-down approach for studying Xenopus l*ae*vis MCM double hexamer formation by Cryo-EM

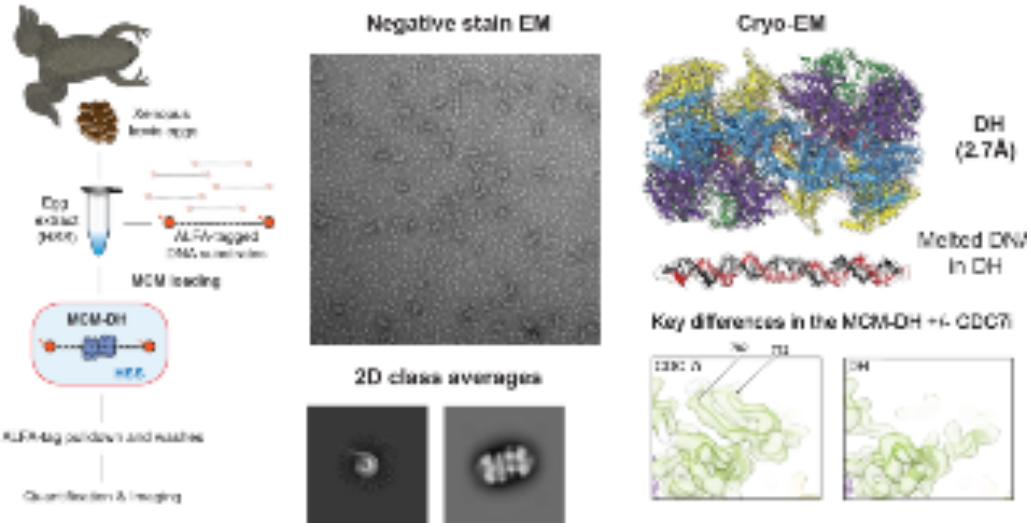
Christl Gaubitz[1], Manil Kanade[1], Ioana Maruntel[1], Marvin Weiler[1], Xueyuan Leng[2], Camilla Colding-Christensen[2], Ivo Hendriks[3], Michael Nielsen[3], Julien Duxin[2] and Thomas Miller[1]*

*[1]Center for Chromosome Stability, Department of Cellular and Molecular Medicine, University of Copenhagen, Copenhagen, Denmark, [2]Biotech Research and Innovation Centre, University of Copenhagen, 2200 Copenhagen, Denmark, [3]Proteomics Program, Novo Nordisk Foundation Center for Protein Research, University of Copenhagen, 2200 Copenhagen, Denmark *Corresponding Author*

DNA replication is a tightly regulated process essential for genome integrity. A key step is origin licensing, where two MCM2-7 helicases are loaded head-to-head onto DNA to form the inactive MCM double hexamer (MCM-DH) the foundation for replisome assembly. The Dbf4-dependent kinase CDC7 initiates replisome assembly by phosphorylating the MCM-DH complex. Traditional structural studies of such large assemblies require multi-step purifications and in vitro reconstitution that strip complexes of their native regulatory context. Here, we present EXPAND (Enrichment of Xenopus Protein Assemblies oN DNA), a novel top-down approach that isolates DNA-bound complexes directly from the near-native environment of Xenopus laevis egg extracts.

Using this system, we efficiently loaded MCM double hexamers (MCM-DH) onto template DNA in Xenopus egg extract. Mass spectrometry showed a 200-fold enrichment of MCMs and generated a proteomic atlas of DNA replication and repair proteins during origin licensing. The reaction was further assessed with negative stain EM to evaluate efficiency by observing loaded MCM-DHs. This allows us to assess the effects of nucleotides, inhibitors, and specific protein depletion on MCM loading. Using our method we solved the ryo-EM structure of the MCM-DH at 2.7 Å resolution, alongside a nucleosome at 2.4 Å, using an unsupervised processing pipeline. The high resolution of the structures enabled the accurate assignment of specific MCM isoforms and de novo model building from the density alone.

To further understand the role of specific phosphorylation, we used a CDC7 inhibitor (CDC7i). We solved the structure of the MCM-DH in the presence of CDC7i to 2.7 Å resolution and observed structural changes in the MCM-DH that may shed light on how CDC7 phosphorylation primes MCM-DHs for replisome assembly. Our work establishes a powerful method to directly visualize the molecular mechanisms of vertebrate DNA replication within a near-native environment.



Cryo-EM data-driven modeling of biomolecular recognition

Nandan Haloi[1], **Elisei Mankov**[2], **Rebecca J. Howard** [1,3], **and Erik Lindahl** [1,3]

[1] *Department of Applied Physics, Science for Life Laboratory, KTH Royal Institute of Technology,*
[2] *Department of Physics, Science for Life Laboratory, Stockholm University,*
[3] *Department of Biochemistry and Biophysics, Science for Life Laboratory, Stockholm University.*

Molecular recognition underlies both biological function and pharmaceutical efficacy. Accurate modeling of ligand binding—including interaction networks, conformational states, and geometries—is essential for understanding function and for guiding drug design. Although recent advances in single-particle cryo-EM have made it possible to resolve complex biomolecular assemblies at near-atomic resolution, ligand densities often remain poorly resolved, limiting structural insight at binding sites (Figure, *left*). Recently, artificial intelligence (AI)-based methods have been developed to model and refine structures based on EM data; however, they primarily focus on proteins and fail when applied to ligands. Here, we exploited the increased power of data-driven research and built an AI model to improve low-resolution ligand maps and refine structural models for small molecules in protein-ligand complexes (Figure, *right*). We further developed integrative AI and molecular simulation approaches to fit atomic ligand models into EM maps. We validated our approach on a set of biomedically relevant protein-ligand complexes, including kinases, GPCRs, and solute transporters, none of which were present in the AI training data. Our work demonstrates the power of integrating developing methods to decode the complex language of molecular recognition and holds promise for advancing both basic science and pharmaceutical innovation.

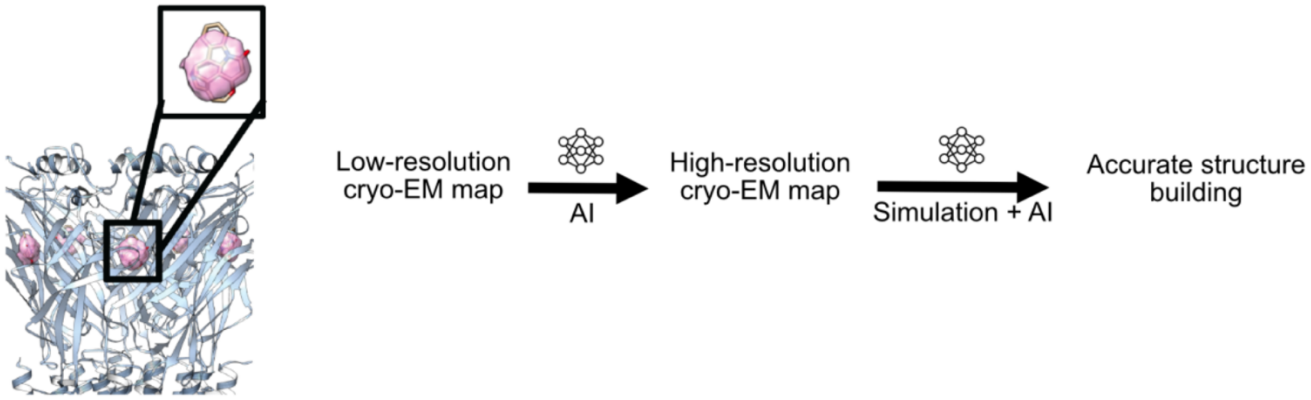


Figure : (*Left*) A depiction of poor ligand density in Cryo-EM maps, using the example of strychnine binding in glycine receptor. Without a careful fitting protocol, deciding the best orientation of the drug in this spherical blob-like density would be very difficult. (*Right*) A workflow developed in this work.

References:
N. Haloi, R. J. Howard, and E. Lindahl, "Cryo-EM ligand building using generative AI and molecular dynamics "*bioRxiv* , doi: https://doi.org/10.1101/2025.02.10.637508

Cryo-ET of influenza A virus infected cells and on-lamella dual-axis tomography

Moritz Wachsmuth-Melm[1,2], Sarah Peterl[1,2], Jana Makroczyová[1,2],Aidan O'Riain[2], Konstantin Fischer[1,2], Sílvia Vale-Costa[3,4,5], Santiago Gomez Melo[2,6], Cornelis Mense[2,6], Liv Zimmermann[1,2], Ulrich S. Schwarz[2,6], Maria João Amorim[3,4], and Petr Chlanda[1,2]

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[5] I3S-Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal

[6] Institute for Theoretical Physics, Heidelberg University, Heidelberg, Germany

Influenza A virus (IAV) is a membrane-enveloped, segmented RNA virus forming pleomorphic virions. Each of the eight segments consists of viral genomic RNA with negative polarity, nucleoproteins, and a tripartite polymerase complex, assembled into a helical vRNP complex. Viral genome replication and vRNP assembly take place in the nucleus. Upon nuclear export, vRNPs undergo Rab11-dependent trafficking to the plasma membrane, where virus assembly is orchestrated by the matrix protein M1 and the two glycoproteins hemagglutinin (HA) and neuraminidase (NA). Along this route, vRNP selection occurs; however, the site of vRNP clustering is not yet fully understood at the molecular level.

To shed light on this critical step in the viral replication cycle, we employed in situ cryo-electron tomography (cryo-ET). We found that HA-containing membranes interact with vRNPs in a Rab11-dependent manner. Three-dimensional segmentations combined with spatial analyses revealed that nearest-neighbour vRNP–vRNP distances are reduced in proximity to HA membranes, suggesting that HA membranes play an important role in vRNP clustering. We further show that the M1 protein forms a large multilayered helical complex in both the nucleus and cytosol, and subtomogram averaging suggests distinct M1–M1 interactions compared to those reported in budding virions. Finally, cryo-ET reveals that M1 layer formation in budding virions precedes membrane attachment and is critical for vRNP bundle formation. Taken together, our data show that vRNP clustering takes place on HA membranes and that the characteristic 7+1 vRNP arrangement occurs concomitantly with virus budding.

In addition, we discuss the feasibility of dual-axis cryo-ET on cryo-lamellae prepared using a trapezoid milling pattern. We show that dual-axis cryo-ET outperforms deep learning–based missing-wedge restoration algorithms. Finally, we employ von Mises stress calculations to optimize lamella stabilit

Balancing user support, throughput and innovation in a multi-user EM facility

Sonja Welsch

Central Electron Microscopy Facility, Max Planck Institute of Biophysics, Frankfurt am Main, Germany

The modes of operation of EM core facilities can vary significantly, ranging from a purely service driven mode - where expert facility staff handle all incoming samples and operate all instruments - to a strict hands-on user mode - where all researchers actively drive all experiments required for their projects.

A hands-on user mode of operation comes with pros and cons: it allows researchers to actively drive their own projects, control experimental conditions independently and it provides researchers with valuable hands-on expertise that can be utilized in their future career. This way of working also allows facility staff members to provide support that is tailored to user needs, typically leading to a more meaningful use of instrument time.
On the other hand, this mode of operation requires facility staff members to dedicate a significant portion of capacities to training and scheduling of newly incoming users and to create and maintain meaningful training documentation. In addition, it inevitably leads to instrument use by researchers who aren't specialists (yet) and to the need for a facility team to support users with instrument optimization and troubleshooting on a daily basis.

I will discuss how the Central Electron Microscopy facility at Max Planck Institute of Biophysics strives to balance the pros and cons of such a hands-on operation mode in a multi-user facility, how we attempt to optimize both quality and quantity of users' results, and to balance innovation and continuity of electron microscopy workflows by close collaboration with facility users as well as soft- and hardware suppliers.

Small molecules restore mutant mitochondrial DNA polymerase activity

Sebastian Valenzuela

Department of Medical Biochemistry and Cell biology

Mammalian mitochondrial DNA (mtDNA) is replicated by DNA polymerase γ (POLγ), a heterotrimeric complex consisting of a catalytic POLγA subunit and two accessory POLγB subunits. More than 300 mutations in *POLG*, the gene encoding the catalytic subunit, have been linked to severe, progressive conditions with high rates of morbidity and mortality, for which no treatment exists. Here we report on the discovery and characterization of PZL-A, a first-in-class small-molecule activator of mtDNA synthesis that is capable of restoring function to the most common mutant variants of POLγ. PZL-A binds to an allosteric site at the interface between the catalytic POLγA subunit and the proximal POLγB subunit, a region that is unaffected by nearly all disease-causing mutations. The compound restores wild-type-like activity to mutant forms of POLγ in vitro and activates mtDNA synthesis in cells from paediatric patients with lethal POLG disease, thereby enhancing biogenesis of the oxidative phosphorylation machinery and cellular respiration. Our work demonstrates that a small molecule can restore function to mutant DNA polymerases, offering a promising avenue for treating *POLG* disorders and other severe conditions linked to depletion of mtDNA.

Uncovering molecular determinants of antibody immunodominance using cryoEM

Aleksandar Antanasijevic

Swiss Federal Institute of Technology in Lausanne

B-cell immunity is an essential part of the adaptive immune system that contributes to pathogen clearance and protection against reinfection. Antibodies, the secreted form of B-cell receptors, recognize foreign molecular features called epitopes. Epitopes are typically protein regions exposed on pathogen surfaces but can also include glycans and lipids. Because some epitopes are more likely to be targeted than others, antibodies develop reproducible response patterns, a phenomenon known as immunodominance. Our research focuses on identifying the molecular determinants that govern these hierarchies, with the goal of understanding what makes certain epitopes preferentially recognized by B cells. In this talk, I will present our cryo-EM studies of antibody–epitope interactions and describe computational approaches we have developed to predict immunodominant sites in different antigens.

Seeing the small move: dissecting flexibility in a hemoglobin-binding receptor from *Staphylococcus aureus*

V. Buoli Comani[1], O. De Bei[2], M. Gragera[3], G. Paris[4], B. F. Luisi[4], M. Marchetti[2], L. Ronda[2,5], B. Campanini[1], S. Faggiano[1,5], S. Bettati[2,5]

[1] *Department of Food and Drug, University of Parma, Italy.* [2] *Department of Medicine and Surgery,University of Parma, , Italy.* [3] *Centro Nacional de Biotecnología (CNB), CSIC, 28049, Madrid, Spain.*[4] *Department of Biochemistry, University of Cambridge UK.* [5] *Institute of Biophysics, National Research Council (CNR), Pisa, Italy.*

Iron acquisition is a key virulence strategy of *Staphylococcus aureus* [1], which scavenges heme from host hemoglobin (Hb) via specialized hemophores of the Isd (iron-regulated surface determinant) system [2]. IsdH, a surface-exposed hemophore composed of three NEAT (NEAr iron Transporter, N) domains, captures heme directly from Hb. Enriching the knowledge of heme acquisition in *S. aureus* with high-resolution structural information will offer a valuable framework for a rational drug design campaign targeting iron uptake in bacterial pathogens.
Here, we used cryo-EM to investigate the structure of the preextraction complex formed between carboxyhemoglobin (HbCO) and the minimal functional construct IsdHN2N3 containing the N2 and N3 NEAT domains. Despite considerable sample heterogeneity, we successfully resolved the structure of several complexes with different stoichiometry at high resolution (below 4 Å). Among them, we reconstructed the structure of the 2to2 complex – consisting of one Hb dimer bound by two IsdHN2N3 – a notable achievement for such a small assembly (~115 kDa). By applying 3D classification without alignment, we further distinguished two 2to2 conformations (Figure 1A), both exhibiting significant flexibility of a single NEAT domain (~15 kDa, red circle).

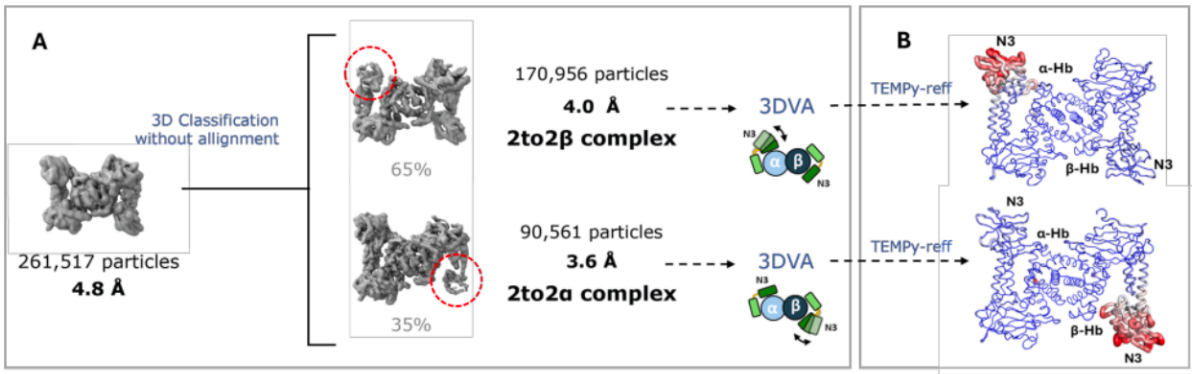

Figure 1. (A) Workflow of cryo-EM data processing of the 2to2 complexes. (B) Structural motion of the complexes, with flexibility visualized by backbone thickness and color-coded B-factors (blue: low, red: high).

To explore this conformational plasticity, we accessed the Instruct-ERIC Flexibility Hub (PID 32912) and employed 3D Variability Analysis (3DVA) [3], DynaMight [4], and ZART [5]. Using 3DVA, PCA identified two major components of motion for which we extrapolated intermediate volumes along these trajectories and fitted atomic models into the resulting maps using TEMPy-reff. [6]. To visualize continuous domain movement, we developed a custom Python script exploiting ProDy [7], providing a clear readout of local flexibility (Figure 1B). These results demonstrate that flexibility analysis tools can be successfully applied to very small regions of proteins.

[1] 10.3390/ijms21062145; [2] 10.1016/j.jinorgbio.2009.09.012;[3]10.1016/j.jsb.2021.107702; [4] 10.1038/s41592-024-02377-5; [5] 10.1016/j.jmb.2023.168088; [6] 10.1038/s41467-023-44593-1; [7]10.1093/bioinformatics/btab187

The Structure and Function of the CCHF Virus Polymerase

Adrian Deng[1], Franziska Guenl[2], Rory Cunnison[3], Loic Carrique[4], Nicole Robb[3], Jonathan M. Grimes[4], Jeremy Keown[1]

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Crimean-Congo haemorrhagic fever virus (CCHFV) is a member of the Nairoviridae family and the cause of localised high fatality human disease. The CCHFV genome consists of single-stranded, negative-sense RNA that is tri-segmented and packaged into ribonucleoprotein complexes. Each ribonucleoprotein complex comprises the viral RNA, multiple copies of the protective nucleoprotein, and a single copy of the viral L-protein (CCHFV-L), which performs genome replication and transcription. CCHFV-L is almost twice the size of other RNA virus L-proteins limiting functional inference from studies on related viruses. Multiple enzymatic functions and several substrate binding domains identifies CCHFV-L an ideal target for antiviral development.

To understand the function of the CCHFV-L we established a protocol for robust recombinant expression and purification of the 4000 amino acid polypeptide. To probe the activity of the endonuclease domain we developed an RNA cleavage assay, validating the full-length CCHFV-L contains a potent endonuclease which we could inactivate through specific mutagenesis. We next developed a cryoEM pipeline and determined high-resolution structures of the viral polymerase. Our structures reveal the architecture of the core catalytic RNA-dependent RNA polymerase domain, describing a ~2200 amino acid domain which approximately twice the size of other segmented negative sense L-protein cores that is stabilised by several divalent cations. Using our endonuclease inhibited CCHFV-L and cryoEM we next revealed how genomic RNA is tethered inside the viral core. Radionucleotide incorporation assays demonstrate the polymerase is highly active on a several of templates, low fidelity, and RdRp activity is not dependent the presence of both promoters. Collectively these results provide the foundations for future work to unravel the molecular basis of CCHFV replication/transcription and for therapeutic development.

The molecular basis of lamin specific chromatin interactions

Ohad Medalia

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In the cell nucleus, chromosomes are anchored to the nuclear lamina, a structure composed primarily of lamins and their binding proteins. The nuclear lamina constitutes a fibrillar layer that is situated beneath the inner nuclear membrane. The nuclear lamina, a structural layer of proteins that bridges between the DNA and the nuclear membrane, impact chromatin organization through the interaction with lamin associated domains (LADs) within the densely packed heterochromatin regions. Employing cryo-focused ion beam (cryo-FIB) milling and cryo-electron tomography (cryo-ET) allowed us to resolve the concentration of nucleosomes at the lamin-chromatin interface. The depletion of lamin A/C is resulting in a reduction of chromatin density at the lamina interface, suggesting the involvement of lamins in a direct interaction with chromatin. Employing cryo-electron microscopy (cryo-EM), we have identified the specific motif of lamin A tail domain that interacts with nucleosomes, distinguishing it from other lamin isoforms. Genome-wide analyses have revealed lamin-dependent macroscopic-scale alterations in gene expression and chromatin remodeling. The present findings offer insights into the dynamic and specific interplay between lamin isoforms and chromatin, thereby providing a more comprehensive understanding of the process of nuclear lamina-chromatin tethering.

New developments in Cryo-EM: Pushing boundaries for *in situ* structural determination and contextual imaging

Itziar Serna Martin

Thermo Fisher Scientific

Cryo-electron microscopy has revolutionised structural biology and our ability to explain with molecular precision how many macromolecules function. However, it is being increasingly recognised that studying these structures in isolation—away from the context of their native cell, tissue, and organism—leaves significant gaps in our understanding. This has reinforced the need to develop techniques that enable in situ structural characterization and volume imaging, bridging cellular biology and structural biology.

The introduction of the latest generation of plasma FIBs to biological sciences has opened the door to the imaging of cells and tissues through Slice & View (serial FIB SEM) both at room and cryo temperatures in a way that achieves optimal quality and resolution. Further combining volumetric imaging approaches with cryo-ET allows insight to be gained across orders of magnitude of scale.

This presentation will highlight the integrated workflow from plasma FIB lamella preparation and serial FIB-SEM volume imaging through to high-resolution data acquisition on the 300 kV TEM Krios 5. Advances in plasma FIB technology that enable precise lamella preparation and large-scale 3D imaging will be showcased, including how new AI-driven adaptive scanning and dynamic auto functions accelerate and expand volume data collection. Finally, we will discuss the latest developments in tomography and single particle analysis data acquisition, demonstrating how these innovations together open new avenues to study biological structures across scales, from cellular landscapes to molecular detail.

Open and closed forms of assembled henipavirus nucleoprotein suggest structural basis of genome access

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Henipaviruses, such as Nipah virus, can cause deadly illness and are designated as WHO blueprint priorities due to their pandemic potential. The RNA genomes of these pathogens are tightly packaged within a protective nucleocapsid consisting of 1000s of copies of the viral nucleoprotein N. To date, it is unclear how the encapsidated RNA is released from N to allow the viral polymerase to read its sequence during transcription. Here, we present the first high-resolution cryo-EM structure of a helical N-RNA assembly from an emerging henipavirus, Langya virus (LayV), at 3.1 Å resolution. We assemble the structure *in vitro* from purified N monomers by incubation with RNA. Using time-resolved fluorescence anisotropy, we show that the assembly efficiency of helical N-RNA from monomers is sequence dependent and prefers 5'-genomic sequences. While lateral contacts between N oligomers have been described previously, we are now able to also identify vertical interactions that are crucial to form the helical nucleocapsid, and test these by site-directed mutagenesis. Further, we solve the cryo-EM structure of a ring-like assembly of LayV N at 2.6 Å resolution. Surprisingly, and in contrast to previous studies, the 13-mer N assembly is devoid of RNA. Structural comparison of the RNA-bound and RNA-free LayV N shows a conformational opening and closing of N domains, even in the assembled state. Our data suggest that N within henipaviral nucleocapsids may undergo local conformational changes, switching between closed and open states, to temporarily allow access to the encapsidated RNA without disrupting the nucleocapsid.

Decoding the nanoscale architecture of intracellular lipid flux

Veijo Tuomas Verneri Salo

Julia Mahamids group, European Molecular Biology Laboratory, Heidelberg, Germany

Abstract: Lipid droplets (LDs) are endoplasmic reticulum (ER)-derived organelles essential for neutral lipid storage and cellular metabolism. Their biogenesis is thought to involve neutral lipid phase separation in the ER bilayer, coordinated by proteins such as the lipodystrophy protein seipin. Yet the molecular mechanism remains unresolved. We combine cryo-correlative light and electron tomography (cryo-CLEM) with genetically encoded nanoparticles (GEMs) to capture the structural timeline of LD maturation in human cells. GEMs enable nanoscale localization of seipin and other factors directly in tomograms. Our data reveal that seipin undergoes a striking conformational switch during LD formation, effectively "opening up" to permit droplet emergence. I will present these findings and highlight ongoing advances in cryo-CLEM workflows for studying dynamic membrane processes.

Simplified High-Pressure Freezing Workflow in Autogrids

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The study of purified proteins, bacteria and monolayered mammalian cells grown on EM-grids has become a routine method in cryo-EM over the last decades. Plunge freezing is the method of choice for such samples and reproducibly preserves biological structures in their native state. However, the study of thicker specimens that exceed the plunge freezing limit of ~10 µm remains challenging. High Pressure Freezing (HPF) is currently the only method that allows preservation of up to 200 µm thick samples in vitreous ice, by freezing samples in metal carriers called planchettes. HPF however is still a rather niche technique. One major drawback is that the freezing success is fairly low, since planchettes were originally not designed to freeze samples on EM grids and therefore don't meet their needs in terms of size, geometry and thickness. This significantly limits the throughput of the technique, as grids easily get damaged or bent during the freezing process. To streamline HPF on EM grids and improve the success rate, we developed a workflow for high pressure freezing samples directly on clipped Autogrids, which can be transferred directly to state-of-the-art cryo-FIB instruments. Therefore, we designed and optimized novel planchettes meant to accommodate already clipped grids, as well as compatible lids that regulate the sample thickness on top of the Autogrid, to enable waffle grid preparation as well as vitrification of tissue samples on grids. Directly freezing clipped grids improves the stability and reduces the grid handling steps required after freezing, as it eliminates potential risks such as grid bending during planchette removal, transferring and clipping steps. Our new Autogrid planchettes thus enable a more straightforward workflow specifically designed for subsequent FIB-milling and cryo-EM imaging.

A high-throughput workflow towards quantitative cryo electron tomography

Sebastian Tacke

Max Planck Institute of Molecular Physiology Structural Biochemistry

Cryo-electron tomography (cryo-ET) offers an unique possibility to study the architecture of cells and protein complexes in their native environment.
However, for clinically relevant specimens such as 3D cultures, organoids, and tissues, current workflows remain slow - preparing lamellae from these thick samples can take hours.

By integrating high ion-beam currents into the milling protocol, we reduced lift-out preparation for thick samples from hours to minutes while preserving lamella quality.
In parallel, we developed a glove box system that protects samples from ice contamination during handling and storage.

Taken together, these innovations enable high-throughput cryo-ET investigations of thick specimens, enabling quantitative analysis of medically relevant samples

Caught in a moment: dynamics of proteins at the nanoscale

Ilaria Testa

KTH Royal Institute of Technology, Stockholm, Sweden

Formation and dissociation of macromolecular complexes is one of the foundations of all cellular processes. However, state-of-the-art techniquedoesn't allow precise, efficient, and specific observation at the same time of arbitrary complex formation, especially in living cells.

We developed novel techniques such as STARSS, event triggered STED and MoNaLISA to study the dynamic nature of molecules in living cells beyond the diffraction limit of light.

By using fluorescence photo-switching, light patterning and real-time images' computation we provide new way to monitor elusive cellular process timely and efficiently with time-lapse super resolution imaging.

Molecular assemblies are further investigated by measuring rotational diffusivity. Rotational diffusion provides direct information on the "mass spectrum" and local environment of molecular complexes in solution and cells. It is usually measured via fluorescence anisotropy (FA) in steady-state and time-resolved (TR-FA) modes, which probe changes in molecular orientation over a time window defined by the fluorescence lifetime, which is 1-5 ns for commonly used fluorophores. FA is widely used within the life sciences for drug screening applications and binding assays due to its molecular specificity and sensitivity, high throughput, and compatibility with microscopy but only for small molecules (~0.1-30 kDa). Conventional TR-FA cannot reveal binding in most of the human proteome, because the molecular complexes are too large, i.e. they tumble too slowly to be distinguished from stationary within the nanosecond-scale time-window defined by the fluorescence lifetime.

We developed a novel approach named Selective Time-resolved Anisotropy with Reversibly Switchable States (STARSS) to bypass the fundamental limits of fluorescence anisotropy measurements in solution and living cells, extending the observable mass range more than three orders of magnitude and increasing the photo-selection accuracy throughfluorescence photo-switching. We used STARSS to investigate viral maturation, chromatin compactization and protein oligomerization in living cells.

Towards elucidating the structure and operating principles of primase-helicase TWINKLE from *Arabidopsis Thaliana*

Mira Dombi

Biotech REsearch and Innovation Centre, University of Chopenhagen

Plant organelles rely on nuclear-encoded proteins for DNA replication. Key components of this machinery in both chloroplasts and mitochondria include the helicase-primase Twinkle and the DNA polymerases Pol1A and Pol1B. Plant Twinkle, a Superfamily 4 (SF4) helicase, comprises an N-terminal primase domain and a C-terminal helicase domain, and assembles into homo-hexamers that unwind DNA in the 5'□3' direction. While some SF4 family members function exclusively as a helicase and rely on a separate primase (e.g., T4 gp41 with gp61), others, like T7 gp4, combine both helicase and primase activities in one polypeptide chain. Similar to its ancestral T7 gp4, atTwinkle uses ATP to unwind DNA and primes lagging-strand synthesis,while the priming activity has been lost in animal mitochondrial Twinkle. Despite its essential role in organellar DNA replication, the mechanisms behind DNA loading, translocation, and priming motif recognition followed by primer synthesis remain poorly understood.
To address these questions, we examined the structure and function of *Arabidopsis thaliana* Twinkle (atTwinkle) using biochemical assays and cryo-electron microscopy. Our results suggest that movements of a single monomer may facilitate atTwinkle self-loading onto DNA by opening the hexameric ring and allowing single-stranded DNA binding. atTwinkle translocates along DNA via a two-step mechanism driven by ATP hydrolysis. In addition, we show that atTwinkle primase activity is supressed in the absence of DNA and that the DNA binding triggers closure of the C-terminal helicase ring and the release of the N-terminal primase domain, suggesting that primer synthesis is tightly coupled to DNA binding and translocation.

Continuous serial electron diffraction (c-SerialED) for studying protein-ligand interactions and enzymatic redox reactions

Xiaodong Zou

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Understanding protein–ligand interactions and enzymatic redox processes requires structural information at high spatial resolution with minimal radiation-induced artefacts. While microcrystal electron diffraction (MicroED) has enabled macromolecular structure determination from sub-micrometre-sized crystals, it remains limited by cumulative radiation damage. Serial electron diffraction (SerialED), which merges single-shot patterns from tenth of thousands of crystals, overcomes this limitation but requires specialized instrumentation for crystal finding and beam control, limiting their accessibility.

We present a continuous SerialED (c-SerialED) protocol that simplifies data acquisition and works on any standard cryo-TEM. c-SerialED data are collected and processed continuously while the crystals on an EM-grid are scanning through continuous stage translation. Our method does not rely on pre-identification of crystals, enabling convenient, fast, and high-resolution c-SerialED data collection with negligible radiation damage. We show atomic-resolution lysozyme structure (to 0.83 Å) with well-resolved hydrogen sites, and high-resolution structure of a *dye-type heme peroxidase* DtpAa (1.3 Å) virtually free of radiation damage. We demonstrate the application of the method for rapid screening of protein–ligand interactions and capturing different redox states of metalloenzymes. c-SerialED thus emerges as a powerful and accessible tool for macromolecular structure determination, offering new possibilities for studying enzymatic function and ligand recognition.

Poster list

The posters will be displayed in **Brashörnan** throughout the symposium. The poster session will be from **16:10 to 17:30 on 1 October**. The evaluation committee will select the best poster, and the winner of the Best Poster Award will be announced during Session 3 on 2 October, at 10:15 (before the coffee break). The prize is kindly sponsored by Thermo Fisher Scientific.

Posters will be mounted the 1st of October upon registration, where they will give you poster board number in Brashörnan room.

Here is a link to the map.

The poster committee will select the best poster to be awarded the "best poster award", which will be announced during session 3 before coffee. The poster prize is kindly sponsored by Thermo Fisher Scientific.

N	Name	Title
1	Adriana Chrenková	Understanding sucrose transport in plants
2	Andreas Brech	Array STEM-tomography used for high volume imaging of hepatocytes and analysis of autophagy
3	Anna Munke	Visualizing a nuclear virus factory in diatoms
4	Anna Toft	Investigating the structure and function of cable bacteria truncated hemoglobins
5	Alexandra Náplavova	The Orphan Protein SLC12A8: Story of Anomalies
6	Alexia Gobet	Structural and functional study of cardiolipin synthases
7	Ayaan Ali	Structural and Functional Insights into A Human Zinc Transporter
8	Bas Leemburg	Cryo-ET of intracellular interactions between Mycobacterium spp. And their human host
9	Beatriz Castro	Toward the cryo-EM structural characterization of TRPA1 with novel antagonists
10	Bianca Korse	Structural basis of GRAF1-mediated membrane tubulation
11	Bina Singh	Role of NS protein in the formation of the Replication Complex in Langat Virus
12	Dariia Kuvshinova	Evaluating Calmodulin Binding Site Occupancy on TRPV5 Using OccuPy
13	Dev Thacker	Evolution of Ab42 fibril polymorphism in AppNL-F mice

14	Erwan Quignon	A deep dive into thick tissue
15	Gabriel Ducrocq	cryoSPHERE: Single-particle heterogeneous reconstruction from cryoEM
16	Himanshu Sharma	Illuminating the mysterious infection strategies of Microsporidia parasites
17	Irwin Selvam	Investigating phospholipid biosynthesis and bioenergetics in Mycobacterium tuberculosis-an integrated structural biology approach
18	Itziar Serna Martin	Cutting through the ice: how plasma FIBs are pushing the boundaries of cryo-EM.
19	Jan Silham	Cryo-EM Structures of the Human 20S Proteasome Bound to Small Molecule Covalent Inhibitors
20	Jane Corwin	Matrix protein as a cornerstone of non-segmented negative-sense RNA virus assembly
21	Jeremy Keown	The Structure and Function of the CCHF Virus Polymerase
22	Johan Unge	MicroED structure determination and developments
23	Johannes Messinger	Cryo-electron microscopy reveals hydrogen positions and water networks in photosystem II
24	Josy ter Beek	A first glimpse of a Gram-positive Type 4 Secrtion system that facilitates the spread of antibiotic resistance
25	Josephine Dannersø	Exploring the Axon Initial Segment by Cryo-ET and expansion microscopy
26	Kevin Homberg	Enhancing Precision and Throughput in Cryo-FIB Milling via an Optimized Correlative Imaging Workflow
27	Kieran Deane-Alder	Optimisations in sample preparation to determine the structure of a Gram-positive type IV secretion system
28	Koen Jurgens	Mechanistic insights of how ataxin-3 bindin is regulated by conformational changes in AAA+ ATPase VCP/p97
29	Léon Schierholz	Structural principles of Directed Evolution: The Structural Model of a Calcium regulated Affinity Binder
30	Liya Mukhamedova	Initial stages of enteroviral infections
31	Lorene Gonnin	Cryo-EM Analysis of a Post-translational Modification Impactating the Helical Symmetry of RSV Nucleocapsid
32	Lukas Peter Feilen	Structure and function of the human Na+/H+ exchanger 6

33	Mads Lykke Justesen	Visualizing the conductive structures of cable bacteria with cryo-electron tomography
34	Manil Kanade	A top-down approach for studying Xenopus l*ae*vis MCM double hexamer formation by Cryo-EM
35	Maia Francesca Vicino	A Copolymer-Based Approach for Structural Characterization of Acid-sensing Ion Channels
36	Marta Siborova	TBA
37	Maxim Rulev	Cryo-EM Uppsala: The Cryo-EM Facily at Uppsala University
38	Miguel Leung	Building structural atlases of giant molecular machines
39	Miriam Weber	Simplified High-Pressure Freezing Workflow in Autogrids
40	Misha Le Claire	In situ cryo electron tomography of Influenza A Virus for the study of viral assembly and export.
41	Mrinalini Ramanan	Just add water: GUVs as a tool for Aerolysin visualization via CryoEM
42	Nandan Haloi	Cryo-EM data-driven modeling of biomolecular recognition
43	Omar De Bei	Decoding Allosteric Regulattion in the Cysteine Synthase Complex: A Cryo-EM Approach to a Dynamic Protein-Protein Interaction
44	Olivia Andén	Vestibular Modulation by Stimulant Derivatives in a Pentameric Ligand-Gated Ion Channel
45	Rooshanie Ejaz	Structure of a contractile injection system in *Salmonella enterica* subsp. *salamae*
46	Sainath Polepalli	Structure-guided mechanistic decoding of *Mtb* Crystathionine ß-Synthase inhibition: A blueprint for rational design of covalent PLP enzyme inhibitors
47	Sara Henriksson	Volume Electron Microscopy by FIB-SEM at Umeå Centre for Electron Microscopy
48	Shekhar Jadhav	Dynamic assembly of a large multidomain ribozome visualized by cryo-electron microscopy
49	Sunanda Chhetry	Structural investigation of bacterial divisome complex
50	Swati Srivastava	Structural and functional analysis of AAA+ATPases enzymes and stress regulatory proteins from mycobacterial tuberculosis ESX-1 secretion system: Novel targets for drug development

51	Szabolcs Bodizs	Chemical mechanism of allosteric and asymmetric dark reversion in a bacterial phytochrome uncovered by cryo-EM.
52	Valeria Buoli Comani	Seeing the small move: dissecting flexibility in a hemoglobin-binding receptor from *Staphylococcus aureus*
53	Veronika Vundrová	Exploring Self-control: Autoinhibition of a Plant Lipid Flippase ALA10-ALIS1
54	Viktoria Svane	Expression and 3D reconstruction of Salmonella FimAfimbriae

Understanding sucrose transport in plants

Adriana Chrenková[1], Camilla G. Andersen[1], Laust Bavnhøj[1], Lorena Zuzic[1,2], Jan H. Driller[1] and Bjørn P. Pedersen[1]

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The SUC/SUT sucrose transporter family in plants plays a crucial role in the process of sucrose import from photosynthetic tissues into the phloem. Characterized by generally low affinity for their substrate, SUC transporters enable the accumulation of high concentrations of sugar in sink organs, such as fruits and seeds.

Members of the SUC/SUT family belong to the larger Major Facilitator superfamily and function as proton-driven symporters. The structural and biochemical characterization of the SUC1 transporter from *Arabidopsis thaliana* provided insights into the mechanism of sucrose binding and identified key active site residues required for proton-driven sucrose uptake.

We aim to further characterize sucrose transport in plants by obtaining structures of additional states of the protein from the transport cycles, including a substrate-bound state. Additionally, we will investigate other *A. thaliana* SUC transporters (SUC2–SUC9), with the hope of gaining a more comprehensive understanding of sucrose transport in plants.

References:

1. Bavnhøj, L., Driller, J.H., Zuzic, L. et al. Structure and sucrose binding mechanism of the plant SUC1 sucrose transporter. Nat. Plants (2023)

Array STEM-tomography used for high volume imaging of hepatocytes and analysis of autophagy

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We are studying a range of intracellular trafficking events using STEM-tomography often in combination with correlative light/electron microscopy (CLEM). STEM tomography in microprobe mode is a method to generate 3d-data from relatively thick biological samples (up to 1.5 micron), which can be done on both plastic embedded samples as well as cryo-fixed samples. Attainable resolutions in microprobe STEM is basically limited by the beam diameter and the preparation method, with useful resolution of around 1-2 nm in plastic embedded material. This resolution is 2-3 times higher than resolution obtainable from most FIB-SEM instruments and therefore presents an attractive choice when highest possible resolution is required. We characterize a variety of autophagic structures in hepatocytes to illustrate this advantage.

The FIB-SEM allows though for acquisition of larger sample volumes and is the method of choice for whole cell imaging and larger volumes. We therefore attempted to increase the possible sample volume to be imaged with STEM-tomography by performing large array tomography. This can be performed automatically using acquisition software such as SerialEM. We here present tomography data from a large array of tilt series (8x9) automatically stitched and aligned that represent a whole hepatocye slice of 1000 nm thickness at high resolution. This imaging technique therefore is an useful addition to whole cell imaging with FIB-SEM as it extends the resolution obtainable to a relatively large sample volume.

Poster #3

Visualizing a nuclear virus factory in diatoms

Johanna Gebhard[1], Kei Kimura[2], Anna Munke[3]

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The regulation of diatoms, a type of single-celled algae, is essential for maintaining healthy ecosystems on Earth [1]. Recent research has revealed that viruses play a key role as natural regulators of diatom populations. Despite their ecological importance, the molecular details of these virus-host interactions remain poorly understood.

A diatom virus of particular interest is the single-stranded DNA (ssDNA) bacilladnavirus, which replicates within the host cell nucleus [2]. In previous work, we determined the capsid structures of two bacilladnaviruses using cryo-EM and SPA, revealing host-specific structural features and evolutionary insights into their origin [3,4]. Additionally, we obtained low resolution reconstructions of the ssDNA genome within the capsids. Surprisingly, they revealed a spooled arrangement previously only observed in double-stranded DNA (dsDNA) viruses that use an NTP-driven motor protein to package their genomes into preformed capsids. However, no motor protein was observed in bacilladnaviruses, suggesting a potentially unique genome packaging and viral assembly mechanism.

While mature, infectious bacilladnaviruses are spherical, rod-shaped viral structures have been observed in the nucleus of infected diatom cells using negative-stain TEM [2], leading us to hypothesize that these rods are involved in viral assembly. To investigate this further, we therefore turned to FIB-SEM and cryo-ET.

The higher resolution and near-native conditions provided by cryo-ET revealed that the rods are, in fact, spherical particles arranged in a highly ordered and condensed manner, and possibly enclosed by membranes.

We will present this ongoing cryo-ET work aimed at visualizing the formation of this nuclear virus factory and characterizing its ultrastructural organization. This may ultimately provide insights into why the bacilladnavirus genome has a dsDNA-like arrangement.

References:

[1] Suttle CA. Viruses in the sea. Nature 2005;437:356–61.
[2] Kimura K, Tomaru Y. Discovery of Two Novel Viruses Expands the Diversity of Single-Stranded DNA and Single-Stranded RNA Viruses Infecting a Cosmopolitan Marine Diatom. Appl Environ Microbiol 2015;81:1120–31.
[3] Munke A, Kimura K, Tomaru Y, Wang H, Yoshida K, Mito S, et al. Primordial Capsid and Spooled ssDNA Genome Structures Unravel Ancestral Events of Eukaryotic Viruses. mBio 2022;13:e00156-22.
[4] Gebhard J, Tomaru Y, Okamoto K, Munke A. Structural diversity and conservation among CRESS-DNA bacilladnaviruses revealed through cryo-EM and computational modelling 2025.

Poster #4

Investigating the structure and function of cable bacteria truncated hemoglobins

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Cable bacteria are filamentous bacteria, with a unique ability to perform long-distance (centimeter) transport of electrons between spatially separated electron donors and acceptors. This is believed to occur through a conduction machinery consisting of periplasmic conductive fibers with putative associated factors involved in delivering to and extracting electrons from the fibers. An interesting protein family suggested to be involved in electron transport in cable bacteria is the pentaheme cytochrome (PHC) family. Certain members of this family have an N-terminal or/and C-terminal truncated hemoglobin (trHb) domain and were suggested to act as an alternative terminal oxidase.
To understand the role of trHbs in terminal oxidase activity of cable bacteria the truncated hemoglobin domains (trHbs) were expressed and purified in high quantities and purity and used for structural and functional studies. The experimental atomic structure of the N-terminal trHb (tN) was previously determined by X-ray crystallography. In contrast, it has so far not been possible to obtain the structure of the C-terminal trHbs (tC). This reason for this was that it was difficult to get crystals large enough for X-ray crystallography and the proteins are too small for single particle analysis by cryo-EM. We were able to produce tiny crystals suitable for microcrystal electron diffraction and are now pursuing the tC structure with this method. The results obtained will be presented.

Poster #5

The Orphan Protein SLC12A8: Story of Anomalies

Alexandra Náplavová[1,2], Rasmus Kock Flygaard1 & Poul Nissen[1,2]

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Our cells are a finely tuned system that relies on tight balance of salt concentration both within them and in their surroundings. To maintain this balance, ion transporters in the cell membrane work endlessly to shuttle ions. Among the diverse array of membrane transporters, a protein family dubbed SLC12 (Solute Carrier family 12) is currently in the spotlight of research – aiding the transport of sodium, potassium and chlorides1,2. Still, one member of this family, known as SLC12A8, remains a mystery, with no known function or structure2.

Based on computational predictions and few available uptake studies3,4, three large differences from the otherwise highly conserved family are expected for SLC12A8: its structure, transport substrate and localization. The predicted disordered region, missing dimerization domain, possible link to the transport of polyamines, and connection to the thyroid gland all pose a question – why is it different?

Therefore, we set to uncover the SLC12A8 function, by solving the molecular details by means of cryo-electron microscopy. As such, we have established expression in mammalian cells and optimised purification process, reaching sufficient yields for upcoming structural studies. The structure determination will require several sample optimisation strategies, as the small size and lack of fiducial of SLC12A8 presents an obstacle, touching on current boundaries of cryo-EM.

1. Trejo, F., Elizalde, S., Mercado, A., Gamba, G. & de los Heros, P. *American Journal of Physiology-Cell Physiology* 325, C921–C939 (2023).
2.Arroyo, J. P., Kahle, K. T. & Gamba, G. *Mol Aspects Med* 34, 288–298 (2013).
3.Grozio, A. *et al. Nat Metab* 1, 47–57 (2019).
4.Daigle, N. D. *et al. J Cell Physiol* 220, 680–689 (2009).

Structural and functional study of cardiolipin synthases

Alexia Gobet[1], **Rasmus Kock Flygaard**[1]

[1] *Molecular Biology and Genetics department, Aarhus University*

Mitochondria are delineated by two lipidic membranes and cardiolipin is one of the major lipid components of its inner membrane. This lipid is found in eukaryotic and prokaryotic organisms and tissues requiring a high level of energy (heart, liver, pancreas, kidney and small instestine) are enriched in cardiolipin. This lipid is composed of two phosphatidylglycerol bridged by one glycerol moiety and give it a conical shape that induces negative curvature to the membrane systems (Figure 1). Cardiolipin is essential for cell survival, but the process of its synthesis is not completely understood. The molecular details remain limited mainly due to the lack of structural knowledges on the cardiolipin synthase (Cls1) involved in the final step of cardiolipin synthesis. Indeed, no Cls1 experimental structure is available so far (Figure 2). I focus on the structure and function of the human Cls1, an integral membrane protein belonging to the CDP-alcohol phosphotransferase (CDP-AP) family1. After overexpression, I screen different detergents and optimize the purification to get a sample suitable for cryo-EM studies and functional assays.

In parallel, I work on native tissue to extract Cls1 and potential protein partners to get protein complexes, using differential centrifugation and density sedimentation methods2. Resolving the structure of cardiolipin synthase could help to decipher the molecular nature of cardiolipin synthesis and understand the link between the structure and the function of the enzyme.

(1) Houtkooper, R. H.; Akbari, H.; van Lenthe, H.; Kulik, W.; Wanders, R. J. A.; Frentzen, M.;Vaz, F. M. Identification and Characterization of Human Cardiolipin Synthase. *FEBS Lett* **2006**, *580* (13), 3059–3064.
(2) Serricchio, M.; Vissa, A.; Kim, P. K.; Yip, C. M.; McQuibban, G. A. Cardiolipin Synthesizing Enzymes Form a Complex That Interacts with Cardiolipin-Dependent Membrane Organizing Proteins. *Biochim Biophys Acta Mol Cell Biol Lipids* **2018**, *1863* (4), 447–457.

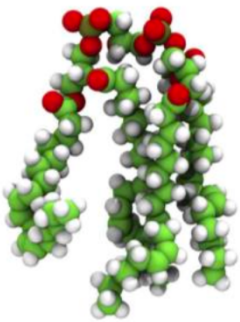
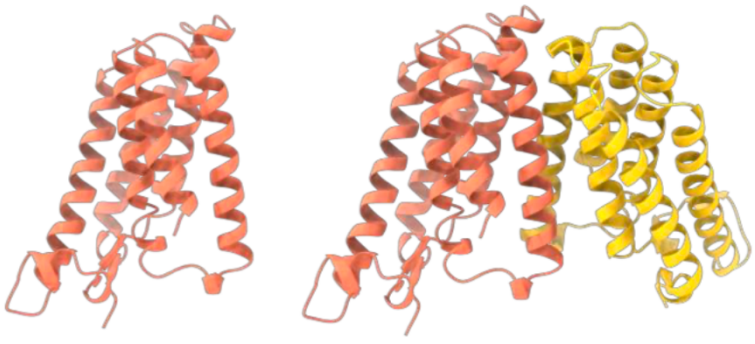


Figure 1: Cardiolipin Figure



2: Monomer (left) and homodimer (right) of Human cardiolipin synthase (AlphaFold 3 predicted models)

Structural and Functional Insights into A Human Zinc Transporter

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Mammalian zinc transporters are critical for maintaining zinc homeostasis in the cell. Two solute carrier families SLC30 (ZnT) and SLC39 (ZIP) are tasked with keeping the free cytosolic zinc concentration in the sub nanomolar range. Of the ZnT efflux transporters, only ZnT3 is localized on synaptic vesicles, implicating it in various neurological diseases such as Alzheimer's disease, schizophrenia, and febrile seizures. Despite recent contributions of ZnT cryoEM structures, low sequence homology and structure diversity within the ZnT family still necessitates elucidating the structural mechanisms by which ZnT3 translocates zinc to the synaptic vesicle and its role in neurological disease. Herein we report cryoEM structures of human ZnT3 with and without mutations in the conserved zinc translocation site at 3.1 Å and 3.8 Å respectively. ZnT3 forms a V-shaped, 84 kDa, homodimeric protein with protomer contact in the transmembrane domain (TMD) and the intracellular cytosolic domain. The transporter was captured in the inward facing conformation with the primary zinc ion coordinated around H108, D112, H238, and D242. This zinc arrangement was broken upon mutating the histidines to glutamines and the aspartic acids to asparagines in the mutant structure. Alongside the conserved translocation site, putative zinc transport sites in the histidine rich loop, the N-terminus, and in the TMD were mutated and were found to abolish zinc transport measured at the cell membrane at physiological pH and pH 6.0. Our findings confirm the long held putative Zn2+/H+ antiporter mechanism for ZnT3, and offer a structural framework for zinc translocation as well as provides insight into zinc dysregulation in cognitive disorders.

Cryo-ET of intracellular interactions between *Mycobacterium spp.* and their human host

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Within the genus *Mycobacterium* are highly pathogenic species, which are the causative agents of diseases such as tuberculosis, leprosy, and so-called 'non-tuberculous mycobacterial (NTM) diseases'.1,2 Mycobacteria canonically cause disease by colonising macrophages, predominantly in the lungs.3 In order to survive and infect their host, mycobacteria rely on large, membrane-bound protein complexes known as Type VII Secretion Systems (T7SS). 4 T7SSs are required to block merging of the macrophage lysosome and phagosome, for translocation to the host cytosol, and for infection of other macrophages, as well as for the uptake of metabolites and nutrients.5 Because of their vital role in both mycobacterial homeostasis and host infection, T7SSs present a potential target for the development of novel treatments against tuberculosis, leprosy, and other life-threatening mycobacterial diseases. It is therefore important that we understand these protein complexes in more detail, and especially in the context of its host environment. Cryo-ET is uniquely suited to help us understand these pathogenic microorganisms within their host cell. The aim of my project is to study T7SSs *in situ*. I will employ large-volume Cryo-ET workflows to image mycobacteria inside human macrophages at different stages of infection, such as during phagocytosis or phagosomal escape. I will also investigate actin 'comet tails'; a type of motility that is T7SS-dependent. The genus *Mycobacterium* is normally considered non-motile, but several *Mycobacterium* species are capable of recruiting host N-WASP actin elongation machinery using a small protein secreted by T7SS ESX-5. By doing this they can propel themselves forward through the host cell.6

References:

1. Gagneux, S. Ecology and evolution of Mycobacterium tuberculosis. *Nat. Rev. Microbiol.* **16**, 202–213 (2018).
2. Johansen, M. D., Herrmann, J.-L. & Kremer, L. Non-tuberculous mycobacteria and the riseof Mycobacterium abscessus. *Nat. Rev. Microbiol.* **18**, 392–407 (2020).
3. Stewart, G. R., Robertson, B. D. & Young, D. B. Tuberculosis: a problem with persistence. *Nat.Rev. Microbiol.* **1**, 97–105 (2003).
4. Roy, S., Ghatak, D., Das, P. & BoseDasgupta, S. ESX secretion system: The gatekeepers of mycobacterial survivability and pathogenesis. *Eur. J. Microbiol. Immunol.* **10**, 202–209 (2020).
5. Gröschel, M. I., Sayes, F., Simeone, R., Majlessi, L. & Brosch, R. ESX secretion systems: mycobacterial evolution to counter host immunity. *Nat. Rev. Microbiol.* **14**, 677–691 (2016).
6. Hill, N. S. & Welch, M. D. A glycine-rich PE_PGRS protein governs mycobacterial actin based motility. *Nat. Commun.* **13**, 3608 (2022).

Toward cryo-EM structural characterization of TRP with novel antagonists

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Transient receptor potential ankyrin-1 (TRPA1) is a widely expressed, non-selective cation channel sensitive to a variety of noxious stimuli. Due to its role as a sensor, this channel participates in a plethora of signaling pathways and has great influence over regulation of Ca2+ levels. TRPA1 has emerged as a therapeutic target for various diseases, including neurological disorders and cancer1,2. Inhibition of Ca2+ influx by channel blockade or by TRPA1 knockout have become potential strategies for disrupting TRPA1 activity for therapeutic benefit. However, the limited understanding of TRPA1´s compound-binding mechanisms and poorly resolved binding pocket structures present substantial challenges for the development of effective modulators. To date, available compounds lack specificity and potency against TRPA1, underscoring the need for improved pharmacological agents 3. Here, we present the pipeline we have established toward the determination of the cryo-EM structures of TRPA1 in complex with a novel class of antagonists. These structures, once determined, will provide additional understanding of TRPA1, particularly regarding its binding pockets and the mechanisms of compound binding.

References:

1. Talavera, K. *et al*. Mammalian Transient Receptor Potential TRPA1 Channels: From Structure
to Disease. *Physiol. Rev.* **100**, 725–803 (2020).
2. Takahashi, N. *et al*. Cancer Cells Co-opt the Neuronal Redox-Sensing Channel TRPA1 to Promote Oxidative-Stress Tolerance. *Cancer Cell* **33**, 985-1003.e7 (2018).
3. Koivisto, A.-P., Belvisi, M. G., Gaudet, R. & Szallasi, A. Advances in TRP channel drug discovery: from target validation to clinical studies. *Nat. Rev. Drug Discov.* **21**, 41–59 (2022).

Structural basis of GRAF1-mediated membrane tubulation

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GTPase Regulator Associated with Focal Adhesion Kinase-1 (GRAF1) is a membrane-remodeling protein that belongs to the large GRAF protein family. Clathrin-independent carriers (CLICs) is a major endocytic pathway, which is distinguished by their characteristic tubular membrane morphology and dependence on GRAF1. GRAF1 associates with membranes through its N-terminal Bin/Amphiphysin/Rsv (BAR) domain and adjacent Pleckstrin Homology (PH) domains. BAR domains are dimeric, crescent-shaped membrane-binding modules that generates membrane curvature through oligomerization driven scaffolding. In addition, GRAF1 contains a central RhoGAP domain and a C-terminal SH3 domain that mediates protein-protein interactions. However, the regulation and stabilization of BAR-mediated membrane remodeling by neighboring domains is poorly understood.

Interestingly, cross-linking experiments with purified GRAF1 truncates (BAR-PH and BAR-PH-GAP) and artificial liposomes indicate that formation of stable oligomers at the membrane is GAP-domain dependent. Moreover, pull-down assays confirm the interaction between GAP and BAR-PH domains. Furthermore, the purified BAR-PH-GAP truncate is sufficient to tubulate FOLCH liposomes in vitro and cryogenic electron microscopy (cryo-EM) reveals a protein coat, possibly with helical symmetry, on the membrane tubes.

Structural insights into the spatial arrangement of BAR, PH, and GAP domains are key to understanding the GRAF1-mediated membrane-sculpting mechanism. Therefore, we aim to determine the structure of purified GRAF1 (BAR-PH-GAP truncate), both in solution and assembled on membrane tubes, using cryo-EM and single particle reconstruction.

Role of NS protein in the formation of the Replication Complex in Langat Virus

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Flaviviruses are positive-sense RNA viruses whose intracellular replication is marked by endoplasmic reticulum (ER) membrane remodelling. The genome replication step is one of the least understood processes in the viral life cycle. Upon flavivirus infection, the viral genome is translated as a single polyprotein comprising both capsid and non-capsid ("non-structural", NS) proteins. The polyprotein, upon protease cleavage, forms inverted buds on the ER, called replication organelles (ROs). ROs are the site of viral genome replication. Inside the membrane bud, the NS proteins co-localise with the viral genomic RNA and bring about its replication. But, how the non-enzymatic NS proteins (NS2A, NS2B, NS4A, NS4B) initiate the formation of ROs or partake in genome replication is yet to be deciphered. Using cryo-ET, we have recently studied the RCs *in situ* in Langat virus, a BSL2 model organism for TBEV. The tomograms highlighted the occurrence of macromolecular densities at the membrane neck of ROs, which on segmentation yielded a molecular mass of approximately 500 kDa. In this study, we investigate the probable role of NS4B in the formation of this 'neck'. NS4B, has been successfully expressed in mammalian and insect expression systems and affinity purified using Flag-tag in the presence of detergent. Also, we have used Fluorescence Size Exclusion Chromatography (FSEC) with a GFP tag to assess the protein's oligomeric state, which aligns well with our AlphaFold multimer model. In the future, we aim to investigate the molecular details of NS4B using cryo-EM and establish its role in the formation of ROs.

Evaluating Calmodulin Binding Site Occupancy on TRPV5 Using OccuPy

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Cryo-EM enables the visualization of macromolecular complexes in multiple conformational or compositional states. However, determining ligand binding site occupancy remains a challenge. *OccuPy* [1] estimates relative occupancy in cryo-EM maps and helps identify and quantify binding sites in heterogeneous datasets.

This study aims to validate the performance of *OccuPy* in detecting calmodulin (CaM) binding site occupancy on TRPV5 using publicly available cryo-EM data. The datasets used are full-length TRPV5 in nanodisc and TRPV5 with CaM bound [2]. Mixed datasets representing different occupancies are constructed by combining randomly selected particles in varying proportions to emulate heterogeneity. *OccuPy* is applied to the reconstructed 3D density maps to estimate CaM binding site occupancy. This seeks to validate current computational methods for ligand detection in cryo-EM by creating controlled occupancy mixtures, to demonstrate OccuPy's ability to quantify compositional heterogeneity, and to suggest future avenues of research.

References:

[1] Forsberg BO, Shah PNM, Burt A. A robust normalized local filter to estimate compositional heterogeneity directly from cryo-EM maps. Nat Commun. 2023. doi:10.1038/s41467-023-41478-1.

[2] Dang S, van Goor MK, Asarnow D, Wang Y, Julius D, Cheng Y, van der Wijst J. Cryo-EM structure of TRPV5 full length in nanodisc. EMPIAR-10254 & EMPIAR-10256. 2019.

Evolution of Ab42 fibril polymorphism in AppNL-F mice

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Advances in high resolution cryoelectron microscopy (cryo-EM) have provided ground-breaking insights into structures of amyloid fibrils *in vitro* and *ex vivo*. Conventionally, *in vitro* fibril structures are solved at the end point of the amyloid self-assembly process, but recent studies on human islet polypeptide (IAPP) and tau have shown that fibril structures change over time along the aggregation process and polymorph distribution varies at each stage. *Ex vivo* patient derived samples have provided great insights into the fibril structures formed in tissue and how they differ from *in vitro* fibril structures of the same peptide. Such *ex vivo* protein structures have also shown the involvement of non-proteinaceous cofactors with disease related amyloid fibrils in the case of a-synuclein, tau, as well as amyloid b 40 and 42. However, what biomolecule contributes to this non-proteinaceous electron density has so far remained unknown. Here, we study the structures of Ab42 fibrils purified *ex vivo* from App^{NL-F} Alzheimer's disease model mouse brains using cryo-EM at three different ages of mice. We identify a novel Ab42 fibril structure that shows the presence of a strong electron density along the length of this fibril structure indicates interaction of Ab42 with biomolecules present in the brain tissue. Similar non-proteinaceous density has previously been observed from E22G Ab42 fibrils purified ex vivo from App^{NLG-F} mouse brains. Using immunogold labelling negative stain EM, we identify this biomolecule to be a glycosaminoglycan, possibly hyaluronic acid, which is present abundantly in the extracellular matrix of the brain. Confocal microscopy also shows co-localization of fluorophore labelled to Ab42 with fluorophore labelled to hyaluronic acid as well as chondroitin sulfate. Both these glycosaminoglycans are present abundantly in the extracellular matrix of the brain, and their interactions with Ab42 can have vital implications in the pathology of Alzheimer's disease.

A deep dive into thick tissue

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The Mononegavirales order comprises non-segmented negative-sense single-stranded RNA viruses such as Ebola virus, Nipah virus, and human Metapneumovirus (hMPV). These viruses utilize liquid membrane-less organelles, named inclusion bodies, which concentrate viral components and serve as sites of both transcription and replication. hMPV is an upper tract respiratory virus responsible for approximately 0.5 million hospital admissions annually with no vaccine or specific treatments available. Previous studies have shown that nucleoprotein (N) and phosphoprotein (P) interact with each other to induce the formation of inclusion bodies. Since no *in situ* structural data exists to date, we aim to study the structure of hMPV-induced inclusions using two models: LLC-MK2 cells, the standard cell line for hMPV infection, and an Air-Liquid Interface (ALI) system that mimics the human respiratory epithelium.

To this end we are developing a novel workflow to obtain *in situ* structures in the ALI system. First, we optimized growth and differentiation conditions for ALI on cryogenic electron microscopy grids. By optimising plunge freezing parameters and fine-tuning DeepFiB milling, we were able to mill through 25-30 µm thick tissue and obtain vitreous lamellas. Our ongoing work focuses on validating this method by obtaining structural data from these lamellas. Successful implementation of this workflow will allow us to obtain new insights in the structural biology of hMPV and other respiratory viruses in an analogous system of the human respiratory epithelium.

cryoSPHERE: Single-particle heterogeneous reconstruction from cryoEM

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The three-dimensional structure of proteins plays a crucial role in determining their function. Protein structure prediction methods, like AlphaFold, offer rapid access to a protein's structure. However, large protein complexes cannot be reliably predicted, and proteins are dynamic, making it important to resolve their full conformational distribution. Single-particle cryo-electron microscopy (cryo-EM) is a powerful tool for determining the structure of large protein complexes. Importantly, the numerous images of a given protein contain underutilized information about conformational heterogeneity. These images are very noisy projections of the protein, and traditional methods for cryo-EM reconstruction are limited to recovering only the one or a few consensus conformations.

Here, we introduce cryoSPHERE, which is a deep learning method that uses a nominal protein structure (e.g., from AlphaFold) as input, learns how to divide it into segments, and moves these segments as approximately rigid bodies to fit the different conformations present in the cryo-EM dataset. This approach provides enough constraints to enable meaningful reconstructions of single protein structural ensembles. We show that cryoSPHERE is very resilient to the high levels of noise typically encountered in experiments, where we see consistent improvements over the current state-of-the-art for heterogeneous reconstruction[1]

Illuminating the mysterious infection strategies of Microsporidia parasites

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Microsporida parasites are obligate intracellular parasites that infect both vertebrate and invertebrate hosts. To initiate infection, environmental spores translocate their infective cytoplasmic content through a thin, hollow superstructure known as the polar tube. Following this infection, the sporoplasms manipulate the host cellular milieu, thereby kicking off their replicative stages, which often leads to exponential growth of the parasite and their release from host cells to further the infection. For host manipulation, the parasites utilize a variety of effector proteins and RNA that are secreted into the host cells; however, their exact nature and mechanism of deployment are poorly understood. Similarly, the molecular architecture of the polar tube is not well resolved due to technical challenges in purifying the polar tube components and the rapid speed of polar tube firing. To address these challenges, our lab employs a combination of biochemical and in situ approaches to study microsporidia infection and host cell remodeling events. Here, we utilize SPA and cryoET to investigate the architecture of the polar tube and intracellular replication events of these parasites.

Investigating phospholipid biosynthesis and bioenergetics in Mycobacterium tuberculosis – an integrated structural biology approach

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Mycobacterium tuberculosis (*Mtb*) is the primary causative agent of tuberculosis (TB) in humans. In 2023 alone, 10.8 million people fell ill with TB and there were 1.3 million deaths. This places TB as the leading cause of death due to a single infectious agent worldwide. Given the prevalence of drug-resistant strains, there is an urgent need for novel therapeutics that are efficacious, well-tolerated and cost-effective. The complex *Mtb* cell envelope is a key determinant of pathogenicity in humans. In *Mtb*, the biosynthesis of cell envelope constituents involves a number of essential enzymes. Structural and functional divergence between human and *Mtb* pathways may allow for selective inhibition of the latter. Our current work involves the characterization of a number of these enzymes using X-ray crystallography, microED and cryo-EM. The introduction of Bedaquiline showed that targeting enzymes involved in *Mtb* bioenergetics was a valid approach. Following on from recent cryo-EM structures of the *M. smegmatis* respiratory supercomplex, we aim to utilise cryo-ET to answer pressing questions about its structure *in vivo*.

Cutting through the ice: how plasma FIBs are pushing the boundaries of cryo-EM

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Over the last few years, cryo-electron microscopy has revolutionised structural biology and our ability to explain with molecular precision how many macromolecules function. However, it is being increasingly recognised that studying these structures in isolation- away from the context of their native cellular, tissue and organism level context, leaves significant gaps in our understanding. This has reinforced the need to develop techniques that are capable of handling larger samples and bigger volumes.

The introduction of a latest generation of plasma FIBs to biological sciences has opened the door to the imaging of samples that have only undergone cryo preservation (as opposed to chemical fixation). This includes both the imaging of cells and tissues through Cryo Slice & View, providing volumetric data with the best Z resolution presently possible, but also the production of lamellae from cell and tissue samples up to 200 µm in thickness. These lamellae can be imaged using cryo-electron tomography (cryo-ET), which is becoming the method of choice for structural characterization of proteins and protein complexes in their native environment. The advances in sample preparation by Focused Ion Beam milling have also been matched by improvements in both software and hardware of cryo-TEMs, namely with the introduction of the latest generation 300kV Krios 5 cryo-TEM. These have made a significant difference to the speed, reproducibility and quality with which tilt series can be acquired.

This talk will explore the latest advancements in the applications of plasma FIBs, highlighting their pivotal role in volume imaging and sample preparation for cryo-electron tomography (cryo-ET). Furthermore, we will discuss the future of cryo techniques and volume imaging, and how their convergence promises to produce higher-quality data and open new avenues for biological research.

Cryo-EM Structures of the Human 20S Proteasome Bound to Small Molecule Covalent Inhibitors

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Targeting the human 20S proteasome, the central unit of protein degradation, is a well-established strategy for cancer therapy, especially against multiple myeloma. However, the limited selectivity and toxicity of current inhibitors hinder their wider therapeutic use. Here, we present two cryo-electron microscopy (Cryo-EM) structures of the human 20S proteasome in complex with two novel covalent small molecule inhibitors, Cp-17 and Carmaphycin B (CpB).

The high-resolution structures reveal the binding modes of these inhibitors within the proteasome's active sites, providing critical insights into their irreversible mechanism of action. Our data highlight key interactions with specific catalytic subunits and residues within active sites of the two β-rings of the 20S proteasome, confirming the compounds' potent inhibitory effects.

These findings not only structurally validate Cp-17 and CpB as promising lead molecules but also offer a structural blueprint for the rational design of next-generation proteasome inhibitors with enhanced specificity and reduced side effects. This research advances our understanding of proteasome inhibition and its therapeutic potential

Poster #20

Matrix protein as a cornerstone of non-segmented negative-sense RNA virus assembly

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The replicative molecular mechanisms of non-segmented negative-sense RNA viruses (nsNSVs) remain regrettably underexplored. These include the recently identified Langya henipavirus, which causes flu-like illness in humans. Related viruses, human and avian metapneumovirus, cause seasonal illness and death every year. All three viruses contain a structural matrix protein (M), which has been shown to be vital to virion formation in other nsNSVs, such as respiratory syncytial virus (RSV), measles, and Nipah virus. It has been demonstrated that M interacts with other structural proteins and cofactors as part of the viral assembly process and constitutes part of the minimum basis for virus-like particle (VLP) formation. In RSV, M coordinates with the viral membrane fusion protein (F) to promote VLP assembly. Measles and Nipah virus M proteins bind membrane phospholipids and undergo a conformational change that deforms the lipid bilayer.
This work applies a structural virology approach to elucidating M's role as a coordinator of nsNSV assembly. The M dimer measures around 100 kDa, near what has historically been the lower limit for particle size in cryo-EM studies. However, advances in cryo-EM data collection and processing techniques allow us to obtain increasingly detailed structural information on increasingly small biomolecules. We aim to gain detailed insight into M's role in viral assembly for nsNSVs through single-particle studies of M in complex with other viral proteins and cofactors, including F and membrane-associated phospholipids of interest.

These single-particle studies naturally require the development of a protocol for purification of recombinantly overexpressed M from different nsNSVs in sufficient quantity for structural studies. M's tendency to self-assemble and its lipid-binding properties pose a particular challenge to obtaining large quantities of non-aggregated pure protein. We have optimized the purification strategy, which has allowed us to begin cryo-EM experiments. Establishing M's role in viral assembly will paint a more detailed picture of the nsNSV replicative cycle, providing a basis for future therapeutic studies targeting these crucial steps of viral assembly.

The Structure and Function of the CCHF Virus Polymerase

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Crimean-Congo haemorrhagic fever virus (CCHFV) is a member of the Nairoviridae family and the cause of localised high fatality human disease. The CCHFV genome consists of single-stranded, negative-sense RNA that is tri-segmented and packaged into ribonucleoprotein complexes. Each ribonucleoprotein complex comprises the viral RNA, multiple copies of the protective nucleoprotein, and a single copy of the viral L-protein (CCHFV-L), which performs genome replication and transcription. CCHFV-L is almost twice the size of other RNA virus L-proteins limiting functional inference from studies on related viruses. Multiple enzymatic functions and several substrate binding domains identifies CCHFV-L an ideal target for antiviral development.

To understand the function of the CCHFV-L we established a protocol for robust recombinant expression and purification of the 4000 amino acid polypeptide. To probe the activity of the endonuclease domain we developed an RNA cleavage assay, validating the full-length CCHFV-L contains a potent endonuclease which we could inactivate through specific mutagenesis. We next developed a cryoEM pipeline and determined high-resolution structures of the viral polymerase. Our structures reveal the architecture of the core catalytic RNA-dependent RNA polymerase domain, describing a ~2200 amino acid domain which approximately twice the size of other segmented negative sense L-protein cores that is stabilised by several divalent cations. Using our endonuclease inhibited CCHFV-L and cryoEM we next revealed how genomic RNA is tethered inside the viral core. Radionucleotide incorporation assays demonstrate the polymerase is highly active on a several of templates, low fidelity, and RdRp activity is not dependent the presence of both promoters. Collectively these results provide the foundations for future work to unravel the molecular basis of CCHFV replication/transcription and for therapeutic development.

MicroED structure determination and developments

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The CryoEM technique MicroED, complimentary to both Single-Particle Analysis and X-ray Crystallography. First implemented in 2023 at Umeå Center for Electron Microscopy (UCEM) it is now an established technique in Umeå. Several targets have been successfully structurally determined using MicroED supporting local, national and international researchers. Small molecule structures can be determined directly from powder samples facilitating analysis of only partially crystallized samples. In addition, UCEM is also setting up protein structure determination from nano-crystals, which are too small for X-ray synchrotron beamlines.

At the same time as hardware and software have been updated at UCEM to support MicroED, for both manual and automatic mode, new local developments of algorithms and software allow for better data to be collected and the use of a more robust workflow ensures that the MicroED analysis at Umeå is unique and enables structure determination of the most difficult samples.

Current focus on protein crystals addresses ways to identify nano-sized crystals in a high-throughput approach, undetectable by conventional light microscopy, that may be suitable for MicroED structure determination. This will be an important contribution to protein crystallography where crystal formation is limited in size.

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

Cryo–electron microscopy reveals hydrogen positions and water networks in photosystem II

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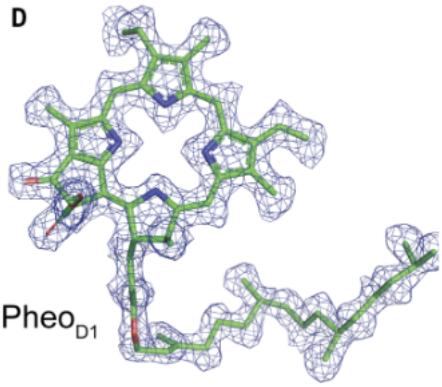
Photosystem II starts the photosynthetic electron transport chain that converts solar energy into chemical energy and thus sustains life on Earth. It catalyzes two chemical reactions: water oxidation to molecular oxygen and plastoquinone reduction. Coupling of electron and proton transfer is crucial for efficiency; however, the molecular basis of these processes remains speculative owing to uncertain water binding sites and the lack of experimentally determined hydrogen positions. We thus collected high-resolution cryo–electron microscopy data of fully hydrated photosystem II from the thermophilic cyanobacterium *Thermosynechococcus vestitus* to a final resolution of 1.71 Å. The structure reveals several previously undetected partially occupied water binding sites and more than half of the hydrogen and proton positions. This clarifies the pathways of substrate water binding and plastoquinone B protonation.



Coulomb density and model of a chlorophyll molecule in the reaction center of Photosystem II.

Reference:

Rana Hussein, André Graça, Jack Forsman, A. Orkun Aydin, Michael Hall, Julia Gaetcke, Petko Chernev, Petra Wendler, Holger Dobbek, Johannes Messinger, Athina Zouni, and Wolfgang P. Schröder (2024) Science 384, 1349 – 1355.

A first glimpse of a Gram-positive Type 4 Secretion system that facilitates the spread of antibiotic resistance

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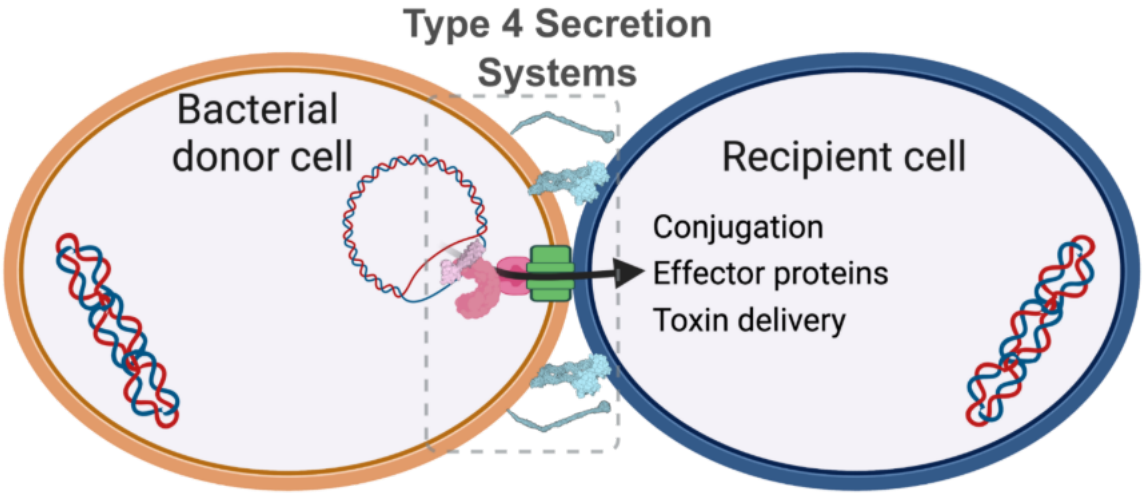
Abstract: Type 4 Secretion Systems (T4SSs) are main drivers for the spread of antibiotic resistance genes and virulence factors in bacteria. T4SSs are versatile, megadalton sized, complexes that facilitate the transfer of proteins and DNA from a donor bacterium to a recipient cell, which can be from a different species. Studies in the past decades have advanced our understanding of how these systems work in Gram-negative bacteria. However, so far little is known about their structure and function in Gram-positive bacteria, while these bacteria account for more than half of all hospital-acquired infections. These bacteria have a much thicker peptidoglycan barrier, that will have to be crossed by the DNA-protein substrate.

Our lab studies the T4SS that is encoded on the conjugative pCF10 plasmid from the commensal Gram-positive bacterium and opportunistic pathogen *Enterococcus faecalis*. By combining x-ray crystallography and electron microscopy with *in vitro* biochemistry and *in vivo* microbiology assays, we have now gained an understanding of multiple processes within our T4SS.

Currently we are working towards the structure of the transport channel. Using new bioinformatics tools, such as AlphaFold3, we have made a hypothetical model for central parts of the channel to guide our future experiments. Via pull-downs from *E. faecalis* and negative stain electron microscopy, we have obtained the first glimpse of a Gram-positive Type 4 Secretion system. We are also optimizing fluorescent labelling techniques to be able to use correlated light and electron microscopy (CLEM) to visualize the T4SSs directly in *E. faecalis* cells.



Exploring the Axon Initial Segment by Cryo-ET and expansion microscopy

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Neuronal signaling is crucial to function of the vast network of neurons that are found in the brain. Essential to this is both ultrastructural compartmentalization of the single cell into e.g. somatodendritic vs axonal compartments, and on a smaller scale, microenvironments in e.g. the plasma membrane allowing for tightly controlled ion-flow. With the aim of studying neuronal function and action potential initiation and regulation, the work presented here focuses on the ultrastructure of the Axon Initial Segment (AIS). The AIS is an approx. 20-60 um long compartment of the axon localized between the soma and distal axon and is crucial to action potential initiation and regulation. Interestingly, an overall tight organization with a periodicity of ~190 nm of the cytoskeleton and numerous related scaffolding- and membrane- proteins has been found in the AIS, and this is of great importance for the specialized function of the AIS.

This project employs two leading imaging methods for elucidating cellular ultrastructure, combining cryo-electron tomography (cryo-ET) and expansion microscopy (ExM). With ExM we work with immunolabelling constituents to study their relative localisation and stoichiometry, overcoming the diffraction limit by physically expanding the sample. To reveal the ultrastructural organization of protein complexes at the AIS in their native environment we employ cryo-ET on labelled and cryo-fixated neurons.

Primary hippocampal neuronal culture was cultured directly on EM-grids and antibody labeling enabled localization the AIS. Cryo-ET data of labeled AIS was collected, and analysis showed great potential for future focused studies on different aspects of the AIS ultrastructure. The 3D information from labelled cryo-ET data in combination with immunofluorescent expansion microscopy studies showed the presence of two ankyrin G (AnkG) molecules pr. 190 nm in the organized AIS scaffold, and the results furthermore highlight the possibilities of combining different imaging methods in studies of a complex ultrastructure as that of the AIS. Preliminary cryo-CLEM and cryo-FIB-milling studies have furthermore been initiated, and a protocol for obtaining high quality cryo-ET data for future structural analysis of ion channels and pumps of the plasma membrane has been established

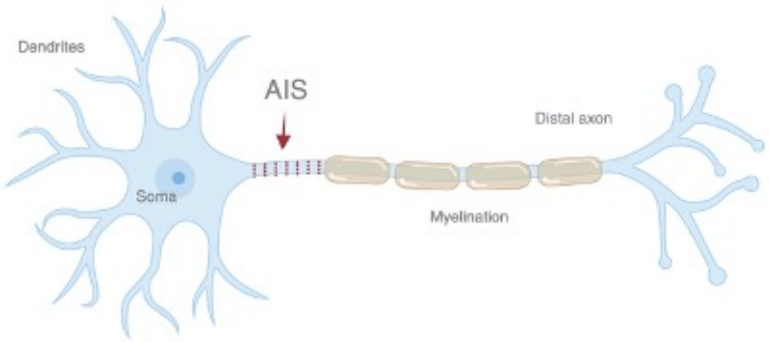

Figure: A schematic overview of a neuron with important compartment highlighted (soma, dendrites, axon, myelination and AIS).

Enhancing Precision and Throughput in Cryo-FIB Milling via an Optimized Correlative Imaging Workflow

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Correlative cryo-focused ion beam (cryo-FIB) milling is a powerful sample preparation technique for *in situ* cryo-electron tomography (cryo-ET), enabling the visualization of cellular components in their native state at near-atomic resolution. A key bottleneck in this workflow, however, remains the efficient and precise correlation between fluorescently-labeled regions of interest (ROIs) and the milling position, particularly for high-throughput applications [1-4].
This challenge often limits the feasibility of studying rare or transient biological events.

To address this challenge, we have developed and implemented a streamlined correlative method utilizing an integrated fluorescence microscope with significantly enhanced capabilities. Our approach leverages an expanded field of view, which accelerates initial sample mapping by providing broader cellular context in a single acquisition. Furthermore, optimizations to the optical path increases the SNR with 50%, improving the precision of ROI identification while minimizing potential phototoxicity through shorter exposure times.

We demonstrate the efficacy of this workflow with two challenging applications. For the serial lift-out of a high-pressure frozen C. elegans, precise fluorescence-guided trench milling resulted in a high success rate, with 27 of 29 prepared lamellae containing the targeted ROI. For on-the-grid milling of primary rat neurons, the method enabled rapid, multi-channel mapping of the entire sample grid and reliable ROI verification in the final, thinned lamella.

These methodological advancements significantly improve the speed, precision, and throughput of the cryo-FIB workflow. By making the identification of ROIs more robust and efficient, this approach facilitates the structural investigation of complex biological questions that were previously intractable, paving the way for deeper insights into cellular architecture and function.

[1] M. Smeets et al. (2021). Micros Today, vol. 29, no. 6, pp. 20–25.
[2] D. Boltje et al. (2022). eLife, vol. 11
[3] L. Wang et al. (2023) Nature Methods, Vol. 20 no. 2 pp. 276-283
[4] J. Yaeng et al. (2023) Microscopy and Microanalysis, vol. 29, no. 1, pp-1055-1057

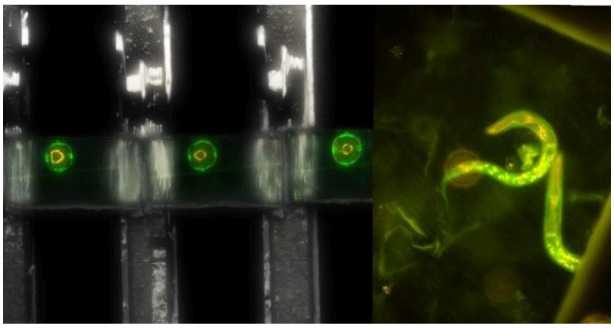

Fig. 1: Correlative serial lift-out workflow of C.elegans

Optimisations in sample preparation to determine the structure of a Gram-positive type IV secretion system

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The spread of antimicrobial resistance (AMR) is driven by the transfer of mobile genetic elements, such as plasmids containing resistance genes, between bacteria. Conjugative type IV secretion systems (T4SS) enable this transfer by transporting substrate-bound single-stranded DNA from donor cells to their recipients.

The recent high-resolution structure of the Gram-negative (G-) T4SS from the R388 plasmid has proven the utility of cryogenic electron microscopy methods (cryo-EM) to obtain vital structural and functional information on these complex molecular machines (1). However, it has so far been challenging to translate methods used on G- systems to those of Gram-positive (G+) organisms, and therefore there is almost no structural information available on assembled conjugative G+ T4SS. In particular, the lack of a stabilising outer membrane complex, and difficulty in establishing heterologous expression, have been limiting factors.

Our lab uses the conjugative T4SS from the pCF10 tetracycline resistance plasmid of *Enterococcus* species as a model G+ system. By engaging in systematic method optimisation, we have established a procedure to natively express, pull-down, and characterise the pCF10 T4SS using EM methods. We also present low-resolution data on its architecture, and hope that these methods will help establish general procedures for the structural biology of G+ T4SS.

Mechanistic insights of how ataxin-3 binding is regulated by conformational changes in AAA+ ATPase VCP/p97

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Ataxin-3 (ATX-3), a deubiquitinase of the Machado-Joseph disease (MJD) protein family1, plays a critical role in regulating protein degradation through its interaction with valosin containing protein (VCP/p97), an essential hexameric ATPase of the AAA+ superfamily2. ATX-3 binds the inter-lobe cleft of p97's N-terminal domain (NTD) via its VCP-binding motif (VBM)3, modulating substrate processing within the ubiquitin-proteasome system (UPS) by remodelling polyubiquitin chains4. Under physiological conditions, this interaction ensures proteostasis by facilitating the clearance of misfolded proteins. However, expansion of ATX-3's polyglutamine (polyQ) tract results in toxic protein aggregation, disruption of p97 function, and the onset of spinocerebellar ataxia type 3 (SCA3). While high-resolution structures of isolated p97 have been resolved, structural characterization of p97 in complex with ATX-3 remains incomplete. Previous work suggests that ATX-3 binding is controlled by specific nucleotide states of p97's D1 ATPase domain which induce up-down conformational changes of the NTD3, but this has yet to be confirmed structurally. In addition, elucidating the structure of p97 bound to both physiological and aggregation-prone forms of ATX-3 is essential for understanding their molecular interplay and how this interaction is disrupted in disease. We structurally unveil the interaction of p97 with healthy ATX-3 13Q (meaning 13 glutamines in the polyQ tract) using cryo-electron microscopy (cryo-EM). We show the VBM binding in detail and how the binding is exclusively possible in the more compact down conformation of p97's NTD. Our high-resolution structures also allow for stoichiometry determination, and analysis of the relation between NTD positioning and nucleotide state in p97.

References

1. Li, X., Liu, H., Fischhaber, P. L. & Tang, T.-S. Toward therapeutic targets for SCA3: Insight into the role of Machado–Joseph disease protein ataxin-3 in misfolded proteins clearance. Prog.Neurobiol. 132, 34–58 (2015).
2. Ogura, T. & Wilkinson, A. J. AAA+ superfamily ATPases: common structure–diverse function. Genes Cells 6, 575–597 (2001).
3. Rao, M. V., Williams, D. R., Cocklin, S. & Loll, P. J. Interaction between the AAA+ ATPase p97 and its cofactor ataxin3 in health and disease: Nucleotide-induced conformational changes regulate cofactor binding. J. Biol. Chem. 292, 18392–18407 (2017).
4. Burnett, B. The polyglutamine neurodegenerative protein ataxin-3 binds polyubiquitylated proteins and has ubiquitin protease activity. Hum. Mol. Genet. 12, 3195–3205 (2003).

Structural principles of Directed Evolution: The Structural Model of a Calcium regulated Affinity Binder

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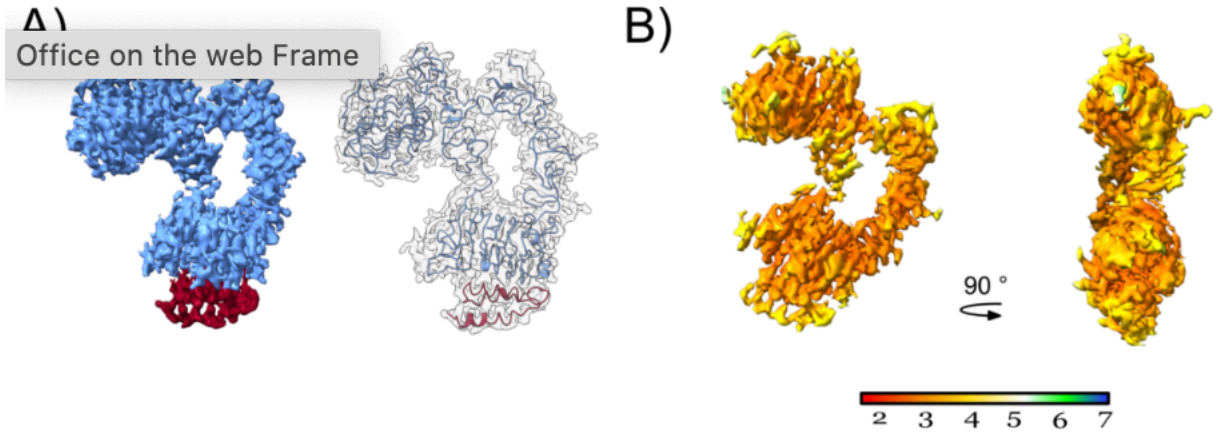

The engineering of protein–drug conjugates is a rapidly advancing field with significant potential for targeted therapies, particularly in oncology. Such approaches rely on the selective recognition of disease-specific biomarkers combined with the efficient delivery and retention of cytotoxic payloads, thereby minimizing off-target effects. CaRA is a small scaffold protein with an intrinsic calcium-binding motif that functions as an environmental switch1. It has been engineered to conditionally bind the epidermal growth factor receptor (EGFR) in the presence of calcium. By exploiting the steep calcium gradient encountered during endocytic trafficking—from the extracellular space into the lysosomal environment—CaRA enables efficient internalization of drug conjugates while permitting non-destructive recycling of EGFR back to the plasma membrane. Here, we present an experimentally validated cryo-EM structural model of the CaRA:EGFR complex, providing molecular insight into the interaction interface. This structure reveals the evolutionary trajectory by which the scaffold was optimized to engage the cytosolic fraction of EGFR with nanomolar affinity, establishing a basis for its application in next-generation targeted therapeutics.

Jönsson M, Mushtaq AU, Nagy TM, von Witting E, Löfblom J, Nam K, Wolf-Watz M, Hober S. Cooperative folding as a molecular switch in an evolved antibody binder. J Biol Chem. 2024 Nov;300(11):107795. doi: 10.1016/j.jbc.2024.107795. Epub 2024 Sep 19. PMID: 39305954; PMCID: PMC11532951.

Initial stages of enteroviral infections

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Enteroviruses are a diverse group of non-enveloped viruses that cause illnesses ranging from the common cold to severe encephalitis. At the initial stage of cell entry, binding to specific receptors, together with endosomal acidification, promotes structural changes in the viral capsid and subsequent genome release. However, for successful delivery into the cytoplasm, the viral genome must overcome two major barriers: the capsid shell and the endosomal membrane.
In our research, we used *in situ* cryo-electron microscopy to visualize the early stages of enteroviral infections. In contrast to prevailing models, our experiments with multiple enteroviruses did not reveal the formation of endosomal pores. Furthermore, particles captured during genome release *in vitro* and *in situ* exhibited partial disassembly of the capsid, suggesting that genome release may occur through a larger opening, allowing passage of the genome without unwinding its secondary structures. Together, these observations indicate that enteroviruses exploit both capsid opening and cell-mediated endosome rupture for efficient genome delivery into the cytoplasm, providing insight into a shared strategy likely employed by other members of the Picornaviridae family.

Cryo-EM Analysis of a Post-translational Modification Impacting the Helical Symmetry of RSV Nucleocapsid

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Respiratory syncytial virus (RSV) is the prototypical member of the *Pneumoviridae family*. Its nonsegmented negative-strand RNA genome is replicated and transcribed by viral ribonucleoprotein complexes, which consist of the helical nucleocapsid (NC) - formed by the genomic RNA coated with the nucleoprotein N - and the associated RNA-dependent RNA polymerase complex. We recently discovered that helical RSV NCs obtained by expression of N in insect cells have a non-canonical helical symmetry, with 16 nucleoproteins displaying different tilts and axial shifts per asymmetric unit spanning circa 1.5 helical turns, and demonstrated the role of the C-terminal arm of the RSV N in this unique assembly (Gonnin et al., Nature Communications 2023). Given that post-translational modifications (PTMs) are widely recognised as rapid and reversible regulators of protein structure, function, and activity, we sought to investigate potential links between PTMs and the helical arrangement of RSV NCs.
Here, we show how a single mutation that mimics a specific PTM of the N-terminal arm of the nucleoprotein dramatically affects nucleocapsid organisation. This ongoing work marks a new step towards elucidating the roles of PTMs in regulating RSV RNA synthesis, as well as the structural origins and functional implications of the non-canonical helical symmetry of RSV nucleocapsids.

Structure and function of the human Na+/H+ exchanger 6

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Sodium/hydrogen exchangers (NHEs) are ubiquitously expressed transport proteins that reside in cellular membranes and play a pivotal role in regulating cellular and organellar pH and ion homeostasis. The human Na+/H+ exchanger 6 (HsNHE6) is a key player in endosomal pH and ion homeostasis, particularly in neurons, where its dysfunction has been linked to neurological disorders such as Christianson syndrome (CS) and Alzheimer's disease (AD). Despite its physiological significance, the molecular mechanism of HsNHE6 has remained elusive.

This study aims to provide a molecular understanding of the ion transport mechanism of HsNHE6, focusing on its structural and functional characteristics and thereby providing insights into its relevance to neurological disorders.

We used an integrative approach combining single particle (SP) cryo-EM, NMR spectroscopy, small-angle X-ray scattering (SAXS), mass spectrometry (MS), and ion transport assays to uncover the molecular basis of HsNHE6 function and its dysfunction in pathological conditions.

We report the first high-resolution SP cryo-EM structure of HsNHE6 at 3.4 Å resolution, revealing its homodimeric architecture with 13 transmembrane (TM) helices per protomer. In our structure, HsNHE6 is in an inward-open conformation with additional densities observed in proximity to side chain residues corresponding to the ion binding site revealing key features of the ion-binding site. By integrating our cryo-EM structure with NMR and SAXS data of the distal and flexible part of the C-terminus, we generated a composite model of the near full-length HsNHE6. Utilizing an ATPase-based liposomal assay, we could probe the activity of HsNHE6, which is capable of transporting Na+ and K+ in liposomes composed of brain polar lipid extracts, confirming its role in endosomal pH and ion homeostasis.

Altogether, our structural and functional findings offer a more detailed mechanistic understanding of HsNHE6 function and pave the way for exploring its role in neurological disorders and potential therapeutic interventions.

Visualizing the conductive structures of cable bacteria with cryo-electron tomography

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Cable bacteria are multicellular filamentous bacteria that found across the globe in underwater sediments. Here, they couple sulfide oxidation and oxygen reduction by the most sophisticated system of biological nanowires known to exist. The electrical system is known to consist of two main structural compartments: A 30-nm bundle of fibrils running through the filaments' periplasm, known as the periplasmic conductive fiber (PCFs); and a complicated network of membrane invaginations present at the boundary between cells, known as the junction lamellae which contain the core lamella sheet (CLS). While the PCF is responsible long-distance electron transport (LDET) across several centimeters, the CLS interconnects the PCF and thereby acts as a fail-safe in case localized damage to the filament. In this study, we employed cryo-electron tomography to determine the *in situ* native structure of these remarkable biological conductors. We show that PCF consists of extremely thin fibrils deemed PCF strand components (PSCs) with a diameter of below 2 nanometers. Energy-dispersive X-ray spectroscopy confirm that the PSCs contain the nickel-sulfur-based cofactor proposed as the conductive component of the PCF. Additionally, we show that the CLS consists of equally narrow plates, called CLS plate components (CPCs), with a thickness also below 2 nanometers that are contained within the junction lamellae of the cell junctions. The extremely thin nature of both PSCs and CPCs is unprecedented among biological nanowires and might allow direct electron hopping between the conductive centers of these two components, as well as to periplasmic cytochromes and other soluble electron transporters.

A top-down approach for studying Xenopus l*ae*vis MCM double hexamer formation by Cryo-EM

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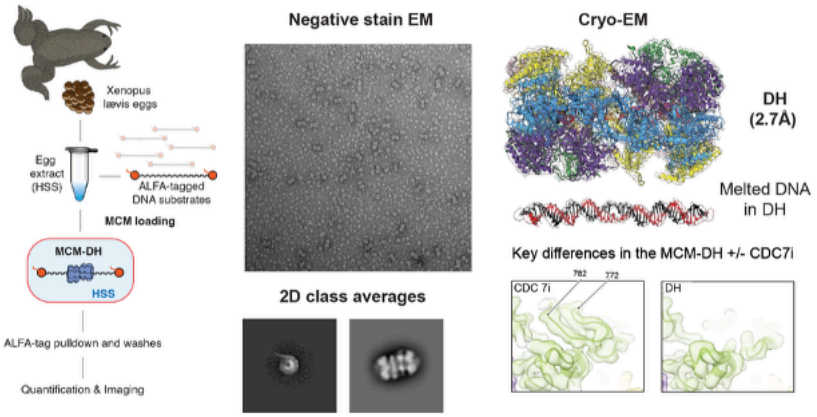
DNA replication is a tightly regulated process essential for genome integrity. A key step is origin licensing, where two MCM2-7 helicases are loaded head-to-head onto DNA to form the inactive MCM double hexamer (MCM-DH) the foundation for replisome assembly. The Dbf4-dependent kinase CDC7 initiates replisome assembly by phosphorylating the MCM-DH complex. Traditional structural studies of such large assemblies require multi-step purifications and in vitro reconstitution that strip complexes of their native regulatory context. Here, we present EXPAND (Enrichment of Xenopus Protein Assemblies oN DNA), a novel top-down approach that isolates DNA-bound complexes directly from the near-native environment of Xenopus laevis egg extracts.

Using this system, we efficiently loaded MCM double hexamers (MCM-DH) onto template DNA in Xenopus egg extract. Mass spectrometry showed a 200-fold enrichment of MCMs and generated a proteomic atlas of DNA replication and repair proteins during origin licensing. The reaction was further assessed with negative stain EM to evaluate efficiency by observing loaded MCM-DHs. This allows us to assess the effects of nucleotides, inhibitors, and specific protein depletion on MCM loading. Using our method we solved the ryo-EM structure of the MCM-DH at 2.7 Å resolution, alongside a nucleosome at 2.4 Å, using an unsupervised processing pipeline. The high resolution of the structures enabled the accurate assignment of specific MCM isoforms and de novo model building from the density alone.

To further understand the role of specific phosphorylation, we used a CDC7 inhibitor (CDC7i). We solved the structure of the MCM-DH in the presence of CDC7i to 2.7 Å resolution and observed structural changes in the MCM-DH that may shed light on how CDC7 phosphorylation primes MCM-DHs for replisome assembly. Our work establishes a powerful method to directly visualize the molecular mechanisms of vertebrate DNA replication within a near-native environment.



A Copolymer-Based Approach for Structural Characterization of Acid-sensing Ion Channels

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Human acid-sensing ion channels (hASICs) are membrane embedded proton-gated channels expressed in the central and peripheral nervous systems. Their dysfunction is linked to pain sensing, loss of memory, neuronal cell death following an ischemic stroke, and to various neurodegenerative diseases. Therefore, these proteins are particularly attractive therapeutic targets for drug development. Structural insights into hASICs are crucial for understanding their function and for guiding rational drug design.

Our aim is to determine the structures of different hASIC isoforms and to investigate how potential drug candidates bind and modulate their function. To achieve this, it is essential to establish a robust purification protocol. However, membrane proteins present a particular challenge, as traditional purification methods often involve the removal of the native lipid environment, potentially disrupting essential native interaction partners and compromising protein stability or function. One alternative approach that preserves the native lipid environment around the membrane protein is using amphiphilic copolymers.

We use MAASTY copolymers as membrane mimetic systems, which are developed in our group and at DTU. MAASTYs consist of methacrylic acid (MAA) and styrene (STY), which are synthesized by reversible addition-fragmentation chain-transfer (RAFT) polymerization. This allows control over the copolymer length. In these first proof-of-concept experiments we demonstrate that MAASTY copolymers enable successful solubilization and purification of various hASIC homologs, and that these are very promising tools for high-resolution structure determination. By preserving the native lipid environment, our approach represents a crucial first step towards the structural and functional characterization of hASICs in different conformational and ligand-bound states laying the foundation for future drug discovery efforts.

Cryo-EM Uppsala: The Cryo-EM Facility at Uppsala University

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Cryo-EM Uppsala is a comprehensive facility that provides tailored cryogenic electron microscopy solutions to users of all experience levels. The platform supports the entire workflow for single particle cryo-EM analysis, offering state-of-the-art instrumentation and expert services for sample preparation, screening, data collection, and advanced image processing. Key instruments include a Mark IV Vitrobot for sample vitrification and a 200kV Glacios electron microscope now equipped with a Falcon IV direct electron detector and Selectris energy filter, enabling high-throughput, automated screening and enhanced data acquisition quality. The facility also hosts a local GPU hub for real-time data processing and 3D model building using leading software packages such as Relion and Cryosparc. Users benefit from hands-on training, flexible support, and a streamlined project submission system, making Cryo-EM Uppsala an essential hub for structural biology research.

Building structural atlases of giant molecular machines

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The microtubule-based axoneme is one of the largest macromolecular complexes in eukaryotic cells. Axonemes drive the rhythmic beating of motile cilia, which are essential for reproduction, development, and homeostasis in mammals. However, available models of mammalian axonemes are incomplete due to challenges imposed by the sheer scale and complexity of this assembly. Here we apply cryo-electron microscopy and cryo-electron tomography to solve structures of axonemal doublet microtubules from diverse mammalian tissues, including sperm flagella and epithelial cilia from the oviduct, brain ventricles, and respiratory tract. We use our cryo-EM maps in conjunction with proteomics and artificial intelligence (AI)-enabled protein structure modelling to build the most complete atomic model of a mammalian sperm axoneme to date. Our model defines the identities, localizations, and interactions of >150 unique proteins, including microtubule-binding proteins, motor proteins, chaperones, and kinases associated with metabolism and signalling **(Fig. 1)**. Our results illustrate how an evolutionarily conserved molecular machine is adapted for diverse functions through the addition of cell type specific protein modules. Our structures serve as molecular atlases for guiding and interpreting genetic and biochemical studies towards understanding the molecular pathology of ciliopathies and infertility, thus exemplifying the tremendous discovery power of modern structural biology.

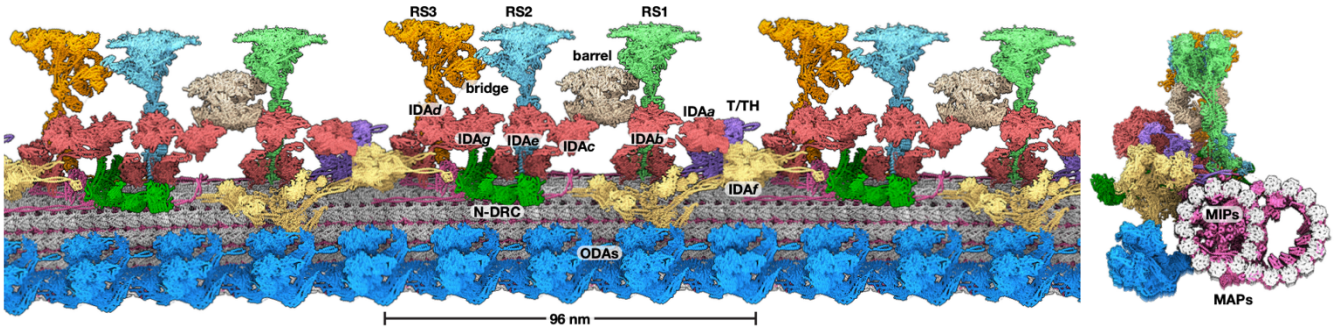

Figure 1. Atomic model of the 96-nm repeat of axonemal doublet microtubules from mammalian sperm flagella. Labels: RSs - radial spokes, IDAs - inner dynein arms, ODAs - outer dynein arms, N-DRC - nexin-dynein regulatory complex, T/TH - tether/tetherhead complex, MIPs - microtubule inner proteins, MAPs - microtubule-associated proteins

Simplified High-Pressure Freezing Workflow in Autogrids

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The study of purified proteins, bacteria and monolayered mammalian cells grown on EM-grids has become a routine method in cryo-EM over the last decades. Plunge freezing is the method of choice for such samples and reproducibly preserves biological structures in their native state. However, the study of thicker specimens that exceed the plunge freezing limit of ~10 µm remains challenging. High Pressure Freezing (HPF) is currently the only method that allows preservation of up to 200 µm thick samples in vitreous ice, by freezing samples in metal carriers called planchettes. HPF however is still a rather niche technique. One major drawback is that the freezing success is fairly low, since planchettes were originally not designed to freeze samples on EM grids and therefore don't meet their needs in terms of size, geometry and thickness. This significantly limits the throughput of the technique, as grids easily get damaged or bent during the freezing process. To streamline HPF on EM grids and improve the success rate, we developed a workflow for high pressure freezing samples directly on clipped Autogrids, which can be transferred directly to state-of-the-art cryo-FIB instruments. Therefore, we designed and optimized novel planchettes meant to accommodate already clipped grids, as well as compatible lids that regulate the sample thickness on top of the Autogrid, to enable waffle grid preparation as well as vitrification of tissue samples on grids. Directly freezing clipped grids improves the stability and reduces the grid handling steps required after freezing, as it eliminates potential risks such as grid bending during planchette removal, transferring and clipping steps. Our new Autogrid planchettes thus enable a more straightforward workflow specifically designed for subsequent FIB-milling and cryo-EM imaging

Just add water: GUVs as a tool for Aerolysin visualization via CryoEM

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Enzymatic processing of proAerolysin (pAel) results in the cleavage of its C-terminal peptide region (CTP) leading to oligomerization, and subsequent pore formation in target membrane environments[1].

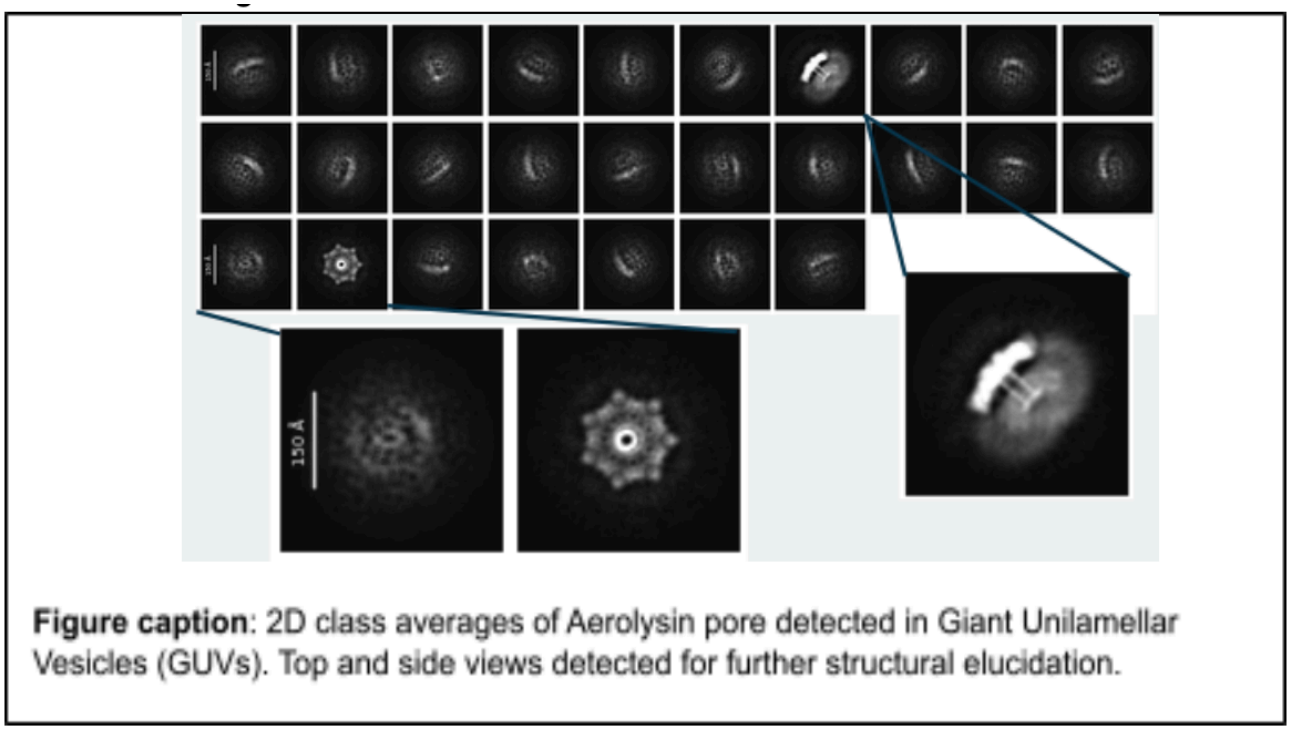

Figure caption: 2D class averages of Aerolysin pore detected in Giant Unilamellar Vesicles (GUVs). Top and side views detected for further structural elucidation.

In structural studies, proteolysis of Aerolysin for CTP removal to study oligomerization mechanisms has largely been mimicked *in vitro* via Trypsin incubation[1]. However, proteolytic cleavage using Trypsin results in fibril formation that impedes both mechanistic and structural characterization of the Aerolysin nanopore[2]. Here, we utilize Giant Unilamellar Vesicles (GUVs) to provide a native membrane environment that promotes Aerolysin pore formation and insertion that simultaneously circumvents the pesky fibril formation challenge. Further structural characterization via cryogenic electron microscopy (CryoEM) resulted in both top and side views of the heptamer, which are currently being employed in ongoing structure refinement of the Aerolysin pore. Future studies would involve the physiological assessment of the GUVs via electrical recordings during pore insertion and analyte trapping.

References: [1]F. G. van der Goot, J. Lakey, F. Pattus, C. M. Kay, O. Sorokine, A. Van Dorsselaer, J. T. Buckley, *Biochemistry* **1992**, *31*, 8566. [2]I. Iacovache, et. al., *Nat. Commun.* **2016**, *7*, 1.

Poster #42

Cryo-EM data-driven modeling of biomolecular recognition

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Molecular recognition underlies both biological function and pharmaceutical efficacy. Accurate modeling of ligand binding—including interaction networks, conformational states, and geometries—is essential for understanding function and for guiding drug design. Although recent advances in single-particle cryo-EM have made it possible to resolve complex biomolecular assemblies at near-atomic resolution, ligand densities often remain poorly resolved, limiting structural insight at binding sites (Figure, *left*). Recently, artificial intelligence (AI)-based methods have been developed to model and refine structures based on EM data; however, they primarily focus on proteins and fail when applied to ligands. Here, we exploited the increased power of data-driven research and built an AI model to improve low-resolution ligand maps and refine structural models for small molecules in protein-ligand complexes (Figure, *right*). We further developed integrative AI and molecular simulation approaches to fit atomic ligand models into EM maps. We validated our approach on a set of biomedically relevant protein-ligand complexes, including kinases, GPCRs, and solute transporters, none of which were present in the AI training data. Our work demonstrates the power of integrating developing methods to decode the complex language of molecular recognition and holds promise for advancing both basic science and pharmaceutical innovation.

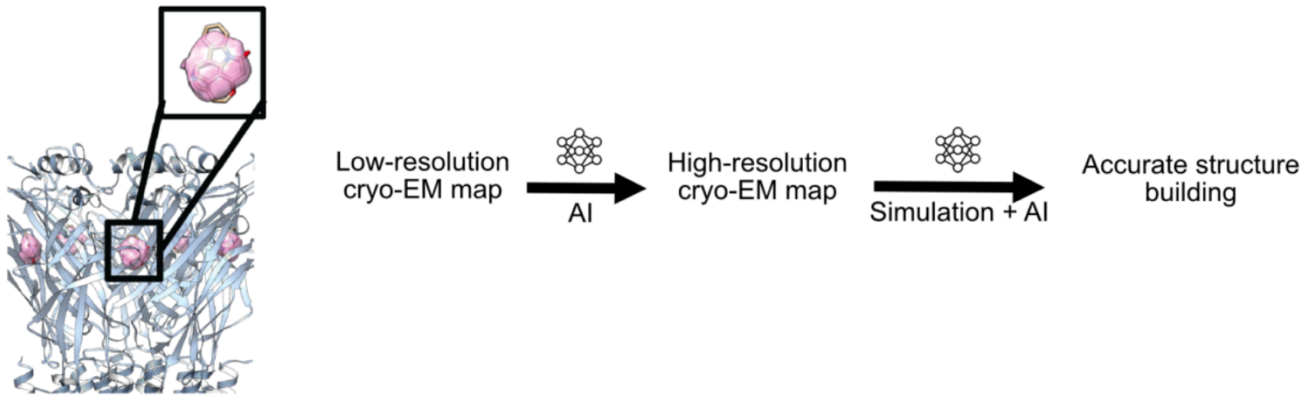

Figure : (*Left*) A depiction of poor ligand density in Cryo-EM maps, using the example of strychnine binding in glycine receptor. Without a careful fitting protocol, deciding the best orientation of the drug in this spherical blob-like density would be very difficult. (*Right*) A workflow developed in this work.

References:

N. Haloi, R. J. Howard, and E. Lindahl, "Cryo-EM ligand building using generative AI and molecular dynamics "*bioRxiv* , doi: https://doi.org/10.1101/2025.02.10.637508

Poster #43

Decoding Allosteric Regulation in the Cysteine Synthase Complex: A Cryo-EM Approach to a Dynamic Protein–Protein Interaction

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The Cysteine Synthase Complex (CSC), composed of serine acetyltransferase (CysE, a homoexamer) and O-acetylserine sulfhydrylase (CysK, a homodimer), is a transient and dynamic protein–protein complex conserved in plants and bacteria [1]. It likely plays a regulatory role in L-cysteine biosynthesis, yet its exact mechanism and biological function remain elusive [2]. Inhibiting CysE and CysK is a promising strategy to develop antibiotics or adjuvants [3,4], but the consequences of targeting CSC assembly are still poorly understood. The lack of high[1]resolution structural data hampers both mechanistic insight and drug development efforts.

We employed an integrative structural biology approach to characterize CSC. Protein painting and Small-Angle X-ray Scattering (SAXS) provided initial information on quaternary organization and interaction surfaces [5]. Hydrogen-deuterium exchange mass spectrometry (HDX-MS) revealed long-range allosteric communication between CysE and CysK upon complex formation [6]. To obtain higher-resolution structural insights, we applied cryo-EM Single Particle Analysis.

Cryo-EM highlighted significant challenges due to compositional and conformational heterogeneity, as well as preferential orientation (likely due to the elongated shape of the particles). These limitations hindered resolution but enabled the identification of discrete structural states, that may underlie structural rearrangements involved in the enzyme allosteric regulation.

These findings support the hypothesis that CSC is not a passive scaffold, but an allosterically regulated assembly. Cryo-EM, applied under near-native conditions, enabled the capture of distinct structural states and proved highly complementary to solution-based methods like HDX[1]MS. This integrative approach strengthens the basis for targeting transient protein–protein interactions in antibiotic development.

[1] Campanini et al., BBA 2015. 10.1016/j.bbapap.2015.02.013; [2] Benoni et al., FEBS Lett. 2017. 10.1002/1873-3468.12630; [3] Annunziato et al., Pharmaceut. 2022. 10.3390/ph15060766; [4] Marchetti et al. Catalysts 2021. 10.3390/catal11060700; [5] Rosa et al., Int. J. Mol. Sci. 2019. 10.3390/ijms20205219; Rosa et al., MCP 2021. 10.1016/j.mcpro.2021.1

Vestibular Modulation by Stimulant Derivatives in a Pentameric Ligand-Gated Ion Channel

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Stocholm University

Allosteric modulation of pentameric ligand-gated ion channels (pLGICs) is critical to the action of neurotransmitters and many psychoactive drugs. However, details of their modulatory mechanisms remain unclear, especially beyond the orthosteric neurotransmitter-binding sites. The recently reported prokaryotic channel sTeLIC, a pH-gated homolog of eukaryotic receptors in the pLGIC family, is thought to be modulated by aromatic compounds via a relatively uncharacterized modulatory site in the extracellular vestibule. Here, by determining new cryo-EM and X-ray structures in closed and open states, we show that psychostimulant derivatives preferentially bind a vestibular pocket in the contracted open-state extracellular domain. Interestingly, this pocket appears to be primed to bind amphiphilic compounds, including reagents commonly used in protein or grid preparation. Oocyte electrophysiology recordings in the absence and presence of targeted mutations verify the positive modulatory effects of psychostimulants and other ligands. This work provides a detailed structure-function mechanism for allosteric potentiation via a noncanonical ligand site, with potential conservation in eukaryotic pLGICs.

Structure of a contractile injection system in *Salmonella enterica* subsp. *salamae*

Rooshanie Ejaz

Novo Nordisk Foundation Center for Potein Research

Extracellular contractile injection systems (eCISs) are phage-derived nanomachines used by bacteria to deliver effectors into target cells. Well-studied examples include the *Photorhabdus asymbiotica* virulence cassettes and the antifeeding prophage from *Serratia entomophila*, which have been engineered for heterologous cargo delivery. Recent genomic analyses identified previously uncharacterized eCIS gene clusters in the opportunistic human pathogen *Salmonella enterica* subspecies *salamae*, but their structure, function, and biotechnological potential remain unexplored. Here, we report a high-resolution cryo-electron microscopy structure of the *S. enterica* eCIS. Our atomic models reveal a unique sheath architecture, an expansive cage-like shell around a central spike, and an associated transmembrane hydrolase. We identify a putative effector encoded within the operon exhibiting periplasmic toxicity and provide evidence that the *S. enterica* eCIS deviates from canonical eCISs by interacting with the inner membrane. Guided by these structural features, we uncover a previously unannotated cluster of contractile injection systems (CISs). Together, our findings expand the known diversity of CISs' structures and functions and lay the groundwork for engineering customizable protein delivery platforms.

Structure-guided mechanistic decoding of *Mtb* Cystathionine β-Synthase inhibition: A blueprint for rational design of covalent PLP enzyme inhibitors

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Pyridoxal-5'-phosphate (PLP)-dependent enzymes are attractive yet underexplored drug targets due to the lack of selective inhibitors and an incomplete understanding of what drives the irreversible inhibition. Among these enzymes, cystathionine β-synthase plays a crucial role in sulfur metabolism and redox regulation in *Mycobacterium tuberculosis* (*Mtb*), a pathogen causing tuberculosis (TB) and responsible for the world's leading threat, HIV-TB co-infection. Despite its biological significance and potential druggability, *Mtb*CBS remains poorly characterised in terms of structure and inhibition. In this study, we present the first high-resolution cryo-EM structures of *Mtb*CBS bound to aminooxyacetic acid (AOAA), a classical PLP enzyme inhibitor and a molecular mimic, unravelling the structural basis of its irreversible binding. We identify two key active-site residues, T75 and Q147, that anchor the PLP-AOAA oxime adduct, locking the enzyme in a catalytically inert state. Site-directed mutagenesis confirms the functional importance of these residues, as their substitution significantly reduces both activity and AOAA-mediated inhibition. To dissect AOAA's potency, we designed and tested molecular mimics lacking specific functional groups. None of the mimics reproduced AOAA's inhibitory profile, indicating the precise positioning group of both the carboxylate group and O1 atom was crucial for covalent adduct formation and enzyme inactivation. These findings were further supported by spectroscopic, biochemical, computational and comparative structural analysis. Together, this work offers the first mechanistic explanation for AOAA's strong inhibition of a PLP enzyme and defines structure-based principles for designing next-generation covalent inhibitors. *Mtb*CBS serves here as a model system, but the active site features were conserved across CBS enzymes, extending the relevance of this study beyond TB. These insights provide a rational blueprint for developing PLP-targeting therapeutics for TB and potentially for other PLP-associated diseases.

(1) Bandyopadhyay, P.; Pramanick, I.; Biswas, R.; Ps, S.; Sreedharan, S.; Singh, S.; Rajmani, R.S.; Laxman, S.; Dutta, S.; Singh, A. S-Adenosylmethionine-Responsive Cystathionine β-Synthase Modulates Sulfur Metabolism and Redox Balance in Mycobacterium Tuberculosis.*Sci Adv* **2022**, *8* (25), eabo0097. https://doi.org/10.1126/sciadv.abo0097.
(2) Polepalli, S.; Roy, A.; Pramanick, I.; Singh, A.; Dutta, S. Understanding Cystathionine Beta Synthase Mechanism of Action and Interaction with Substrates, Product, Inhibitors, Allosteric Modulators. *Biophysical Journal* **2024**, *123* (3), 134a–135a. https://doi.org/10.1016/j.bpj.2023.11.925.

Volume Electron Microscopy by FIB-SEM at Umeå Centre for Electron Microscopy

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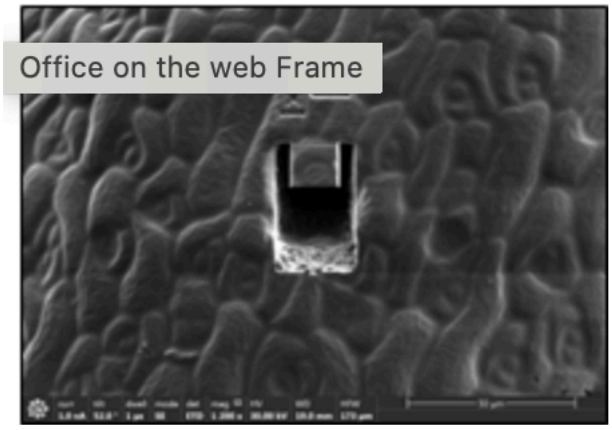
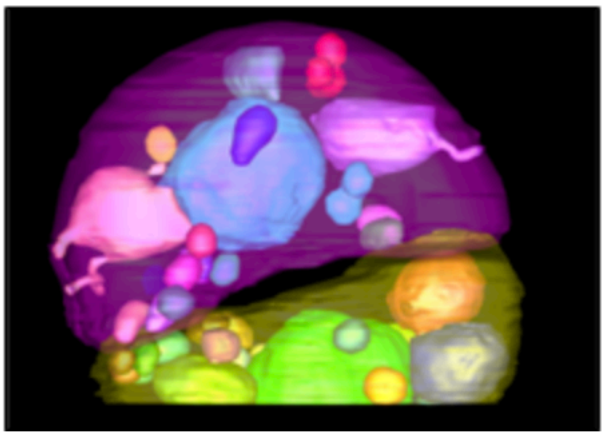
Umeå Centre for Electron Microscopy is the EM-facility at Umeå University in Sweden. We support local and national users with a variety of EM techniques such as SEM, TEM, FIB-SEM, SPA, cryoET and microED. We offer our users volume electron microscopy by FIB-SEM with full service on both sample preparation (conventional fixation and HPF+FS) and volume acquisition with our Scios instrument (Thermo Fisher Scientific). This is a national facility and part of SciLifeLab Integrated Microscopy Technologies.





Volume electron microscopy by FIB-SEM is a new technique, part of the emerging field of volumeEM. This technique can acquire a volume of a sample at excellent isometric resolution (down to 5nm) and large volumes of >10um. A Focused Ion Beam Scanning Electron Microscope (FIB-SEM) is equipped with both an electron beam and an ion beam (Ga+-ions). When a sample is placed at the coincidence point, the same area will be visible with both beams. This area is then consecutively milled with the ion beam and imaged by the electron beam. With a dedicated automated software, a series of 2D electron images is constructed to form a high-resolution 3D-volume of several tens of micrometers in dimensions. Such a volume is then aligned and segmented with a dedicated software to build a detailed model of the given volume.

Dynamic assembly of a large multidomain ribozyme visualized by cryo-electron microscopy

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Many RNAs rely on their 3D structures for function. While acquiring functional 3D structures, certain RNAs form misfolded, non-functional states (‘kinetic traps’). Instead, other RNAs sequentially assemble over pre-folded scaffolds into their functional conformations. Elucidating the principles of RNA sequential assembly is thus important to understand how RNAs avoid the formation of misfolded, non-functional states.

Integrating single-particle electron cryomicroscopy (cryo-EM), image processing, in solution small-angle X-ray scattering (SAXS), EM-driven molecular dynamics (MD) simulations, structure-based mutagenesis and enzymatic assays, we have visualized the sequential multidomain assembly of a self-splicing ribozyme of biomedical and bioengineering significance. Our work reveals an unanticipated dynamic interplay of helical subdomains in the ribozyme’s 5’-terminal scaffold, which acts as a gate to control the docking of 3’-terminal domains. We identify specific conserved and functionally important secondary structure motifs as the key players for orchestrating the energetically inexpensive conformational changes that lead to the productive formation of the catalytic pocket.

Our work provides an unprecedented molecular movie of a large multidomain RNA assembling into its functionally active conformation and establishes a basis for understanding how RNA avoids the formation of non-functional ‘kinetic traps’.

Structural investigation of bacterial divisome complex

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The bacterial divisome complex is a multi—protein complex that catalyzes the process of cytokinesis. FtsZ, a tubulin-like protein, is the main constituent of this complex. The FtsZ subunits polymerise in the presence of GTP to form ring-like structures, called the Z-ring. Further, the assembly of this Z-ring is mediated by various additional factors. One such factor is the septum-forming protein called SepF. SepF is recognized as a protein that binds to membranes and interacts with FtsZ filaments, thereby linking it closely to the bacterial cell membrane. Interestingly, unlike most bacteria, *Streptomyces coelicolor,* our organism of interest, harbours 3 variants of SepF proteins, namely SepF, SepF2 and SepF3. Previous research has emphasized the significance of these SepF variants in the hyphal growth of *Streptomyces* species. However, detailed structural information on this topic remains limited. In this work, we aim to elucidate the cryo- EM structures of recombinantly expressed and purified FtsZ in complex with different SepF variants. Additionally, we examine the membrane-binding properties of these proteins to better understand their functional roles.

Structural and functional analysis of AAA+ ATPases enzymes and stress regulatory proteins from mycobacterial tuberculosis ESX-1 secretion system: Novel targets for drug development

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Tuberculosis, primarily caused by *Mycobacterium tuberculosis*, stands out as a significant infectious disease. The ESX-1 secretion mechanism plays a crucial role in the bacterium's virulence. Within this system, enzymes facilitate the secretion of virulence proteins like ESAT-6 and CFP-10 (EsxAB heterodimer), crucial for invading the host, avoiding phagosomes, and manipulating the host's immune response. The RD1 region of *Mycobacterium* codes for EccC proteins that provide energy for the translocation of these virulent proteins. EccC protein is composed of two polypeptides, which are AAA+ ATPase enzymes. Additionally, the regulation of this ESX-1 system is done by the *espACD* operon of Mycobacterium tuberculosis, which in turn is regulated by the Two-component system proteins, MprA and MprB. These two proteins regulate the expression of genes encoding ESX-1 system proteins.

We have successfully expressed and purified the two polypeptides constituting the EccC protein, along with their respective substrates. The ATPase activity, substrate binding mechanism and low-resolution structure of EccC along with EsxAB were also identified. However, the EccC proteins form oligomers (Mw >400 kDa) and thus complicate crystallization. We have also purified MprA and MprB proteins and done their binding analysis to elucidate the mechanism of binding. The objective of this study is to elucidate the cryo-EM structure of the EccC protein and its substrate, which promises vital insights into the secretion mechanism of virulence proteins. Additionally, we are also planning for structure analysis of MprAB complex through cryo-EM along with the ESX-1 promoter DNA which will provide details about the regulation of ESX-1 genes, and their conformational dynamics. Controlling the expression of ESX-1 genes through MprAB will impact tuberculosis pathogenesis.

Chemical mechanism of allosteric and asymmetric dark reversion in a bacterial phytochrome uncovered by cryo-EM.

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Uppsala University

Phytochromes are light-sensitive proteins found in plants, fungi, and bacteria. They switch between two functional states, Pr and Pfr, distinguished by E/Z isomers of their bilin chromophore. The chromophore can photoswitch between these states, but also thermally react in darkness. Given the high activation energy of the isomerization reaction in solution, it remains unclear how this reaction can proceed in the dark within the phytochrome. Here, we present time-resolved single-particle cryo-EM structures of the *Pseudomonas aeruginosa* bacteriophytochrome (*Pa*BphP) captured at multiple time points during dark reversion from Pr to Pfr. We identify structural asymmetries in the precursor Pr state stretching from the homodimer interface to a conserved histidine (H277) next to the bilin and find that these lead to a strong imbalance of the dark reversion reaction rate of the two protomers. Supported by molecular modelling, we conclude that small, protomer-dependent changes of the conserved histidine control the hydrogen-bonding network around the chromophore, thereby exerting control over the activation energy of the isomerization reaction. This mechanism explains how phytochromes can thermally dark revert and how allosteric control is asserted; and it provides a structural framework for tuning phytochrome signaling lifetimes in optogenetic applications.

Seeing the small move: dissecting flexibility in a hemoglobin-binding receptor from *Staphylococcus aureus*

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Iron acquisition is a key virulence strategy of *Staphylococcus aureus* [1], which scavenges heme from host hemoglobin (Hb) via specialized hemophores of the Isd (iron-regulated surface determinant) system [2]. IsdH, a surface-exposed hemophore composed of three NEAT (NEAr iron Transporter, N) domains, captures heme directly from Hb. Enriching the knowledge of heme acquisition in *S. aureus* with high-resolution structural information will offer a valuable framework for a rational drug design campaign targeting iron uptake in bacterial pathogens. Here, we used cryo-EM to investigate the structure of the preextraction complex formed between carboxyhemoglobin (HbCO) and the minimal functional construct IsdHN2N3 containing the N2 and N3 NEAT domains. Despite considerable sample heterogeneity, we successfully resolved the structure of several complexes with different stoichiometry at high resolution (below 4 Å). Among them, we reconstructed the structure of the 2to2 complex – consisting of one Hb dimer bound by two IsdHN2N3 – a notable achievement for such a small assembly (~115 kDa). By applying 3D classification without alignment, we further distinguished two 2to2 conformations (Figure 1A), both exhibiting significant flexibility of a single NEAT domain (~15 kDa, red circle).

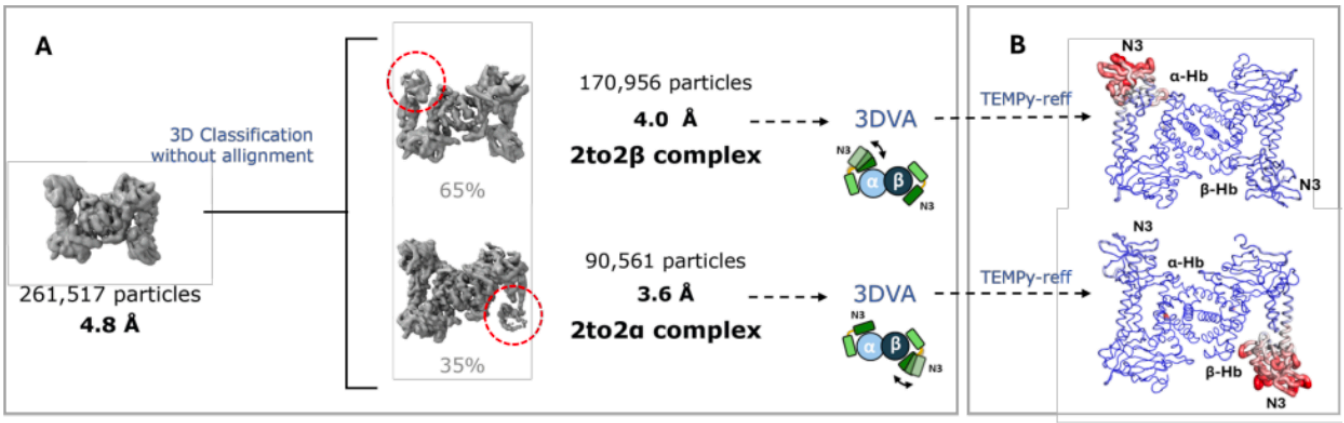

Figure 1. (A) Workflow of cryo-EM data processing of the 2to2 complexes. (B) Structural motion of the complexes, with flexibility visualized by backbone thickness and color-coded B-factors (blue: low, red: high).

To explore this conformational plasticity, we accessed the Instruct-ERIC Flexibility Hub (PID 32912) and employed 3D Variability Analysis (3DVA) [3], DynaMight [4], and ZART [5]. Using 3DVA, PCA identified two major components of motion for which we extrapolated intermediate volumes along these trajectories and fitted atomic models into the resulting maps using TEMPy-reff. [6]. To visualize continuous domain movement, we developed a custom Python script exploiting ProDy [7], providing a clear readout of local flexibility (Figure 1B). These results demonstrate that flexibility analysis tools can be successfully applied to very small regions of proteins.

[1]10.3390/ijms21062145;[2]10.1016/j.jinorgbio.2009.09.012;[3] 10.1016/j.jsb.2021.107702; [4] 10.1038/s41592-024-02377-5; [5] 10.1016/j.jmb.2023.168088; [6] 10.1038/s41467-023-44593-1; [7] 10.1093/bioinformatics/btab187

Exploring Self-control: Autoinhibition of a Plant Lipid Flippase ALA10-ALIS1

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A distinct lipid composition in each leaflet of a lipid bilayer determines the physicochemical and biological properties of cellular membranes1. Flippases (P4-ATPases) contribute to maintaining lipid asymmetry by ATP-driven inward lipid transport and are essential for processes like vesicle formation and cell signaling2. While human and yeast flippases are relatively well-studied, plant flippases remain structurally unexplored. A mechanism involving interaction of their termini with the ATP-binding region, locking the flippase in a specific conformation, was observed for both yeast (e.g. Drs2p-Cdc50p), and human (e.g. ATP8B1-CDC50A) flippases. In these, the C-terminus is a major autoinhibitory factor3,4. Here, we investigate ALA10-ALIS1 from *Arabidopsis thaliana*, a lipid flippase with broad substrate specificity. ALA10-ALIS1 is upregulated by both biotic and abiotic stresses, therefore, its precise regulation is necessary5. Our preliminary cryo-EM data suggest that ALA10-ALIS1 adopts an E2P autoinhibited conformation similar to autoinhibited Drs2p-Cdc50p and ATP8B1-CDC50A. Surprisingly, that is executed solely through ALA10's N-terminal tail. A similar architecture is predicted for several other plant flippases (e.g. ALA7-ALIS5, AlphaFold3), correlating with sequence conservation in that region. That points toward more universal, plant-flippase-specific, and so far undescribed regulatory mechanism of plant flippases.

1) Levental, I., Lyman, E. (2023). *Nat. Rev. Mol. Cell Biol.*, *24*(2), 107–122.
2) Tanaka, K. et al. (2011). *The Journal of Biochemistry*, *149*(2), 131–143.
3) Timcenko, M., Lyons, J. A., Januliene, D., et al. (2019). *Nature*, *571*(7765), 366–370.
4) Dieudonné, T., et al. (2022). *eLife*, *11*.
5) López-Marqués, R. L. (2021). *Nature Plants*, *7*(9), 1188–1199.

Expression and 3D reconstruction of Salmonella FimAfimbriae

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The Fim-operon from Salmonella Enterica is responsible for expression of one of the most well-studied fimbriae. This operon encodes for FimA, FimI, FimC, FimD, sfmH, and FimF, where the major subunit, FimA, constitutes the assembled fimbriae. Here, the entire Fim-operon was expressed in a bald E. coli strain, with a construct utilizing a Tet-on system. Transmission electron microscopy studies showed 20 nm flagella and 8 nm rigid FimA-filaments both protruding from the cells and found broken and released in the cell medium in large quantities. Data was collected with cryo-electron microscopy, and CryoSparc was used for helical reconstruction of the fimbriae, leading to a solved structure with a resolution of 2.67 Å. On this model each subunit can be differentiated, and both size and shape match with the AlphaFold model of FimA.

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FEEDBACK

After the meeting, we would greatly appreciate **your feedback** and suggestions for the next cryoNET Symposium! The feedback form will be available on the conference webpage.

FEEDBACK