Oral Presentation

160 Structural characterization of the active and inactive conformations of the vasopressin V2 receptor using cryo-EM
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434 Molecular mechanism of a bacterial Retron
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798 STRUCTURAL CHARACTERIZATION OF FZD7, THE IMPORTANCE OF WATER NETWORK AND CHOLESTEROL FOR CLASS F GPCRS
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839 Bag1 has a key role in the Hsp70-assisted, proteasome-mediated degradation pathway
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916 The structural basis for energy extraction from air by Mycobacterium smegmatis

Ashleigh Kropp[1], Hari Venugopal[2], Chris Greening[1], Rhys Grinter[3]
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Poster Presentation

19 Using the YOLO DeepLearning algorithm to quantify the rAAV empty/filled ratio from Cryo-EM imaging
Manon Mirgaux[1], William Amadou[1], Louise Conrard[1], Egor Zindy[1], Ayse Betul Oktay[1], Professor David Perez-Morga[1], Professor Olivier Debeir[1]
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451 Structure and Function of Fructose 6-phosphate aldolase
Prof Hans Hebert[1], Dr Eda Sönmez[2], Dr Pasi Purhonen[1], Prof Mikael Widersten[2]
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797 Structural recognition and stabilization of tyrosine hydroxylase by the J-domain protein DNAJC12
Mrs. Mary Dayne S. Tai[1], Mrs. Jimena Munatner[2], Mrs. Lissette Ochoa[2], Mrs. Marte I. Flydal[1,3], Mrs. Lorea Velasco[4], Mr. César Santiago[2], Mr. Kunwar Jung-KC[1,5], Mr. Svein I. Støve[1,5], Mr. Fernando Moro[4], Mr. Juha P. Kallio[1], Mr. Arturo Muga[4], Mr. José María Valpuesta[2], Mr. Jorge Cuéllar[2], Mrs. Aurora Martínez[1,5]
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896 Cryo-EM Reveals RECQ5's Regulatory Role in RNAPII-Mediated Transcription
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951 Cryo-EM structure of the HD6 defensin helical assembly
Dr. Roman Kamyshinsky[1], Lev Khmelnitsky[2], Rishir Kalepu[2], Dr. Nadav Elad[1], Prof. Deborah Fass[2]
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964 Molecular insights into the biogenesis of box H/ACA snoRNPs
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1054 Structural characterisation of a phage-like bacteriocin from Pseudomonas sp. by cryo-Electron Microscopy
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1156 Low-dose cryo-electron ptychography of proteins at sub-nanometer resolution
Berk Kucukoglu[1], Dr. Inayathulla Mohammed[1], Dr. Ricardo C. Guerrero-Ferreira[1,2], Dr. Stephanie M. Ribet[3], Dr. Georgios Varnavides[3,4], Max Leo Leidl[5,6], Dr. Kelvin Lau[7], Dr. Sergey Nazarov[8], Dr. Alexander Myasnikov[8], Dr. Carsten Sachse[5,9,10], Dr. Knut Müller-Caspary[6], Dr. Colin Ophus[3], Dr. Henning Stahlberg[1]
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Late Poster Presentation

1232 Structural organization of the native Neisseria meningitids PilQ environment through an MS-EM approach
Dr. David Fernandez-Martinez[1], Dr. Lucienne Nouchikian[2], Sylvie Goussard[1], Prof. Pierre-Yves Renard[3], Dr. Cyrille Sabot[3], Dr. Martial Rey[2], Dr. Julia Chamot-Rooke[2], Dr. Guillaume Dumenil[3]
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1235 Global BioImaging: Imaging Networks Accelerate Collaboration, Exchange and Innovation in Imaging Science
Dr Yara Reis[1], Dr Gleb Grebnev[1], Dr Deniz Saltukoglu[1], Dr Sophie Winter[1], Dr Antje Keppler[1,2]
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1270 Structural studies of the cross-link mutant, ABC transporter BmrA by cryo electron microscopy
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Structural characterization of the active and inactive conformations of the vasopressin V2 receptor using cryo-EM

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LS-09, Lecture Theater 4, august 27, 2024, 14:00 - 16:00

Background:
The antidiuretic hormone arginine-vasopressin (AVP) binds to the kidney V2 receptor (V2R) triggering Gs protein coupling and thereby activating adenylyl cyclase. First, the AVP-V2R-Gs signaling complex regulates body water balance and solute transport in mammals, and exhibits physiological effects throughout the body. In addition, arrestins also interact with this G protein-coupled receptor (GPCR) to stop G protein activation and to initiate other key signaling pathways.
Dysfunctions in this GPCR lead to clinical disorders, ranging from dysregulation of water balance such as the syndrome of inappropriate antidiuretic hormone secretion (SIADH, associated to many forms of cancer), congestive heart failure, hepatic cirrhosis, to urine disorders (incontinence, nocturia). Moreover, V2R loss-of-function or constitutively active mutations lead to two rare genetic diseases: (a) the congenital Nephrogenic Diabetes Insipidus (cNDI) characterized by excessive urine voiding, and (b) the nephrogenic syndrome of inappropriate antidiuresis (NSIAD) characterized by excessive water loading and hyponatremia. Unfortunately, despite the global prevalence of kidney-related diseases, there is currently no safe prescribed drug on the market for V2R-related disorders. The nonpeptide antagonist Tolvaptan approved for treating hyponatremia (SAMSCA®) and recently for polycystic kidney disease (PKD, the most frequent Mendelian inherited disorder affecting million people worldwide), has hepatotoxic effects. Discovery of therapeutic compounds based on structural information may have a direct impact on millions of citizens. To that purpose, determining both the active and inactive conformations of the V2R is crucial for a comprehensive understanding of the complete conformational changes responsible for the receptor activation or inhibition. Defining the molecular mechanisms occurring in the core of the V2R during these changes is essential for a complete understanding of its function, and to ultimately propose innovative drugs based on rational and structural data.

Methods:
By combining biochemistry and structural biology, we have successfully purified the V2R in complex with either Gs protein or βarrestin1, and structurally characterized the complexes using cryo-EM. Determining structures of the inactive states remains a challenging endeavor due to intricate biochemistry, their dynamic nature, their small size and sophisticated activation mechanism. Given this, the V2R has been modified by introducing a BRIL (cytochrome b562 RIL, 15 kDa) module in the third intracellular loop (ICL3) of the receptor. It is a promising strategy, as a Fab anti-BRIL and an anti-Fab nanobody have been designed to significantly increase the size of the receptor for cryo-EM (~100 kDa).

Results:
We have recently reported the cryo–electron microscopy (cryo-EM) active structures of the wild-type AVP V2R in complex with either Gs protein and βarrestin1. First, the cryo-EM characterization of the AVP-V2R-Gs allowed us to describe three structures, mostly based on the positioning of the Gs relative to the receptor. Those structures reveal an original receptor-Gs interface in which the Gαs

subunit penetrates deep into the active V2R. The structures help to explain how V2R R137H or R137L/C variants can lead to cNDI and NSIAD. Then, the AVP-V2R-βarrestin1 structure reveals an atypical position of βarrestin1 compared to previously described GPCR-arrestin assemblies, associated with an original V2R/βarrestin1 interface involving all receptor intracellular loops. Phosphorylated sites of the V2R carboxyl terminus are clearly identified and interact extensively with the βarrestin1 N-lobe, in agreement with structural data obtained with chimeric or synthetic systems. Finally, in the core conformation, the βarrestin1 finger loop inserts into the intracellular cavity of V2R and overlaps the same binding space than the Gs α5 helix, explaining the desensitization mechanism of the V2R.

We are actually characterizing the V2R into its inactive state, using different types of antagonists. They fully antagonize cAMP signal, arrestin recruitment and MAP kinase phosphorylation associated to V2R activation. Preliminary cryo-EM data demonstrate the potential of the V2R-Bril construction to reach high resolution for the inactive state of the receptor. Resolving the cryo-EM conformation of the antagonist-V2R complex will constitutes the first characterization of an inactive structure of the V2R.

Conclusion:
Elucidating high-resolution structure of different states of the receptor using cryo-electron microscopy will allow to apply structure-based drug design to discover effective innovative drug compounds using computational approaches. In addition to bring those receptors to drug design initiatives, our data will provide new insights into GPCR's function.

Keywords:

Cryo-EM, V2R, structure-based drug design

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Molecular mechanism of a bacterial Retron

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LS-09, Lecture Theater 4, august 27, 2024, 14:00 - 16:00

Background incl. aims:
Retrons are reverse transcriptase-carrying prokaryotic immune systems that protect bacteria against phages. Retrons are formed of three components: A reverse transcriptase (RT), a non-coding RNA (ncRNA), and one or several effector proteins with diverse enzymatic activities. Typically, the RT synthesizes a multi-copy single-stranded DNA (msDNA) from the ncRNA template, a feature that has been exploited for gene editing and synthetic biology applications. Upon phage infection, the effectors induce cell dormancy or death, preventing the phage from spreading throughout the population – a phenomenon known as abortive infection (Abi). To avoid cellular toxicity in the absence of infection, Retron effectors are frequently kept in low-activity states, and RT and ncRNA/msDNA are believed to play a neutralizing role in this process. This work aims to elucidate the molecular events underlying the immune response by a Retron.
Methods:
We combine structural biology (CryoEM), biochemistry, mass spectrometry, and bacterial genetics to address the research question.
Results:
Here, we reveal the molecular mechanism of a Retron. First, we characterized the activity of the effector in vitro and during phage infection. CryoEM structures of the Retron complex illustrate that the msDNA stabilizes the effector in a low-activity state. Interestingly, msDNA's mutations induce the release of the effector from the complex and cause toxicity, underscoring the msDNA role in immunity. In addition, we identified and characterized a phage-encoded Retron inhibitor that suffices to offset the immune response.
Conclusion:
Collectively, our work outlines the structural basis of the Retron defense system and highlights the intricate interplay between bacterial defense systems and phages.

Keywords:

Bacterial Immune System, Retron, CryoEM

Reference:

Carabias et al. (2024) Manuscript under review.

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Structure and mechanism of Zorya anti-phage defense system

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LS-09, Lecture Theater 4, august 27, 2024, 14:00 - 16:00

Zorya is a recently identified and widely distributed bacterial immune system, which protects against phage invasion. It consists of a predicted membrane-embedded complex (ZorAB) and soluble components that differ among Zorya subtypes, notably ZorC and ZorD, in type I Zorya systems. Here, we reveal the molecular basis of the Zorya defense system using cryo-electron microscopy, mutagenesis, fluorescence microscopy, proteomics, and functional studies. We demonstrate that ZorAB shares the stoichiometry of other 5:2 inner membrane ion-driven rotary motors. Additionally, ZorA5B2 features a dimeric ZorB peptidoglycan binding domain and a pentameric α-helical coiled-coil tail made of ZorA that projects approximately 700 Å into the cytoplasm. We further characterize the structure and function of the soluble Zorya components, ZorC and ZorD, and find that they harbour DNA binding and nuclease activity, respectively. Comprehensive functional and mutational analyses demonstrates that all Zorya components work in concert to protect bacterial cells against invading phages. We present evidence that ZorAB operates as an ion-driven motor that becomes activated and anchors to the cell wall upon sensing of cell envelope perturbations during phage invasion. Subsequently, ZorAB transfers the phage invasion signal through the ZorA cytoplasmic tail to the soluble effectors, which function to prevent phage propagation. In summary, our study elucidates the foundational mechanisms of Zorya function and reveals a novel triggering signal for the rapid activation of an anti-phage defense system.

Keywords:

bacterial defense-system, rotary-motor, ion-channel, cryo-EM

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STRUCTURAL CHARACTERIZATION OF FZD7, THE IMPORTANCE OF WATER NETWORK AND CHOLESTEROL FOR CLASS F GPCRS

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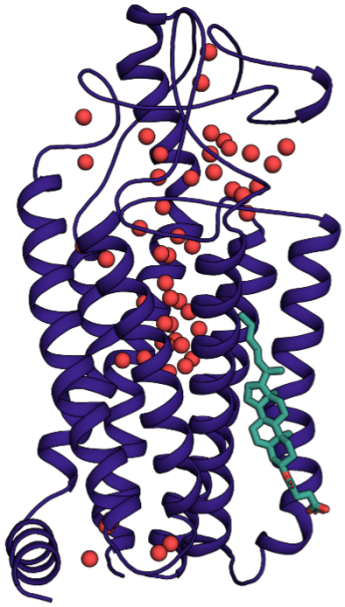
LS-09, Lecture Theater 4, august 27, 2024, 14:00 - 16:00

Background
For more than 30 years, since the discovery of WNTs, WNT/β-catenin and planar cell polarity signaling has formed the basis for what we understand to be the primary output of the interaction between the protein ligands of the WNT family and their receptors known as FZDs (ten isoforms: FZD1-10). FZD7 is one of the best characterized receptors within the family and plays a critical role in many biological processes including migration of mesendoderm cells during development and renewal of intestinal stem cells in adults. Moreover, FZD7 has been highlighted for its involvement in tumor development predominantly in the gastrointestinal tract. This research aims to provide a better understanding of FZDs in general with a highlight on FZD7 by combining structural, computational, and pharmacological tools.

Methods
In this study, we apply a combination of conventional cryo-electron microscopy (cryo-EM) single particle analysis, MD simulations, and phylogenetic analysis to draw FZD family-wide conclusions on structural aspects and mechanisms of FZD activation. These data are complemented with pharmacological experiments employing genetically encoded biosensors to functionally validate our structural findings. This comprehensive approach provides us with insights into the function of FZD7 specifically, as well as FZDs in a broader context.

Results
We report the structure of inactive FZD7, without any stabilizing mutations, determined by cryo-EM. This allowed us to provide a direct comparison with the G protein bound FZD7 and to confirm previously identified residues involved in G protein binding mechanism.
We characterized a fluctuating water pocket in the core of the receptor important for FZD7 dynamics and used a phylogenetic analysis to define conserved residue defining the water pocket base that remains hermetic upon G protein binding unlike what is observed in Class A GPCRs. Molecular dynamics simulations were then used to investigate the temporal distribution of those water molecules and their importance for potential conformational changes in FZD7. Additionally, we discovered lipids that interact with the receptor core and a conserved cholesterol binding site. This site plays a pivotal role in the association of FZD7 with a transducer protein, Dishevelled (DVL), and in the initiation of downstream signaling and the formation of signalosomes.

Conclusion
We provided a high-resolution structure of FZD7 and defined functionally relevant features of FZDs dynamic and signaling.



Keywords:

GPCRs, FZDs, FZD7, Cryo-EM

Reference:

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Bag1 has a key role in the Hsp70-assisted, proteasome-mediated degradation pathway

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LS-09, Lecture Theater 4, august 27, 2024, 14:00 - 16:00

Eukaryotic cells maintain cellular proteostasis through intricate protein quality control systems that orchestrate chaperone-mediated protein folding and protein degradation systems, preventing potential harm from accumulated misfolded proteins. The chaperone system attempts to refold the abnormal proteins and solubilize aggregated proteins; if unsuccessful, these aberrant proteins are removed through the protein degradation system.
In the ubiquitin-proteasome system (UPS), the degradation process is initiated by the recognition of ubiquitinated substrates by the 19S regulatory particle (RP), which is then followed by unfolding and translocation of substrates in the 20S catalytic particle (CP) that executes protein degradation. Aside from the canonical subunits, proteasome function is finely tuned by cofactors that contain ubiquitin-like (UBL) domains which are involved in recruiting substrates to the 26S proteasome.
On the other hand, Bag1 has been shown to interact with Hsc70/Hsp70 to modulate the chaperone activities. Through an ATP-driven conformational cycle, Hsp70 can recognize misfolded proteins, promote refolding, prevent protein aggregation, and resolubilize protein aggregates. Despite their many different roles, all members of the Hsp70 family contain two highly conserved structural domains: the substrate-binding domain (SBD) and the nucleotide-binding domain (NBD). The ADP/ATP switch is catalyzed by a group of cochaperones called nucleotide exchange factors (NEF), which bind to the NBD and favor ADP release from the active site and ATP re-uptake. Bag1 is one of such NEF and contains both UBL and BAG domains and interacts with the 26S proteasome through the UBL domain to degrade unfolded proteins. However, how cochaperone Bag1 bridges between protein folding and degradation systems, and how Bag1 enhances degradation of unfolded proteins remain unanswered.
In this work, using cryoelectron microscopy (cryoEM) and different biochemical and biophysical techniques, we have revealed that Bag1 plays a key role in Hsp70-mediated, proteasome-dependent protein degradation, not only by physically linking Hsp70 to the proteasome (through its subunit Rpn1), thus promoting protein delivery to the latter but also by inducing a series of conformational changes in the 19S that facilitate the client protein degradation.

Keywords:

Chaperone-mediated-degradation, Proteasome, Hsp70, Bag-1, CryoEM

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The structural basis for energy extraction from air by Mycobacterium smegmatis

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LS-09, Lecture Theater 4, august 27, 2024, 14:00 - 16:00

Background
Mycobacterium smegmatis is a heterotrophic, obligately aerobic soil-dwelling bacterium that requires organic carbon for growth. Due to fierce competition for resources in the soil, the bacterium often experiences deprivation of both oxygen and organic carbon sources. To cope with this, M. smegmatis has evolved to persist through metabolic flexibility, upregulating enzymes, such as the high-affinity [NiFe] hydrogenase Huc, that enable it to utilise electron donors and acceptors other than organic carbon and molecular oxygen. Huc provides electrons from H_2 to the cytochrome bcc-aa3 oxidase super complex via the quinone pool, which results in the generation of the proton motive force. The molecular details underpinning hydrogen oxidation in M. smegmatis were unknown. We aimed to characterise how Huc oxidises hydrogen and how the electrons liberated from this process are transferred to the respiratory chain.
Methods
In this work, we purified Huc natively from an M. smegmatis strain and visualised it by cryo-electron microscopy (cryo-EM). We used a wide range of techniques to characterise the enzymatic capabilities of this unique enzyme. Utilising an H_2 sensing electrode and gas chromatography, we quantitated the kinetics of hydrogen oxidation by Huc and the specificity of Huc to different electron acceptor molecules. Further molecular dynamics simulations and mass spectrometry enabled us to identify the specific electron acceptor substrate of Huc. To characterise how this enzyme differs from other low-affinity hydrogenases, we employed spectroscopic and electrochemical techniques.
Results
Our cryo-EM maps of Huc revealed an impressive enzymatic structure with electron density maps at a maximum resolution of 1.52 Å. Uniquely, Huc is an octamer of the large and small enzymatic subunits, with an additional novel membrane-associated central stalk. We showed that this stalk, called HucM, is critical for transporting the electron acceptor molecule menaquinone directly from the bacterial membrane to the enzyme. This long-range quinone transport is a unique feature of Huc. Further, we demonstrated for the first time a very high-affinity purified enzyme capable of oxidising atmospheric H_2 in isolation from the respiratory chain. In addition, we demonstrated that, unlike many other [NiFe] hydrogenases, Huc is insensitive to inhibition by oxygen.
Conclusions
This work represents the first molecular characterisation of a member of a novel family of hydrogenases capable of scavenging H_2 at atmospheric concentrations. This discovery will provide insights for the investigation and development of hydrogenase-based fuel cells that can operate at atmospheric levels of oxygen and use very small amounts of hydrogen to produce energy.

Keywords:

CryoEM, microbiology, enzymes, metabolism

Reference:

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Using the YOLO DeepLearning algorithm to quantify the rAAV empty/filled ratio from Cryo-EM imaging

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Poster Group 1

Recognized as one of the most common vehicles for gene therapy, rAAVs offer numerous advantages such as low pathogenicity, the ability to precisely redistribute the genome, high tissue dispersal, high infectivity, and long-term persistence in cells [1, 2, 3]. The rAAVs are small (25 nm diameter), single-stranded DNA parvoviruses that are non-infectious for humans [4]. Their capsids are made up of an assembly of 60 viral proteins with icosahedral symmetry and contains the genome. When the rAAV enters the cell, it releases the genetic material contained in the capsid, leading to gene therapy treatment. At present, the rAAV production methods used for clinical trials inevitably lead to a mixture of empty and filled virions, present in varying proportions [5]. Consequently, empty vectors (without genetic material) or vectors with erroneous genetic information (partial or double genetic material) co-exist with vectors containing the correct genetic information (called filled vectors). These empty vectors are unable to provide therapeutic effect and acts as an impurity that can generate an immune response or inhibiting transduction, while partially/doubled filled vectors can cause a dysfunctional gene and lead to side effects. It is therefore important for pharmaceutical research to quantify the empty/filled ratio before bringing a drug to market. [6]

There are several ancestral characterization methods for integrity analyses and the quantification of empty/full ratio of rAAVs. Among these methods, cryo-EM microscopy is of particular interest for quantification, as it enables particles to be visualized without the addition of dyes. The contrast is therefore linked to the atomic composition of the particles: the presence of the genome means that filled particles are more contrasted, as empty and intermediate classes can then be determined. To use the power of cryo-EM to determine the level of empty and filled capsids, it is important to develop software tools for post-acquisition image processing. This tool development faces several challenges stemming from the nature of cryo-EM imaging. Sample purity, cryo-plunging conditions, spatial projection and acquisition parameters are all variable parameters that explain the difficulty of development of computer-aided detection methods for accurate capsid detection. As a result, many parameters such as contrast, size, gray scale values and texture must be defined by the user, given the dependence of these characteristics on image background noise and image quality.

In this study, we present a new home-made method for detecting and quantification of empty/full ratio of rAAV capsides in cryo-TEM images. First, the capsides are detected with a popular deep learning architecture, YOLO. Then, the detected rAAVs are analysed according to their image intensity projections and assigned a fullness score. The accuracy of this methodology is then compared to Relion determination, depending on various factors including sample purity, concentration, batch or even variation in plunging and acquisition conditions. These new methodological developments open the door to faster, more precise quantification of rAAVs for pharmaceutical research.

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Keywords:

rAAV, cryo-EM, quantification, deep learning

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Cryo–electron microscopy unveils the gating mechanism of the human Kir2.1 channel

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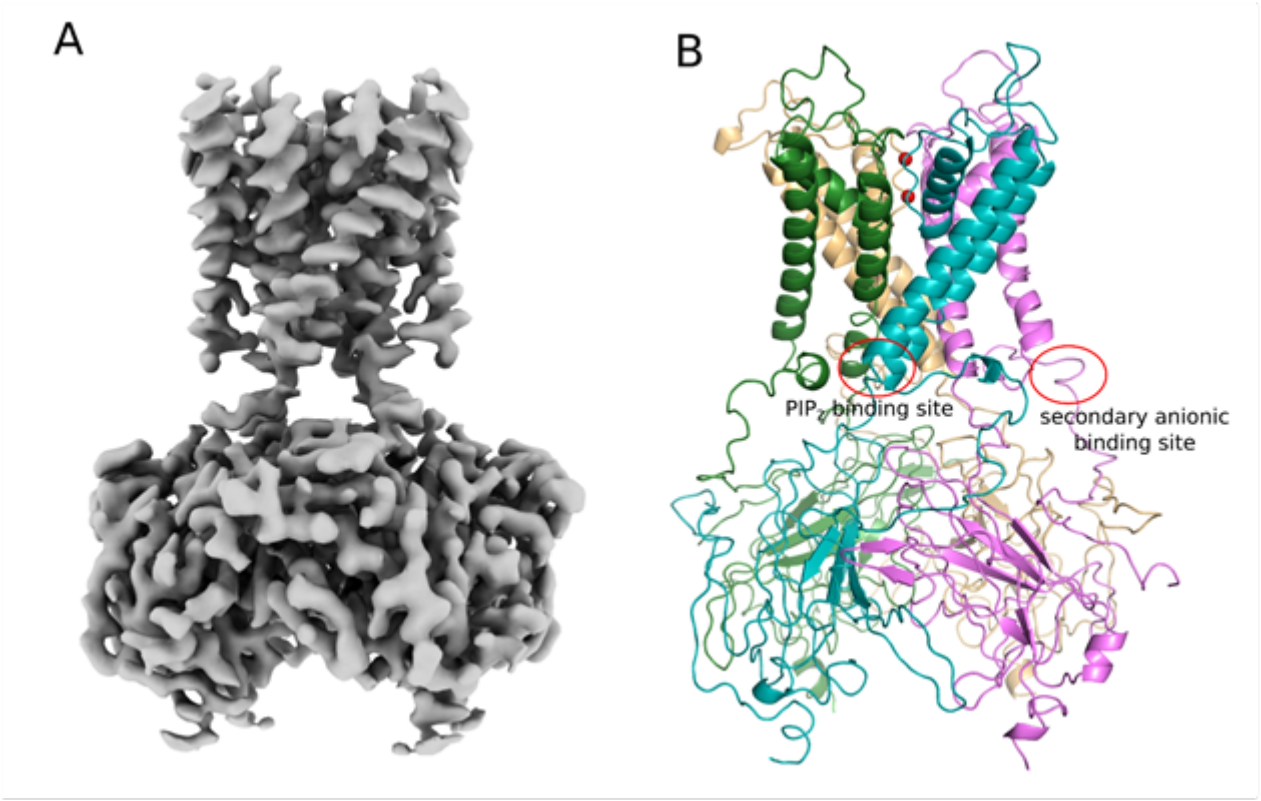
Poster Group 1

Background
Inward-rectifier potassium (Kir) channels are a group of integral membrane proteins that selectively control the permeation of K+ ions across cell membranes. The small outward K+ current through Kir channels controls the resting membrane potential and membrane excitability, regulates cardiac and neuronal electrical activities, couples insulin secretion to blood glucose levels, and maintains electrolyte balance1. All Kir channels are tetramers and share characteristic structural features. They have a canonical pore-forming transmembrane domain (TMD) made of two transmembrane helices separated by a K+ ion selectivity filter and a large cytoplasmic domain (CTD) containing both N and C termini. The CTD extends the ion conduction pathway and provides docking sites for regulatory ions, proteins, and ligands2. The strong inward-rectification mechanism results from a block on the cytoplasmic side of the channels by endogenous polyamines and Mg2+ that plug the channel pore at depolarized potentials. The blockers are then removed from the pore when the K+ ions flow into the cell at hyperpolarized potentials. In addition to being inwardly rectifying, the gating of Kir2.1 channels are selectively activated by the lipid phosphatidylinositol 4,5-bisphosphate (PIP2). The physiological importance of these channels is underpinned by the fact that mutations in these proteins cause a wide range of pathologies. In previous published work, we used cryo–electron microscopy (cryo-EM) combined with image analysis to elucidate the structure of a human Kir channel, Kir2.1 in the closed state3. Furthermore, computational investigations reveal crucial conformational movements, including compaction of the structure and opening movements at the interface between the TMD and CTD, which could facilitate the binding of PIP2. In order to understand the gating mechanism, both structures of Kir2.1 in the closed and open state are needed.
Methods
A total of 9944 micrographs were collected on a Titan Krios G4 microscope operated at 300 kV equipped with Falcon4 and SelectrisX image filter. After a visual inspection to remove poor-quality micrographs, the movies were motion-corrected and dose-weighted (MotionCor2) and contrast function parameters were estimated (CTFFIND4) on 6961 selected micrographs. A total of 781,076 particles were automated picked (SPHIRE-crYOLO). The extracted particles were subject to one round of 2D and 3D classifications (RELION). A map containing 187,153 particles were subjected to 3D Auto-Refine, CTF-refinement and particle polishing. The polished particles were submited to a 3D non-uniform refinement (cryoSPARC)
Results
Here we present the cryo-EM structure of the human Kir2.1 channel complexed to PIP2 in the open state (Fig.). and provide the final cryo-EM map of the Kir2.1/PIP2 complex at 2.85 Å resolution. Preliminary atomic structure of Kir2.1/PIP2 complex was build from the cryo-EM map and revealed the PIP2 binding site.
Conclusion
Comparative structural analysis of the Kir2.1/PIP2 complex (open state) with apo-Kir2.1 (closed state) and in silico studies reveal the structural changes that lead to channel opening. Moreover, the structure of the Kir2.1/PIP2 complex highlights the role of secondary anionic binding site for channel

opening. These data will help to understand the pathological mechanisms associated with mutations in the Ki2.1 channel



Keywords:

Potassium channel, gating, in silico

Reference:

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Structure and Function of Fructose 6-phosphate aldolase

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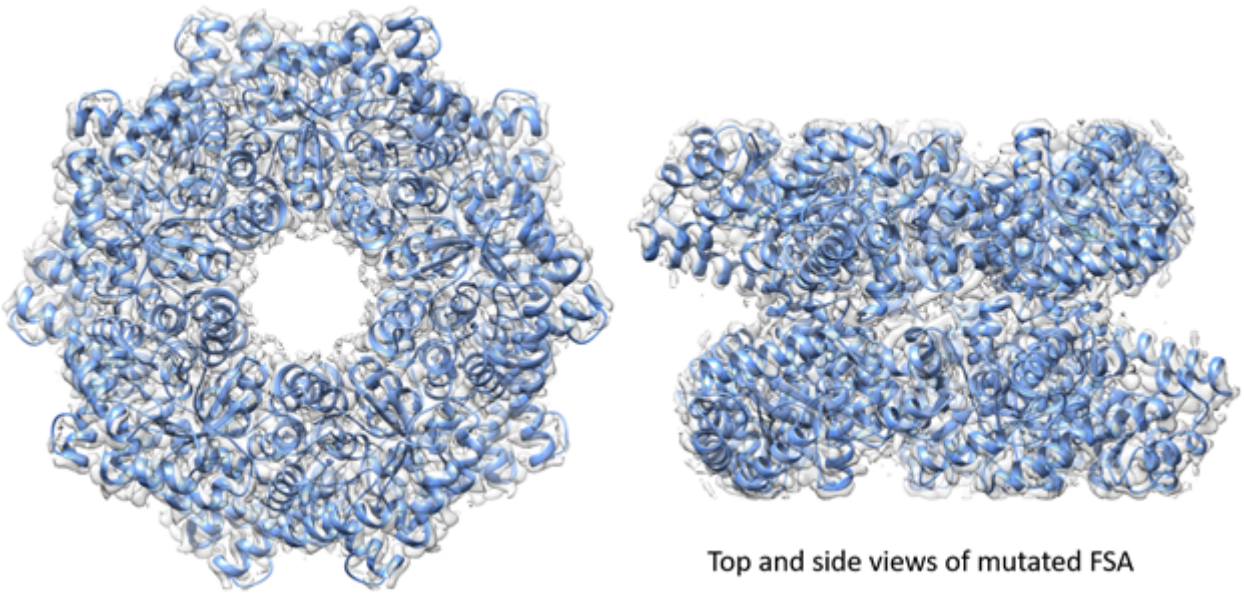
Poster Group 1

Background
Fructose 6-phosphate aldolase (FSA) catalyzes formation of fructose 6-phosphate from dihydroxyacetone and D-glyceraldehyde 3-phosphate via an aldolization reaction (1).
Methods
Cryo-EM structures of FSA from E. coli were obtained of the wild type enzyme and a mutant, L107C/A129G/R134V/L163C/S166G (FSAm), that had been identified as having substantially improved activity(2).
Results
Both proteins form D5 symmetrical decamers and X-ray structures (pdb: 7qxf, 1l6w) could be used for model building(2,3). FSAm samples contained the substrate 2-hydroxyacetophenone and the map had density corresponding to the crucial iminium reaction intermediate captured in the active site. The refined model showed a modification of a lysine residue (Lys85), 2-imino-2-phenylethanol-lysine not previously described in the Chemical Component Dictionary of the pdb database. Moreover, the side chain of the critical residue Y131 was rotated 100 degrees shifting the position of the phenolic group 3 Å. A water molecule participating in a hydrogen bonding network at the active site and thought to be involved in proton relay was shifted similarly.
Conclusion
The new structure can guide identification of additional FSA variants that display improved carboligation activities with 2-hydroxyacetophenone and phenylacetaldehyde.
Keywords
Catalysis, Fructose 6-phosphate aldolase, cryo-EM



Top and side views of mutated FSA

Keywords:

Catalysis, Fructose 6-phosphate aldolase, cryo-EM

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Structural recognition and stabilization of tyrosine hydroxylase by the J-domain protein DNAJC12

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Poster Group 1

Pathogenic variants of the J-domain protein and Hsp70 cochaperone DNAJC12 cause parkinsonism, which seems associated with a defective interaction of DNAJC12 with tyrosine hydroxylase (TH), the rate limiting enzyme in dopamine synthesis. Here, we report the characterization of TH:DNAJC12 complex formation, showing that DNAJC12 binding stabilizes TH and delays its time-dependent aggregation in an Hsp70-independent manner, while maintaining TH activity and regulatory inhibition by dopamine. Interestingly, although DNAJC12 alone is less efficient than other DNAJs to activate Hsc70, the TH:DNAJC12 complex efficiently stimulates its ATPase activity. Cryoelectron microscopy reveals two DNAJC12 monomers bound per TH tetramer, each embracing one of the two regulatory domain dimers, leaving all active sites available for substrate and dopamine interaction. Biochemical data confirm the key role of the DNAJC12 last eight residues in TH binding, explaining the molecular disease mechanism of C-terminal truncated DNAJC12 variants.

Keywords:

DNAJC12, Hsp40, Hsp/Hsc70, tyrosine-hydroxylase, tyrosine-hydroxylase-deficiency

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Cryo-EM Reveals RECQ5's Regulatory Role in RNAPII-Mediated Transcription

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Poster Group 1

Background

In eukaryotic nuclei, the synthesis of mRNA is carried out by RNA polymerase II (RNAPII), a crucial enzyme in transcriptional processes. RECQ5 helicase, a general elongation factor, associates with RNAPII and controls its movement along genes. Despite its significance, the precise mechanism through which RECQ5 regulates RNAPII movement remained unknown.

Methods

We employed a combination of cryo-electron microscopy methods to investigate the interaction between RNAPII and RECQ5. These methods included single-particle analysis (SPA), correlative light-electron microscopy (CLEM), and subtomogram averaging (STA).

Results

Here, we present the details of the interaction between RECQ5 and RNAPII determined by cryo-electron microscopy. SPA reconstruction with a resolution of 3 Å revealed near-atomic level details of the interaction between the RECQ5 helix and RNAPII DNA. Additionally, cryo-EM imaging showed that the RNAPII with RECQ5 complex formed large, spherical objects resembling condensates. CLEM confirmed the presence of both fluorescently labeled RNAPII and RECQ5 within these objects. STA revealed the organization of condensates, further enhancing our understanding of their functional assembly. The subtomogram averaging model of RNAPII with RECQ5 reached a resolution of 7 Å, confirming the observed interaction between the RECQ5 helix and RNAPII DNA across analytical methods.

Conclusions

Our study emphasizes RECQ5's crucial role in modulating transcriptional processes by using the brake-helix to regulate RNAPII movement along genes. Additionally, we discovered that condensates contain tens to hundreds of RNAPII molecules with RECQ5. The study provides valuable insights into how transcriptional machinery functions in eukaryotic nuclei.

Keywords:

RNApolymeraseII, Tomography, Clem, SPA

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Cryo-EM structure of the HD6 defensin helical assembly

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Poster Group 1

Defensins are small antimicrobial proteins produced by animals, plants, and fungi. HD6 is a human defensin secreted into the small intestine. Unlike other defensins, HD6 is not directly bactericidal but rather self assembles into «nanonets» [1] thought to physically trap bacteria and prevent them from penetrating the intestinal epithelium. The existing model for the nanonet filament structure, a spiral of HD6 dimers proposed based on an HD6 crystal structure, does not fully explain the long persistence length (microns) of HD6 filaments in vitro [2]. To resolve this apparent discrepancy, we determined structures of HD6 nanonet filaments using SPA cryo-EM.

HD6 filaments were produced in phosphate buffer according to a previous protocol [2]. The filaments demonstrated polymorphous behavior, so data were collected at different protein concentrations to favor different polymorphs. Specifically, low concentrations yielded predominantly thin filaments ~40 Å in diameter. Higher concentrations yielded thicker filaments ~80 Å in diameter. Cryo-EM data were collected on a Talos Arctica (Thermo Fisher Scientific) operated at 200 kV. EER movies were collected using EPU software in AFIS mode on a Falcon 4i direct detector at a nominal magnification of 190kx, corresponding to a physical pixel size of 0.73 Å. Image processing was performed using cryoSPARC v4 [3].

HD6 thin filaments were found to consist of stacked pairs of dimers spiraling along the helix axis with apparent D2 symmetry. Strong densities were observed along the axis and were interpreted as phosphate ions, each coordinated by multiple copies of a histidine, H5, in HD6. Mutation of H5 abolished filamentation. Once maps of sufficient resolution were obtained, a symmetry break became apparent: the two spirals of dimers in the thin filaments were found to be related by two-fold symmetry perpendicular to the helix axis, but not by true two-fold symmetry along the axis as would have been expected for D2. The repeating unit of the thin filament consists of two stacked HD6 dimers, with one protomer out of the four displaying a loop in a slightly different conformation than in the other three molecules. The thick filaments, in turn, formed by wrapping two additional HD6 dimer spirals around the thin filament without coordination of additional phosphate. The outer spirals adopt D2 symmetry and show helical rise and twist that differ from the inner filament.

The robust multiple micron length HD6 filaments are explained by the fact that two or more HD6 dimers constitute each level of the supramolecular assembly. Moreover, phosphate plays a structural role in stabilizing the filaments. HD6 helical polymorphs arise from an optional wrapping of additional stacked-dimer spirals around the phosphate-coordinating inner filament, and a symmetry mismatch exists between inner and outer spirals.

Keywords:

Cryo-EM, SPA, Defensins, HD6

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Molecular insights into the biogenesis of box H/ACA snoRNPs

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Poster Group 1

Molecular insights into the biogenesis of box H/ACA snoRNPs
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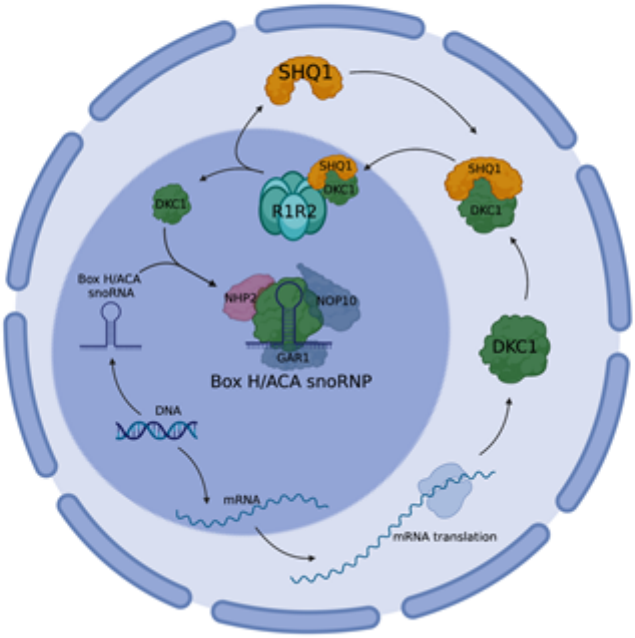
Box H/ACA snoRNPs are protein-RNA complexes composed of NOP10, GAR1, and the catalytically active DKC1. The latter is known to interact with small non-coding RNAs containing the Box H/ACA motif. Guided by the RNA, the complex finds its target RNAs and converts the nuclear base uridine to pseudouridine, resulting in increased stability of the target RNA due to an additional hydrogen bond. Pseudouridines are vital for various cellular functions, such as protein translation, as they undergo frequent post-transcriptional modifications in ribosomal RNAs.
However, it is still poorly understood how the Box H/ACA snoRNP complexes and their individual protein precursors are assembled. SHQ1, a chaperone-like protein, is proposed to prevent DKC1 from binding to nonspecific ribonuceotides during its assembly in the cytosol and subsequently guide the complex to the nucleus, where it interacts with the AAA-ATPase complex RuvBL1/2. These ATPases are known to interact with PIH1D1 and RPAP3 proteins to form the R2TP complex, which is described to play a role in the assembly of multiple macromolecular complexes. Thus, the R2TP complex could be responsible for sequestering SHQ1 from DKC1, allowing DKC1 to interact with Box H/ACA snoRNAs and ultimately assemble the mature snoRNP.
The aim of this work is to elucidate the maturation of the Box H/ACA snoRNP by characterizing its precursor complexes composed of SHQ1, DKC1, and RuvBL1/2. This was achieved using size exclusion chromatography, dynamic light scattering, differential scanning fluorimetry, surface plasmon resonance, mass photometry, cross-linking mass spectrometry, and cryo-EM.
We were able to confirm the interaction between RuvBL1/2 and DKC1 or SHQ1. Additionally, we purified and stabilized the SHQ1:RuvBL1/2 complex and identified multiple complex species. Revealing the interaction site between the AAA ATPases and SHQ1 helped to reconstruct an initial 3D model of the complex's structure processing the movies collected in Cryo-EM experiments.
The biochemical and biophysical analysis of the interaction between R1R2 and SHQ1 allowed us to identify an additional electron density below the unique and regulartory domain II of the hexameric R1R2 complexes. However, our Cryo-EM reconstruction shows high flexibility for the complex, which is why mild cross-linking using GraFix might to be a valid option to determine a high resolution 3D structure.



Keywords:

snoRNPs, PAQosome, Cryo-EM, SPR, Chaperones

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Structural characterisation of a phage-like bacteriocin from Pseudomonas sp. by cryo-Electron Microscopy

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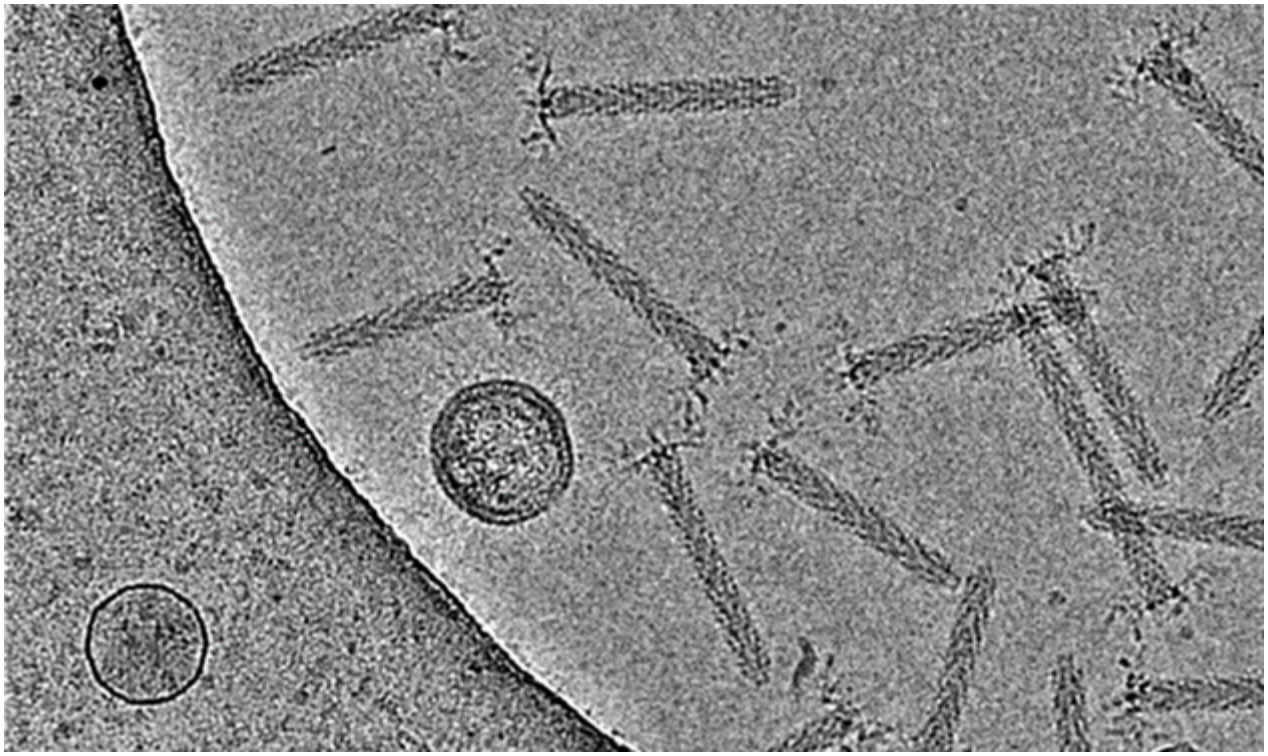
Poster Group 1

Background incl. aims
Antibiotic resistance is a global health crisis with the ever growing need to develop novel antibiotics and strategies to treat resistant infections. Bacteriophage therapy is often highlighted as an alternative approach due to its high specificity to kill a certain bacterial strain. However, bacteriophage propagate through a replication cycle within the target bacterium, with the potential to generate mutations with detrimental consequences. Strains of Pseudomonas sp. produce phage tail-like bacteriocins (PTLBs) which have evolved from bacteriophage. Although sharing many similarities, they differ from bacteriophage lacking a capsid and therefore the ability to replicate. These unique features highlight the potential of PTLBs as an alternative therapy to treat bacterial infections as they can be tittered to a specific dose. However, for PTLBs to be implemented as a bactericidal treatment, further information is needed regarding their structure, mechanism of action and how they recognise their target strains. We have isolated a contracting PTLB from an environmental strain of P. veronii and determined its structure by cryo-EM.

Methods
The PTLB was purified by ammonium sulphate precipitation and visualised by cryo-EM. The structure of the PTLB was determined using a combination approach of single particle and helical analysis.

Results
The structure was determined of a new clade of contracting PTLBs in both its uncontracted and contracted states. We also identified the lack of a ‘ruler protein’ for the purified PTLBs, observing varying lengths in the collected micrographs.

Conclusion
We solved the structure of a novel contracting PTLB and show that it shares structural similarities with the previously characterised contractile nanomachine from P. aeruginosa. We also observe that inconsistent lengths of PTLBs does not appear to affect the lethality of the PTLB to its target strain.



Keywords:

Bacteriocin, filament, cryo-EM

Reference:

Carim, S. et al. ISME J 15, 2289–2305 (2021)

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Low-dose cryo-electron ptychography of proteins at sub-nanometer resolution

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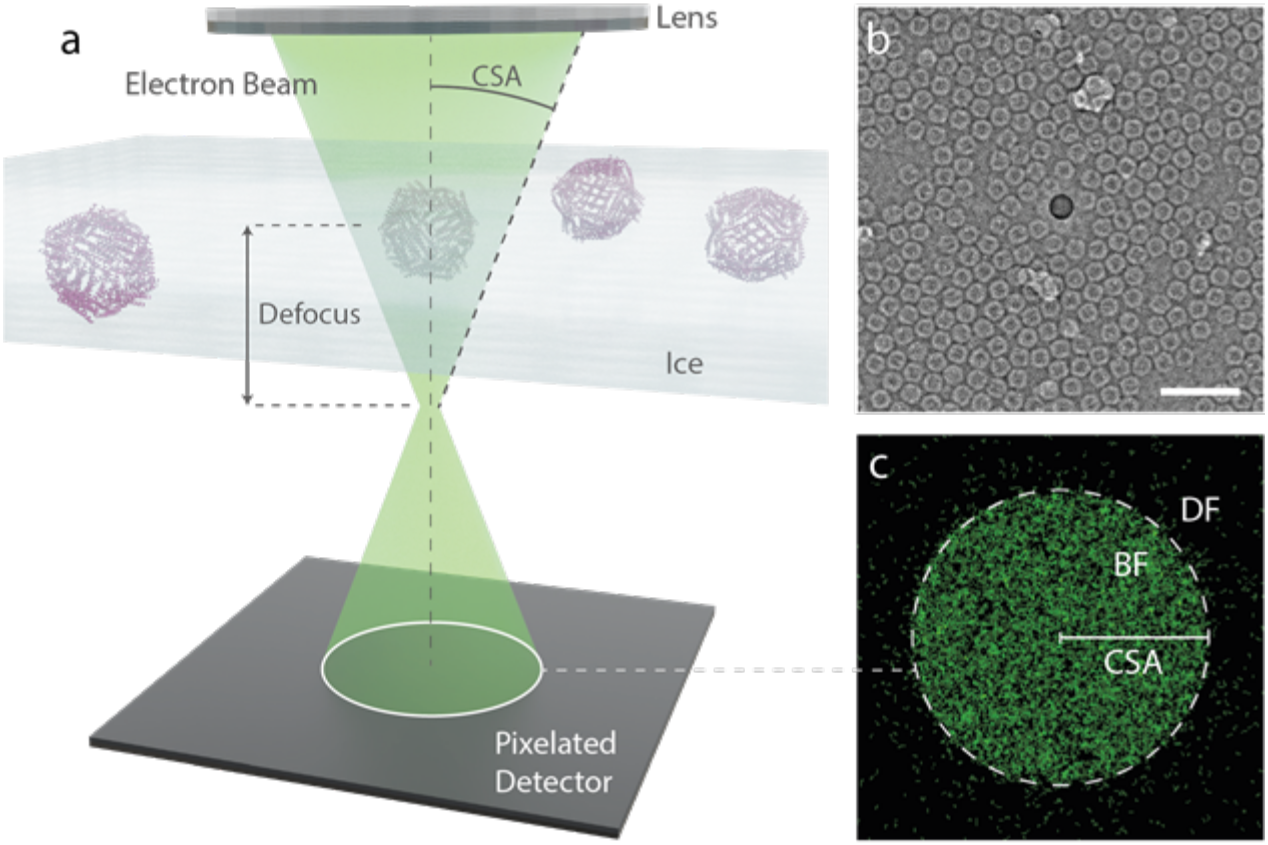
Poster Group 1

The field of cryogenic electron microscopy (cryo-EM) has seen remarkable advancements, enabling researchers to visualize biomolecular structures with ångström-level resolution. While conventional transmission electron microscopy (CTEM) combined with single-particle analysis (SPA) has played a crucial role in these developments, it faces challenges with very small proteins and large biomolecules. Specifically, small proteins often exhibit insufficient contrast, and large biomolecules can produce opaque images due to multiple scattering.
Electron ptychography, a technique derived from 4D scanning transmission electron microscopy (4D-STEM), presents promising solutions to these issues. It enhances contrast through phase retrieval, aiding in imaging small proteins, and manages multiple scattering effects, which is vital for examining thicker specimens. Electron ptychography has already proven to be a powerful method for room temperature material science samples, achieving unprecedented resolutions[1]. However, the application of STEM-related techniques to biological specimens is relatively rare[2,3], and ptychographic imaging at low-dose conditions (less than 50 e/Å²) remains largely unexplored.
In our study, we applied this technique to frozen hydrated single protein samples and achieved sub-nanometer resolution with a relatively small number of particles, yielding micrographs of enhanced quality (signal-to-noise ratio, SNR). The scattering potential of single protein particles was iteratively reconstructed using the open-source software, py4DSTEM[4]. We present structures of apoferritin at 5.8 Å using 11,552 particles, a phi92 sheath structure at 8.4 Å using 1,600 particles, and a tobacco mosaic virus at 6.4 Å using 2,120 particles.
The application of ptychography to biological specimens, particularly when integrated within the SPA framework for smaller frozen hydrated protein particles or combined with tomography tilt series for thicker samples (e.g., bacteria, tissue), holds great promise.



Keywords:

4D-STEM, ptychography, cryo-EM, low-dose

Reference:

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Structural organization of the native Neisseria meningitids PilQ environment through an MS-EM approach

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Poster Group 1

Background incl. aims
Neisseria meningitidis are Gram-negative bacteria responsible for life threatening sepsis and meningitis and are human-exclusive pathogens. Disease is driven by polymeric fibers at the surface of bacteria called type-IV pili (T4P), assembled by a multi-protein molecular machine known as type-IV pilus machinery (T4PM). Only the outer membrane and peptidoglycan segment of the T4PM, the PilQ secretin, allows the T4P to be secreted to the extracellular medium and thus it is of great interest for therapeutical approaches and bacterial model design. Because PilQ is reported to be widely different among bacteria, the structure and assembly of this secretin in Neisseria is not known. We therefore aim to solve not only the high-resolution model of PilQ but also describe the proteins involved in its assembly. We finally use this information to develop a piliation-trapped bacteria for cryo-ET.

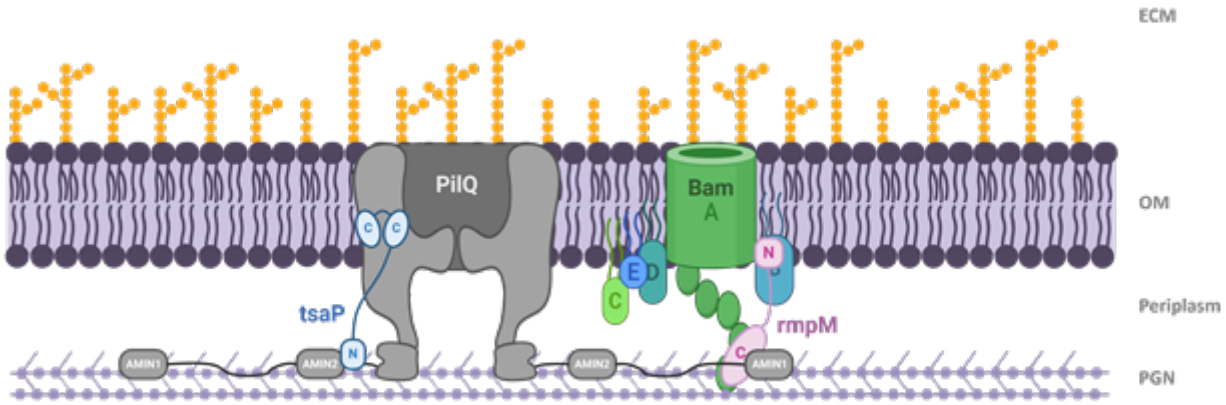
Methods
For electron microscopy purposes, PilQ was natively purified from N. meningitidis (Strain 8013) ΔsiaD under Biosecurity II conditions. Proteins were loaded into custom carbon-coated Au-CFlat grids and images were collected in a Titan Krios microscope equipped with a Falcon 4i camera (Thermo Fischer Scientific), then processed using diverse processing software, most notably Relion 4 and CryoSPARC 4. Modelling and refinement of PilQ and its associated proteins was performed with Coot and Phenix using information from MS, Alphafold2, deep-etch EM and cryo-EM. Peptidoglycan-binding and protein-protein interaction assays were carried out to support the working model.
Mass spectrometry was performed on cross-linked samples of N. meningitidis pellets harvested from plate cultures. Membranes were crosslinked using UV-aided click-chemistry with NNP9 and digested. Eluted peptides from Photocleavable alkyne agarose beads were analyzed by nanoLC-MS/MS using a Vanquish Neo UHPLC system coupled to an Orbitrap Eclipse Tribrid mass spectrometer fitted with an EASY-Spray Source (Thermo Scientific). Peptides were separated on an EASY-Spray PepMap Neo.

Results
Our cryo-EM map of native NM PilQ at 2.14 Å resolution allowed to fully build an atomic model of all domains of the protein, including its highly-dynamic gate but excluding the flexible AMIN1 and AMIN2 peptidoglycan region. It is a 14-fold symmetry dynamic molecule as opposed to the previously reported C12. This structure allowed to design NM strains with locked conformations of the T4PM for subtomogram averaging purposes.
Both in vitro and crosslink data, combined with knowledge from literature allowed to build the AMIN regions, which tend form dimers, thus pointing to a heptamer-of-dimers structure. Similarly, MS data on NM membranes pointed to three proteins that interacted with PilQ in-situ: TsaP, RmpM and BamA. This data, along with PGN-binding and BLI assays, allowed to build a working model of the assembly. Crosslinks of interactor-deficient mutant strains also enabled to describe the functionality of the interacting proteins in the assembly of PilQ. TsaP dimerizes PilQ protomers and anchors them

to the peptidoglycan, while BamA interacts with RmpM which in turn localizes the PilQ protomers and keeps the secretin on top of the peptidoglycan.

Conclusion

Neisseria meningitidis PilQ is a 14-mer secretin, assembled as a heptamer-of-dimers, driven by TsaP and the propensity of the AMIN region to dimerize. Transitory interactions with BamA and RmpM help localize protomers for oligomerization and position the secretin on top of the peptidoglycan. The structure of PilQ shows a highly-dynamic gate that can be blocked with mutations, thus enabling T4PM-driven piliation steps to be studied in-situ by cryo-electron tomography. Further understanding piliation may enable novel approaches to inhibiting the process and thus, disease.



Keywords:

Secretin, gram-negative, peptidoglycan, cryo-EM, cross-linking

Reference:

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Global BioImaging: Imaging Networks Accelerate Collaboration, Exchange and Innovation in Imaging Science

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Poster Group 1

Global BioImaging mobilizes imaging scientists and facilities from 60 countries and 13 networks, accelerating resource exchange and championing the global impact of imaging. Through inclusive programs, Global BioImaging transcends barriers to foster innovation and community building under the banner of "Imaging 4 All," spanning bioimaging, biomedical, and material sciences.

Global BioImaging offers comprehensive training, job shadowing, and advocacy to empower imaging scientists and influence policy. Collaborative papers and working groups advance topics from career pathways to infrastructure development. Key discussions focus on fair career paths, funder communication, network building, training courses, showcasing core values, and data management.

Imaging networks connect researchers with advanced instruments, enhancing data quality, fostering collaboration, and improving resource efficiency. Shared facilities reduce costs and expand access to cutting-edge technologies.

The synergy between open access and imaging networks drives scientific progress. Open access disseminates high-quality data widely, while imaging networks contribute to openly accessible research, enhancing reproducibility, fostering global collaboration, and driving technological advancements.

Global BioImaging and partner imaging networks are reshaping the scientific community by democratizing knowledge and providing unparalleled tools for exploration and analysis. As anticipation grows for the annual meeting in Japan (#GBI_EoE2024), Global BioImaging continues to accelerate exchange and foster collaboration in imaging science.

Keywords:

#GlobalBioImaging #ImagingScience
#OpenAccess #Training #Innovation

Reference:

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(2) https://doi.org/10.5281/zenodo.10057024
(3) https://doi.org/10.5281/zenodo.10591588

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Structural studies of the cross-link mutant, ABC transporter BmrA by cryo electron microscopy

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Poster Group 1

ATP-binding cassette (ABC) transporters are the membrane proteins that use the energy from ATP hydrolysis to undergo some conformational changes and transport a variety of substrates across biological membranes. The flexible nature of these transporters enables them to adopt various conformation, hence accommodate different substrates. Certain ABC transporters provide multidrug and antimicrobial resistance in cancer cells and pathogenic microorganisms.
These transporters mainly function by alternating between inward-facing and outward facing conformations. ATP binding creates an outward-facing conformation that facilitates drug release, while ATP hydrolysis resets the transporter to its inward-facing conformation. The opening and closing conformation of the nucleotide-binding domains (NBDs) directly cause movements in the transmembrane helices, leading to changes in the substrate-binding pocket. Various degrees of the opening and closing of the nucleotide binding domain has been reported.
Although the amount of the structural information has been increased over the past years, there are still some missing information.
To study the effect of the separation of the NBDs on catalytic cycle, we introduced a Cysteine near the C terminal of the NBD. The structural impact of the mutation on conformational changes of the transporter was investigated by cryo electron microscopy. The mutant did not affect the function of the BmrA and there was no structural difference between mutant and WT BmrA. Two structures were highly similar, showing intermediate opening between their NBDs while their C-terminal extremities remain in close proximity. This data suggests that the NBDs of BmrA function with a tweezers-like motion.