Rhine Knee Meeting 2023

Report of Abstracts

https://indico.psi.ch/e/14717

Abstract ID : **356**

Host lattice display: a method for the analysis of biomolecules.

Content

Crystallography requires crystals to amplify the diffraction of photons. In host lattice display (HLD) the molecule of interest is arranged in a regular assembly by means of a host lattice, which is a porous crystal lattice that can be obtained under established conditions. For imbedding the molecule of interest in the host lattice we used designed ankyrin repeat proteins that are fused to a bulky scaffold protein. The method was initially developed to study relatively short peptides, but in principle it is applicable even to large macromolecules. In practice HLD is limited by the temperature factor gradient of the assembly, which is a feature of the host lattice design, and the occupancy of the target molecule.

Ernst P, Plückthun A, Mittl PRE. (2019) Sci Rep 9:15199.
Kiss C, Gall FM, Dreier B, Adams M, Riedl R, Plückthun A, Mittl PRE. (2022) Acta Cryst D78:1439–1450.

Primary author: MITTL, Peer (Universitaet Zuerich)

Presenter: MITTL, Peer (Universitaet Zuerich)

Track Classification: 3 Methods for structure determination (X-ray, cryoEM)

Contribution Type: Oral presentation

Status: SUBMITTED

Submitted by **MITTL, Peer** on **Monday, 11 September 2023**

Abstract ID : **357**

Crystal structure of archaeal IF5A-DHS complex reveals insights into the hypusination mechanism

Content

The translation factor IF5A is a highly conserved protein in Eukarya and Archea carrying a unique hypusine post-translational modification. Hypusination of IF5A requires the Deoxyhypusine Synthase (DHS) enzyme which transfers the butylamine moiety from spermidine to IF5A using NAD as a cofactor. IF5A is a key player in protein synthesis and its overexpression in cancer drives migration and metastasis, while inhibiting hypusination exerts anti-proliferative effects. Hypusination pathway of IF5A is therefore an attractive new therapeutic target. We have solved the 2.0 Å X-ray structure of the archeal DHS-IF5A complex, showing a hetero-octameric architecture and providing a detailed view of the complex active site including the hypusination loop. These structural data are complemented by biophysical data in order to provide insights into hypusination reaction's catalytic mechanism.

Primary author: ENNIFAR, Eric (CNRS)

Presenter: ENNIFAR, Eric (CNRS)

Track Classification: 5 Molecular machines

Contribution Type: Oral presentation

Status: SUBMITTED

Submitted by **ENNIFAR, Eric** on **Tuesday, 12 September 2023**

Abstract ID : **358**

Exploring Canavanyl-tRNAArg Deacylase (CtdA): a novel tRNA editing enzyme responsible for the removal of mischarged canavanine from arginine tRNA

Content

Proteins, which are responsible at large for the functionalities and skeletal architectures of the living cell, are heteropolymers composed of twenty standard proteogenic amino acids.However, over 500 non-proteogenic amino acids exist in nature. One such example, canavanine an antimetabolite of L-arginine, is present in legume plants. Canavanine is a sole source of carbon and nitrogen P.canavaninivorans, symbiotic bacteria in legume plants. However, in this bacterium, canavanine is also incorporated in proteins as its arginyl-tRNA synthetase can load tRNAArg with both canavanine and arginine. Recently, a canavanyl-tRNAArg deacetylase (CtdA) has been discovered, that removes canavanine from canavanyl-tRNAArg and prevents the incorporation of this antimetabolite in proteins. We have now elucidated the crystal structure of CtdA to 1.52 Å resolution and studied its active site and complexation of canavanine and arginine using bioinformatics, docking methods and site-directed mutagenesis.

Primary author: TABAGARI, Nino (Master student)

Presenter: TABAGARI, Nino (Master student)

Track Classification: 6 Ligand studies

Contribution Type: Poster

Comments:

Full authors list : Nino Tabagari1, Jennifer Fleming1, Franziskus Hauth2, Jörg Hartig2, Olga Mayans1 1 Department of Biology & 2 Department of Chemistry, University of Konstanz, Konstanz

Status: SUBMITTED

Submitted by **TABAGARI, Nino** on **Wednesday, 13 September 2023**

Abstract ID : **359**

Investigation into mechanical signalling of titin-like kinases in the muscle sarcomere

Content

Three parameters are critical for modulation of cell activity: chemical signals, electrical signals and mechanical forces. While chemical and electrical signalling is well studied, the cell's property to respond to mechanical forces is not fully understood. Filamentous proteins like obscurin in the muscular cytoskeleton experience stretching forces. Obscurin contains the C-terminally located kinase PK1, a pseudokinase with scaffolding function, which undergoes conformational changes during stretch, potentially exposing cryptic binding sites. Fluorescently-tagged nanobodies will be designed to target those cryptic binding sites in PK1, validating theoretical models of kinase mechanical loading in vivo.

Primary author: MARTIN, Isabel (University of Konstanz)

Presenter: MARTIN, Isabel (University of Konstanz)

Track Classification: 6 Ligand studies

Contribution Type: Poster

Comments:

Isabel Martin (1), Christof Hauck (1), Guy Benian (2), Olga Mayans (1)

(1) Department of Biology, University of Konstanz, 78464 Konstanz, Germany. (2) Department of Pathology Research, Emory University, Atlanta, GA 30322, U.S.A.

Status: SUBMITTED

Submitted by **MARTIN, Isabel** on **Thursday, 14 September 2023**

Abstract ID : **360**

Tandem Obscurin Kinases and their role in mechanosensing and signalling

Content

Muscle activity is a costly and mechanically challenging cellular process that must be tightly regulated to prevent cellular damage and adjust tissue performance to force demand. Regulation requires that mechanical signals generated during activity are sensed inside the contractile units of muscle. Proposed mechanosensors are a group of elastic-filamentous proteins embedded in the muscle. The least studied are Obscurin proteins, which have a C-terminal dual-kinase region. We investigate the structure of these kinases in the fly and nematode and try with a combination of biochemical and computational approaches to deduce the structure-function relation in respect to mechanosensing for those. The N-terminal kinases are inactive unlike the C-terminals. We find that these two interact even though being 200aa apart. Therefore, we conclude that these kinases have evolved to form a gradually-regulated-mechanosensing unit to sense and integrate the mechanical load during muscle activity.

Primary author: DORENDORF, Till (University of Konstannz)

Presenter: DORENDORF, Till (University of Konstannz)

Track Classification: 5 Molecular machines

Contribution Type: Poster

Comments:

Co-authors: Thomas Zacharchenko2, Anja Katzemich3, Belinda Bullard3, Guy Benian4, Olga Mayans1 1 Department of Biology, University of Konstanz, Konstanz, Germany 2 Institute of Integrative Biology, University of Liverpool, Liverpool, UK 3 Department of Biology, University of York, York, UK 4 Department of Pathology, Emory University, Atlanta, Georgia, USA.

Status: SUBMITTED

Submitted by **DORENDORF, Till** on **Monday, 18 September 2023**

Abstract ID : **361**

An optimised and simplified CHO-based transient gene expression system for protein target optimisation and for research material production.

Content

Transient gene expression (TGE) is used in protein engineering, structural analysis, study of functional principles and to develop more efficient protein-based prophylactic or therapeutic agents. Examples are shared. The CHO4Tx® technology was simplified significantly for easy handling, eliminating error prone steps, from reagent/DNA interaction to cell handling in order to improve reproducibility and yield. The transfection-inducing chemicals are part of a medium, the transfection medium (TM). It can be used off the shelf. In addition, nucleic acids can be provided to the container, before adding cells in TM to it. Subsequently, a production medium (PM) is just added and cells are incubated for an appropriate time until harvest of product from the supernatant of the chemically defined media (no serum). The optimized CHO4Tx® cells are part of the system with a detailed protocol for handling. In comparative studies, the CHO4Tx® system shows superior results over others.

Primary author: PUGINIER, Jérôme (Magellan Biologics)

Presenter: PUGINIER, Jérôme (Magellan Biologics)

Track Classification: 2 In situ structural biology

Contribution Type: Oral presentation

Comments:

The lecture would be done by Prof. Florian Wurm, possibly on October 11th. The authors are: Florian M. Wurm1,2,3*, Artur Rodrigues1, Vanessa Fernandes1, Maria. J. Wurm1,2** 1Magellan Biologics and Consulting LDA, Torres Novas, Portugal 2ExcellGene SA, Monthey, Switzerland 3Swiss Federal Institute of Technology Lausanne (EPFL) *corresponding and presenting author

Status: SUBMITTED

Submitted by **PUGINIER, Jérôme** on **Tuesday, 19 September 2023**

Abstract ID : **362**

Characterization of the active state of the sarcomeric twitchin kinase

Content

The cytoskeleton is an essential and extensive component of the eukaryotic cell, where it plays a key role in generating, transmitting and responding to mechanical strain. Mechanosensing has been studied in twitchin kinase (TwcK) from *C. elegans*, which is a domain within the titin-like, giant filamentous protein twitchin of the specialized contractile cytoskeleton of muscle, the sarcomere. TwcK has two tail extensions that pack tightly against the kinase, thereby inhibiting phosphortransfer activity. Tail unfolding appears induced by the stretch emerging in the sarcomere during muscle function, leading to the activation of its catalysis. Recently, we have succeeded in characterizing by X-ray crystallography the 3D-structure of uninhibited TwcK lacking regulatory flanking domains bound to the ATP-analogue staurosporine and a peptide model substrate. Structural data are complemented with high-pressure 31P NMR spectroscopy and biochemical assays to explore pressure-induced activation.

Primary author: GRAVENHORST, Peter (University of Konstanz)

Presenter: GRAVENHORST, Peter (University of Konstanz)

Track Classification: 5 Molecular machines

Contribution Type: Poster

Comments:

Co-Authors: Frederic Berner2, Michael Kovermann2, Olga Mayans1 1 Department of Biology, University of Konstanz, Germany; 2 Department of Chemistry, University of Konstanz, Germany

Status: SUBMITTED

Submitted by **GRAVENHORST, Peter** on **Wednesday, 20 September 2023**

Abstract ID : **363**

Tubulin as a target for drug discovery: challenges, opportunities, and threats

Content

The microtubule cytoskeleton is vital for cell division, intracellular trafficking, and the maintenance of cell shape and motility. Because microtubules are implicated in such essential cellular activities, compounds interfering with microtubule function have been developed as cytotoxic agents for basic research, cancer treatment, and parasite control. Microtubule-targeting agents (MTAs) promote cell death by suppressing microtubule dynamics. Despite being among the most important medical weapons in cancer treatment, their use faces challenges related to toxicity and resistance. Recent advances in MTA structural analysis have revealed insights into their interactions with microtubules and their building block, tubulin. In my lecture, I will highlight MTA development milestones, discuss our current knowledge of their mechanisms of action, and suggest avenues for future research in MTA structural biology and drug discovery.

Primary author: Dr PROTA, Andrea (PSI - Paul Scherrer Institut)

Presenter: Dr PROTA, Andrea (PSI - Paul Scherrer Institut)

Track Classification: 6 Ligand studies

Contribution Type: Oral presentation

Comments:

keynote lecture

Status: SUBMITTED

Submitted by **Dr PROTA, Andrea** on **Wednesday, 20 September 2023**

Abstract ID : **364**

Targeting the oncoprotein Golph3

Content

Golgi phosphorylated protein 3 (Golph3) regulates the Golgi's transport of glycosylating enzymes within the glycosphingolipid (GSL) synthetic pathway. Golph3 associates with the COPI coat and binds to GSL enzymes, facilitating their incorporation into COPI vesicles. Overexpressed in many cancers, Golph3 alters GSL metabolism, promoting cancer cell proliferation. Despite its clinical relevance, its molecular function lacks characterization. Our study focuses on Golph3's interaction with the Lactosyl Ceramide Synthase (LCS) tail. Employing HDX-MS, NMR, crystallography, and mutagenic studies, we identified residues crucial for this interaction. The LCS binding site resides on Golph3's negative surface. Additionally, we investigate Golph3's interaction with the COPI coat. Our aim is to create an atomistic model of the complex between Golph3, COPI, and GSL biosynthetic enzymes. This work offers potential insights for designing inhibitors targeting Golph3's oncogenic activity.

Primary author: THEODOROPOULOU, Anastasia (EPFL - EPF Lausanne)

Presenter: THEODOROPOULOU, Anastasia (EPFL - EPF Lausanne)

Track Classification: 3 Methods for structure determination (X-ray, cryoEM)

Contribution Type: Oral presentation

Status: SUBMITTED

Submitted by **THEODOROPOULOU, Anastasia** on **Thursday, 21 September 2023**

Abstract ID : **365**

Using integrative structural biology for the identification of novel aerolysin-like pore forming toxins for nanopore sensing

Content

Pore-forming toxins (PFTs) have emerged as a valuable tool for nanopore sequencing. PFTs are secreted as soluble proteins and only upon membrane binding oligomerize into transmembrane pores. One of the well characterized PFTs used for nanopore sensing is aerolysin. However, pores with different characteristics from aerolysin such as size and charge might be more suitable for other applications. Currently. most pores, including aerolysin, are characterized under non-physiological conditions using detergents. Lipid nanodisc present an alternative, which allows to characterize membrane proteins in their lipid environment. Here, we used the Alphafold database to identify new pore candidates with unknown properties by structural search. Pore candidates are further expressed and characterized using various biophysical methods and their lipid preferences are being analyzed. In a last step, we structurally characterized a few pores, including aerolysin, in polymer lipid nanodiscs by cryo-EM.

Primary author: ANTON, Jana Susanne (EPFL - EPF Lausanne)

Presenter: ANTON, Jana Susanne (EPFL - EPF Lausanne)

Track Classification: 3 Methods for structure determination (X-ray, cryoEM)

Contribution Type: Poster

Status: SUBMITTED

Submitted by **ANTON, Jana Susanne** on **Thursday, 21 September 2023**

Abstract ID : **366**

Catalytic cycling of human mitochondrial Lon protease

Content

The human mitochondrial Lon protease (LonP1) regulates mitochondrial health by removing redundant proteins from the mitochondrial matrix. Malfunctions are linked to several diseases and homologues are found in all kingdoms of life. LonP1 also binds mitochondrial DNA, plays a vital role in mitochondrial DNA maintenance, and regulates non-damaged proteins.

Cryoelectron microscopy revealed LonP1 in eight different conformations, which we ordered into a molecular movie showing how LonP1 may unfold proteins. In addition, we identified conserved residues that might form a hitherto unreported proteolytic site.

Building on this, we are currently working on substrate recognition and DNA binding by LonP1 and have thus found another catalytic state. Using fusion proteins, we intend to find out more about the start of the degradation process and solve the structure of LonP1 in complex with a substrate. By that we might also find out more about the utility of a second catalytic site.

Primary author: SCHENCK, Niko

Presenter: SCHENCK, Niko

Track Classification: 5 Molecular machines

Contribution Type: Oral presentation

Status: SUBMITTED

Submitted by **SCHENCK, niko** on **Thursday, 21 September 2023**

Abstract ID : **367**

Regulation of the μ-opioid receptor by a nanobody antagonist

Content

The μ-opioid receptor (μOR) is the molecular target of opioid pain medicine. Due to severe side effects caused by currently available opioids, there is considerable interest in developing novel modulators of μOR signaling. Nanobodies are emerging as promising therapeutics with distinct and often superior properties. Here, we present a novel nanobody (NbE) that binds to the μOR and acts as an antagonist. We functionally characterize NbE as extracellular μOR ligand and determine the structure of the NbE-μOR complex. The structure reveals extensive interactions of NbE with the orthosteric ligand binding pocket of the μOR and its extracellular loops. Based on a variable NbE β-hairpin loop we design and test peptide mimetics that retain μOR antagonism. Future research will focus on computationally optimised nanobodies and their peptides. We are aiming to identify high-affinity nanobodies (in the picomolar range), determine their structure, and characterise their binding behaviour.

Primary author: SCHAEFFNER, Ilse (Universitaet de Geneve)

Presenter: SCHAEFFNER, Ilse (Universitaet de Geneve)

Track Classification: 4 Membrane proteins

Contribution Type: Poster

Status: SUBMITTED

Submitted by **SCHAEFFNER, Ilse** on **Tuesday, 26 September 2023**

Abstract ID : **368**

A Revolutionary Method for Labelling in-situ Proteins

Content

Cryo-electron tomography (CryoET) is an incredible imaging technique. Recently, combining CryoET with sub-tomogram averaging has pushed the resolution to 3-4 Å. However, one challenge is locating proteins in live cells using CryoET. We used the FKBP and FRB system, which involves two tags that bind together when incubated with rapamycin. The FKBP tag links to the target protein, while the FRB tag links to a large protein to create a marker. We chose ferritin as the marker protein because its complex is large, about 10-12 nm, and it binds with iron to create a good contrast in cryo-EM pictures. After adding rapamycin to the cell medium, the ferritin with iron indicates the location and identity of the target protein. This approach could be used in CryoET and sub-tomogram averaging to analyze the in-situ structure of proteins in future. E

Primary author: WANG, Chang (Institute of Anatomy)

Presenter: WANG, Chang (Institute of Anatomy)

Track Classification: 3 Methods for structure determination (X-ray, cryoEM)

Contribution Type: Poster

Status: SUBMITTED

Submitted by **WANG, Chang** on **Wednesday, 27 September 2023**

Abstract ID : **369**

Advancing Protein Complex Analysis with AI and Electron Diffraction

Content

In our research group, we focus on improving the analysis of large protein complexes using electron diffraction. Our aim is to achieve higher resolution for flexible proteins, surpassing the limitations of current methods.

One major challenge we face is the vast amount of data generated by our equipment, which is crucial for structural analysis. This makes automated data triage essential, in order to filter out the useful data from the junk, and I will share my novel approach that uses deep learning. I will also present our breakthrough in efficient data compression. These breakthroughs address the need for efficient data management in electron diffraction studies. We are further developing novel algorithms based on deep learning to interpret electron diffraction and extract valuable structural information.

Although we are still in the early stages of directly determining protein structures through electron diffraction, we are confident in its potential to surpass current methods.

Primary authors: ABRAHAMS, Jan-Pieter; MATINYAN, Senik

Presenter: MATINYAN, Senik

Track Classification: 1 Artificial intelligence in structural Biology

Contribution Type: Oral presentation

Status: SUBMITTED

Submitted by **MATINYAN, Senik** on **Friday, 29 September 2023**

Abstract ID : **370**

Mapping venom-antivenom interactions by cryoEM

Content

Snake bite envenoming is a global health threat causing ~2.7M cases annually and disproportionately affecting rural population in developing countries. Current treatment strategies based on polyclonal antibodies (pAbs) display limitations such as low efficacy, limited cross-reactivity and adverse reactions. Novel antivenom solutions are needed to address these shortcomings. However, antivenom research is impeded by the lack of structural data due to heterogenous composition, coupled with lack of sequence and paratope information of pAbs. Herein, we apply cryoEM-based polyclonal epitope mapping for identifying neutralizing epitopes against different toxin families. In our proof-of-concept study with snake venom L-Amino-Acid Oxidase, we studied how pAbs from commercially available antivenom, EchiTAbG, engage this toxin. Together with bioinformatic analyses, structural and functional data, our study demonstrates how epitope specificity correlates with neutralization and cross-reactivity.

Primary author: KUMAR, Kiruthika (École Polytechnique Fédérale de Lausanne (EPFL))

Presenter: KUMAR, Kiruthika (École Polytechnique Fédérale de Lausanne (EPFL))

Track Classification: 3 Methods for structure determination (X-ray, cryoEM)

Contribution Type: Poster

Status: SUBMITTED

Submitted by **KUMAR, Kiruthika** on **Saturday, 30 September 2023**

Abstract ID : **371**

Encasing carbon fixation: the pyshell in diatoms

Content

Diatoms are algae which are responsible for about 20% of all carbon fixation. In algae, carbon fixation occurs in a dedicated liquid phase separated organelle called the pyrenoid. Here we reveal the in situ architecture of the diatom pyrenoid using cryo-electron tomography and show that it's liquid phase separated matrix encased in a lattice-like protein sheath. Using in vivo photo-crosslinking to catalogue components of diatom pyrenoid we identified a pyrenoid shell (PyShell) protein, which we localized to the pyrenoid periphery. Disruption of PyShell expression resulted in the absence of this protein sheath, altered pyrenoid morphology, impaired growth and reduced carbon fixation efficiency, demonstrating how the PyShell plays a crucial guiding role in establishing pyrenoid architecture. Combining in vitro single particle analysis and in situ subtomogram averaging of the pyrenoid's protein sheath we build an atomic model of the PyShell and framework for CO2 assimilation in the ocean.

Primary author: DEMULDER, Manon (Biozentrum Basel)

Presenter: DEMULDER, Manon (Biozentrum Basel)

Track Classification: 2 In situ structural biology

Contribution Type: Oral presentation

Comments:

Authors list: Ginga Shimakawa, Manon Demulder, Serena Flori, Akihiro Kawamoto, Yoshinori Tsuji, Hermanus Nawaly, Atsuko Tanaka , Rei Tohda, Tadayoshi Ota, Hiroaki Matsui, Natsumi Morishima, Ryosuke Okubo, Wojciech Wietrzynski, Lorenz Lamm, Ricardo D. Righetto, Clarisse Uwizeye, Benoit Gallet, Pierre-Henri Jouneau, Christoph Gerle, Genji Kurisu, Giovanni Finazzi, Benjamin D. Engel, Yusuke Matsuda

Status: SUBMITTED

Submitted by **DEMULDER, Manon** on **Tuesday, 3 October 2023**

Abstract ID : **372**

High Resolution Investigation of Mitochondrial Molecular Architecture using Cryo-Electron Tomography

Content

In the Engel lab, we help develop and use Focused Ion Beam (FIB) milling combined with cryo-electron tomography (cryo-ET) to unravel the native organization of molecular complexes directly inside cells. We apply cryo-ET to investigate organelle biology, focused on photosynthetic organisms. In eukaryotic cells, mitochondria are essential components that act as metabolic hubs and powerhouses, producing energy through aerobic respiration. Using a combination of biochemistry, high-resolution cryo-electron microscopy (cryo-EM), and cryo-ET, we study the molecular diversity of mitochondrial complexes across photosynthetic lineages.

To investigate mitochondrial complexes in their native organization, we acquired a large dataset at high magnification of the green alga *Chlamydomonas reinhardtii*, the main unicellular alga used in the lab. This allowed to identified the major soluble and membrane proteins of mitochondria, hinting at how membrane architecture is shaped by molecular complexes.

Primary author: WALTZ, Florent (University of Basel - Biozentrum)

Presenter: WALTZ, Florent (University of Basel - Biozentrum)

Track Classification: 4 Membrane proteins

Contribution Type: Oral presentation

Status: SUBMITTED

Submitted by **WALTZ, Florent** on **Tuesday, 3 October 2023**

Abstract ID : **373**

Self-assembling nanoparticles as carriers for HIV vaccine candidates

Content

Two-component, self-assembling nanoparticles represent a versatile platform for multivalent presentation of viral antigens. Computational design of protein nanoparticles with differing sizes and geometries enables combination with antigens of choice to test novel multimerization concepts in immunization strategies where the goal is to improve the induction and maturation of neutralizing antibody lineages. Here, Rosetta design was used to engineer tetrahedral, octahedral, and icosahedral nanoparticles presenting trimeric HIV envelope glycoprotein (Env) ectodomain antigens. Following detailed biophysical and structural characterization, the designed constructs were evaluated in vivo for their immunogenic properties. In this talk I will be discussing our findings illustrating some of the strengths and weaknesses of this vaccine design approach.

Primary author: ANTANASIJEVIC, Aleksandar (EPFL - EPF Lausanne)

Presenter: ANTANASIJEVIC, Aleksandar (EPFL - EPF Lausanne)

Track Classification: 5 Molecular machines

Contribution Type: Oral presentation

Status: SUBMITTED

Submitted by **ANTANASIJEVIC, Aleksandar** on **Wednesday, 4 October 2023**

Abstract ID : **374**

Focused classifications and refinements in high-resolution single particle cryo-EM analysis

Content

Recent advances in cryo-EM and image processing provide new opportunities to analyse drug targets at high resolution. However, structural heterogeneity limits resolution in many cases, hence restricting the level at which structural details can be analysed and drug design be performed. As structural disorder is not spread throughout the entire structure of a macromolecular complex but instead is found in certain regions that move with respect to others and covering molecular scales from domain conformational changes up to the level of side chain conformations in ligand binding pockets, it is possible to focus the attention on those regions and the associated relative movements. Here we show how the usage of focused classifications and refinements provide insights into global conformational arrangements, exemplified on the human ribosome and on the cannabinoid GPCR, and how they can improve the local map resolution from an essentially disordered region the 2 Å resolution range.

Primary author: BARCHET, Charles (IGBMC)

Presenter: BARCHET, Charles (IGBMC)

Track Classification: 3 Methods for structure determination (X-ray, cryoEM)

Contribution Type: Poster

Status: SUBMITTED

Submitted by **BARCHET, Charles** on **Friday, 6 October 2023**

Abstract ID : **375**

Plasmid recognition by the SMC JET ATPase-nuclease

Content

SMC complexes play crucial roles in chromosome folding and DNA immunity by folding DNA into loops by an active process called DNA loop extrusion. Prokaryotic SMC JET complexes limit the spread of circular plasmids through plasmid DNA cleavage; yet the mechanisms for target recognition are unresolved. We determine structures of JET complexes in the absence of DNA and with plasmid DNA substrates by cryo-electron microscopy. Structures of plasmid-bound JetABC reveal two presumably stalled SMC motor units that are drastically rearranged from the resting state, together entrapping a U-shaped DNA segment, which is further converted to kinked V-shaped substrate by JetD nuclease binding. Our findings uncover mechanical bending of residual unextruded DNA as principle for non-self DNA recognition and molecular signature for plasmid cleavage.

Primary author: GRUBER, Stephan (University of Lausanne, Department of Fundamental Microbiology)

Presenter: GRUBER, Stephan (University of Lausanne, Department of Fundamental Microbiology)

Track Classification: 5 Molecular machines

Contribution Type: Poster

Status: SUBMITTED

Submitted by **GRUBER, Stephan** on **Friday, 6 October 2023**

Abstract ID : **376**

Improved performance with CRYO ARM II series

Content

According to EMDB and PDB data bases the number of data entries (solved structures) in the last 10 years (2011 –2021) has been grown almost in an exponential manner by 23-fold. This is an enormous increase which is a result of several advances in cryo electron microscopy technology. JEOL is one of the largest manufacturers of electron microscopes worldwide and from early on contributed to the current success in the field of cryo electron microscopy.

Since the early 1980th JEOL has released the 8th generation of cryo TEM's the CRYO ARM series. Basically, two version are available comprising unique features like a cold field emission source which was pioneered by the CRYO ARM series for the cryo electron microscopy community as well as in-column energy filters and outstanding resolution performance.

Primary author: KATZMANN, Emanuel (JEOL (Germany) GmbH)

Presenter: KATZMANN, Emanuel (JEOL (Germany) GmbH)

Track Classification: 3 Methods for structure determination (X-ray, cryoEM)

Contribution Type: Poster

Status: SUBMITTED

Submitted by **KATZMANN, Emanuel** on **Friday, 6 October 2023**

Abstract ID : **377**

EPFL's Protein Production and Structure Core Facility

Content

"From protein production to 3D structural and biophysical characterization, all in one location: The Protein Production and Structure Core Facility (PTPSP) at EPFL in Switzerland is unique in Europe. It operates as a hub in Protein Sciences in general and Integrative Structural Biology, offering top-notch expertise, 'à la carte' services, and high-end instruments. An expert team advises, trains, and connects academic users in production, purification, and biophysical and structural characterizations of macromolecules, such as enzymes, membrane proteins, or antibodies. All of this is possible thanks to close contacts with outstanding facilities, such as the Dubochet Center for Imaging for cryoEM and the Swiss Light Source for X-ray diffraction located in Villigen."

Primary author: DUHOO, Yoan (EPFL)

Presenter: DUHOO, Yoan (EPFL)

Track Classification: 3 Methods for structure determination (X-ray, cryoEM)

Contribution Type: Poster

Comments:

Authors: Yoan Duhoo(♪), Kelvin Lau(♪), Florence Pojer(♪). (♪): Protein Production and Structure Core Facility, School of Life Sciences, Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland.

Status: SUBMITTED

Submitted by **DUHOO, Yoan** on **Friday, 6 October 2023**

Abstract ID : **378**

Cryo-electron microscopy of synapses

Content

Our lab is focusing on the structure and function relationship of synapses. I will present what we have discovered so far and how we are working towards solving some limitations cryo-electron tomography.

Primary author: ZUBER, Benoit (University of Bern)

Presenter: ZUBER, Benoit (University of Bern)

Track Classification: 2 In situ structural biology

Contribution Type: Oral presentation

Status: SUBMITTED

Submitted by **ZUBER, Benoit** on **Friday, 6 October 2023**

Abstract ID : **380**

Examining the mechanism for dual chaperone/protease function of human LonP1

Content

The human Lon protease (LonP1) is essential for maintaining mitochondrial homeostasis. While the protease function of LonP1 is well-established, the evidence of LonP1 chaperone activity consists of observations of aggregates in the mitochondrial matrix upon inhibition of LonP1, and the mechanism of the LonP1 chaperone function remains elusive.
Using dynamic light scattering to monitor aggregation in an in vitro expression system, we show that LonP1 prevents aggregation of a newly synthesized protein substrate. We find that a mutation in the N-domain of LonP1 with decreased chaperone function in vivo (Besse, 2020, Mitochondrion), prevents aggregation in our system, though slightly slower. We also find that disordered regions in the N-domain decrease the protease activity.
Our results are in support of the evidence of LonP1 having chaperone activity in addition to its protease activity and indicate that structural features of the N-domain are essential for this dual function.

Primary author: ROESGAARD, Mette (Universität Basel)

Co-author: ABRAHAMS, Jan-Pieter

Presenter: ROESGAARD, Mette (Universität Basel)

Track Classification: 5 Molecular machines

Contribution Type: Poster

Status: SUBMITTED

Submitted by **ROESGAARD, Mette** on **Monday, 9 October 2023**

Abstract ID : **381**

Quantifying Electron Radiation Damage in biological Samples: Insight from Fluorescence Analysis

Content

Electron microscopy has shown a great potential in structure elucidation and imaging of biological samples due to the strong interaction of electrons with matter. However, as a major drawback, this radiation affects and breaks the bonds within the studied sample. Thus, preserving the native state of a protein while reaching high resolution remains a significant challenge. We aim to quantify electron radiation damage during diffraction data collection by establishing the relationship between the reduction of intrinsic properties and the increase in structural diversity. Additionally, we seek to optimize crystallization strategies to obtain thinner specimens ranging from tens to hundreds of nanometers, enabling high-quality diffraction datasets. This pilot experiment focuses on a specific class of proteins usually considered to track cellular behaviour and tag molecules: fluorescent proteins.

Primary author: BEN MERIEM, AmatassalÃ¢m (UniversitÃ© de BÃ¢le)

Presenter: BEN MERIEM, AmatassalÃ¢m (UniversitÃ© de BÃ¢le)

Track Classification: 2 In situ structural biology

Contribution Type: Poster

Comments:

Ben Meriem, Amatassalâm [1],[2], Abrahams, Jan Pieter [1],[2] [1] Basel University, Department of Structural Biology & Biophysics, Biozentrum, Basel, Switzerland

Status: SUBMITTED

Submitted by **BEN MERIEM, AmatassalÃ¢m** on **Monday, 9 October 2023**