2nd LINXS Symposium on Integrative Structural Biology

Report of Abstracts

Abstract ID : **26**

LP3 and DEMAX

Content

LP3 and DEMAX
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Proteins are diverse molecules and of enormous importance to life on earth. They have a multitude of different functions in all organisms and can work as enzymes, gene regulators, structural components, transporters, and receptors. In disease, most drugs act on proteins. It is therefore expected that the structures and mechanisms of proteins are prominent topics in life science research.
Access to both state-of-the-art X-ray (MAX IV) and neutron sources (ESS) will increase the capacity for innovation in the life sciences. To enable efficient use of these unique and powerful facilities by Lund researchers, Lund University hosts the protein production platform, LP3 (www.lu.se/lp3). LP3 assists users with: 1) Recombinant protein production, 2) biophysical protein characterisation 3) High-throughput crystallization and structure determination, and 4) Stable isotope labelling and bio-deuteration of biological macromolecules.
Since 2016, the DEuteration and MAcromolecular Xtallization (DEMAX) platform of the European Spallation Source ERIC (ESS) is co-localized with LP3. DEMAX and LP3 are coordinating in their efforts(1-4) to develop cost-effective production of deuterated proteins for macromolecular crystallography, enable crystallization of interesting proteins for neutron work, and for the production of labelled proteins/lipids for neutron reflectometry.
For more information and access see: www.lu.se/lp3

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Presenter(s) : KNECHT, Wolfgang (Lund University)

Submitted by **KNECHT, Wolfgang** on **Tuesday 04 June 2019**

Abstract ID : **27**

Structural transitions in protein-surfactant complexes studied by contrast-variation SANS and protein NMR

Content

The interaction between surfactants and soluble proteins have been the goal of many investigations in the past due to the high fundamental and applied scientific relevance. Due to the high complexity of these phenomena, recent investigations have applied a holistic approach that often combines spectroscopy methods, calorimetric analysis, simulations, and/or structural investigations.[1] X-ray scattering techniques in particular have been used to explore the characteristics of the surfactant-protein complexes from a structural perspective. However, detailed structural models are rather scarce and a general theory of the interactions between proteins and amphiphiles in solution is yet missing.

In this project we implement protein nuclear magnetic resonance (NMR) and contrast variation small-angle neutron scattering (SANS) to study the interactions between simple, prototypical surfactants (anionic, cationic and non-ionic) and soluble proteins. Protein NMR has been used to investigate changes in the local dynamics of specific regions of the protein upon surfactant addition, providing information on the protein domains that are susceptible of interacting with the amphiphiles. The use of the contrast variation SANS enables to discriminate the contribution from different components of the system to the scattering, enhancing the level of detail obtained for specific parts of the protein-surfactant complex. Thus, isotopic labelling allowed to follow the formation of protein surfactant complexes, as well as structural changes occurring in the protein backbone when surfactants are present. Combining these advanced methods with in-house techniques (fluorescence spectroscopy, circular dichroism and isothermal titration calorimetry), we aim to elucidate the fundamental science behind the interaction of surfactants with proteins, and how the presence of amphiphiles perturb the structure and dynamics of the macromolecules.

[1] D. Otzen, Biochim. Biophys. Acta, 2011, 1814, 562-591.

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Status: SUBMITTED

Submitted by **SANCHEZ-FERNANDEZ, Adrian** on **Wednesday 03 July 2019**

Abstract ID : **28**

Structure and substrate specificity of the tryptophan biosynthesis enzyme IGPS from Pseudomonas aeruginosa

Content

In bacteria, tryptophan synthesis is performed by the enzymes encoded in the trp operon. The product of the trpC gene, indole-3-glycerol phosphate synthase (IGPS) catalyzes the indole-forming reaction of tryptophan synthesis. The reaction mechanism includes a decarboxylation step of the substrate 1-(o-carboxyphenylamino) 1-deoxyribulose 5-phosphate (CdRP). The decarboxylation has been assumed to constitute an essential step of the mechanism since no activity with the decarboxylated variant of the substrate, phenylaminodeoxyribulosephosphate (PAdRP), was observed in an early study on IGPS from Escherichia coli (Smith and Yanofsky, 1962).

In this study, we demonstrate enzyme-catalyzed formation of the native product IGP from decarboxylated substrate PAdRP using IGPS from Pseudomonas aeruginosa. Moreover, the crystal structure of P. aeruginosa IGPS in complex with a substrate analogue was solved to 2.1 Å resolution. By structural comparison to E.coli IGPS (Wilmanns et al., 1992), we provide structure-based hypotheses on the difference in substrate specificity between the E.coli and P. aeruginosa homologs.

References:
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Wilmanns, M. et al. (1992) 'Three-dimensional structure of the bifunctional enzyme phosphoribosylanthranilate isomerase: Indoleglycerolphosphate synthase from Escherichia coli refined at 2.0 Å resolution', Journal of Molecular Biology, 223(2), pp. 477–507.

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Status: SUBMITTED

Submitted by **SÖDERHOLM, Annika** on **Tuesday 20 August 2019**

Abstract ID : **29**

Structural insight into Gram-positive Type 4 Secretion Systems

Content

Multidrug resistance in bacteria, originating from conjugative gene transfer, is an increasingly common problem in today's world. The majority of bacteria that causes hospital infections are of gram-positive origin, but so far very little is known about their conjugation systems. To remedy this, we aim to determine the molecular structure and function of conjugation complexes belonging to Type IV Secretion Systems (T4SSs) from gram-positive bacteria. This will lead to a deeper insight into one of the main processes responsible for horizontal gene transfer events, including the spread of antibiotic resistance genes in bacteria.

We study the proteins involved in forming the T4SS biochemically, structurally and biophysically. Since gram-positive T4SSs are very dissimilar from their gram-negative counterparts, little can be deduced from the few gram-negative systems so far studied. Furthermore, they occur in a number of pathogens, such as enterococci, streptococci and staphylococci. Another aspect that makes gram-positive T4SSs interesting is that they are used to efficiently transfer not only antibiotic resistance, but also virulence factors.

These megadalton sized systems are built up by i) extracellular adhesion proteins, ii) membrane channel proteins and iii) intracellular DNA processing proteins. Here, I will present our current understanding of the T4SS originating from the conjugative plasmid pCF10 of Enterococcus faecalis. Our work on this system combines molecular biology, biochemistry, X-ray crystallography and Electron Microscopy. This has so far allowed us to determine structures and understand some of the functions of the adhesion proteins as well as part of the DNA processing proteins, which will highlight both major differences and similarities between the gram-positive and gram-negative systems.

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Status: SUBMITTED

Submitted by **BERNTSSON, Ronnie** on **Thursday 22 August 2019**

Abstract ID : **30**

Structural study of synaptic adhesion-like molecules (SALMs)

Content

Abstract
Synaptic adhesion molecules play a crucial role in the regulation of synapse development and maintenance. Recently, several families of leucine rich repeat domain containing synaptic adhesion molecules have been characterized, including netrin G-ligands, leucine rich repeat transmembrane (LRRTM) neuronal protein family, and the synaptic adhesion-like molecules (SALM) protein family. Most of these are expressed at the excitatory glutamatergic synapses in post-synaptic cells, and dysfunctions of these genes are genetically linked with cognitive disorders. My PhD study is focused in the structural and functional characterization of proteins under SALM family. The SALM contains SALM1-5 proteins. SALM2, SALM3&5 have been shown to bind to the presynaptic receptor protein tyrosine phosphatase (RPTP) family ligands. We have solved the crystal structure of SALM5 LRR-Ig domain construct and we have done biophysical studies that verify the crystallographic results and binding of the ligand RPTPs. Currently, I am working to understand the role of SALM and RPTP tran-synaptic interaction and their role in synaptogenesis. Structural studies of synaptic adhesion proteins and synaptic complexes will help us to understand in detail the function of synaptic complexes in synapse formation and regulation.

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Status: SUBMITTED

Submitted by **KARKI, Sudeep** on **Wednesday 28 August 2019**

Abstract ID : **31**

Tracking Ca2+ ATPase Intermediates in Real-Time by X-ray Solution Scattering

Content

Sarco/endoplasmic reticulum Ca2+ ATPase (SERCA) transporters regulate calcium signaling by active calcium ion reuptake to internal stores. Several of the structural transitions associated with transport have been characterized by X-ray crystallography, but critical intermediates of the inward-outward switching are missing. We combined time-resolved X-ray solution scattering (TR-XSS) experiments and molecular dynamics (MD) simulations for real-time tracking of concerted SERCA reaction-cycle dynamics in the native membrane. The TR-XSS pre-pulse model differed in the domain arrangement compared to Ca2E1 crystal structures. A 1.5 ms intermediate showed closure of the cytosolic domains typical of Ca2+- and ATP-bound E1 states. A subsequent transient state with a 13 ms rise-time showed a novel actuator (A) domain arrangement that exposes the ADP-binding site after phosphorylation. Hence, the obtained TR-XSS models determine the relative timing of so-far elusive domain rearrangements in a native environment.

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Submitted by **ANDERSSON, Magnus** on **Tuesday 03 September 2019**

Abstract ID : **32**

How to increase protein stability

Content

More and more proteins are being utilised in e.g. medicinal-, food- and biotechnologies where high degree of robustness is required. This calls for improved and high-throughput screens for stability-inducing mutations. Traditionally, potentially stabilising mutations have been introduced to enzymes sometimes guided by computational predictions. New variants with increased stability are often identified by high-throughput screens based on enzymatic activity. Consequently, the screens are enzyme-specific rather than universal.
Developing a universal, high-throughput pipeline for optimising and monitoring protein stability in vivo offers an efficient approach to identify stabilising mutations without the need for develop screens for each enzyme. We combine directed evolution with a Fluorescence-Activated Cell Sorting (FACS)-based reporter system for monitoring protein stability.
We have designed an E. coli expression system for co-expression of super-folder Green Fluorescent Protein (sf-GFP) under control of a DnaK promoter and the protein of interest (POI) tagged with Red Fluorescent Protein (tag-RFP). The chaperone DnaK is part of the heatshock response in E. coli. Hence, elevated levels of green fluorescence indicate a less stable protein, whereas red fluorescence serves as a reporter of abundance of POI-tagRFP in the cell. Consequently, cells harbouring POI variants with higher stability can be identified by FACS based on increased red-to-green fluorescent ratio.
In a test of eight l repressor variants (Tm= <10 - 61 ℃) we found a correlation between melting temperature and ratio of red-to-green fluorescence indicating that limited induction of the DnaK promotor is a good indicator of protein stability in vivo. To validate the pipeline, comprehensive site-saturation libraries of five model proteins will be created and subjected to FACS and next generation sequencing for identification of stabilising mutations.

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Status: SUBMITTED

Submitted by **GRUNNING, Åsa** on **Thursday 05 September 2019**

Abstract ID : **33**

UrdA: structural characterization of a novel enzyme

Content

Urocanate reductase (UrdA) is a bacterial enzyme that was first characterized in 2012 and shown to reduce urocanic acid resulting in a product imidazole propionate (1). Unlike similar enzymes fumarate reductases, UrdA hasn't been well investigated. Besides being an interesting novel enzyme enabling bacteria to grow in anaerobic conditions with urocanic acid as electron acceptor (1), UrdA was shown to play a significant role in human gut microbiota, as imidazole propionate levels are increased in people with type 2 diabetes and it further affects glucose metabolism (2).

Two domain construct of UrdA, consisting of a FAD binding and a mobile domain were successfully expressed, purified and crystallized. Four X-ray structures were obtained depicting different states of the enzyme: ADP bound, FAD bound, substrate/FAD bound and in complex with product/FAD. The data reveals the overall structural arrangement of the enzyme as well as the substrate binding mode and conformational changes.

The role of UrdA in imidazole propionate production in relation to type 2 diabetes makes the first structure of the UrdA of particular importance to our understanding of this enzyme.

References

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Submitted by **GRUNNING, Åsa** on **Thursday 05 September 2019**

Abstract ID : **34**

Novel algorithms for integrative structural biology

Content

In my talk I will present our approach for modeling macromolecular flexibility of large molecular assemblies and how it can be combined with sparse experimental data obtained with small-angle and cross-linking experiments.

Large macromolecular machines, such as proteins and their complexes, are typically very flexible at physiological conditions, and this flexibility is important for their structure and function. Computationally, it can be often approximated with just a few collective coordinates, which can be computed e.g. using the Normal Mode Analysis (NMA). NMA determines low-frequency motions at a very low computational cost and these are particularly interesting to the structural biology community because they are commonly assumed to give insight into protein function and dynamics [1].

One of the challenges in the community is the explanation of solution small-angle scattering profiles. Very recently, we designed a computational scheme that uses the nonlinear normal modes [2] as a low-dimensional representation of the protein motion subspace and optimizes protein structures guided by the SAXS and SANS profiles [3,4]. For example, in the CASP12 and CASP13 exercises, this scheme obtained best models for some (3 out of 9 in CASP12) SAXS-assisted targets [5,6]. Overall, the flexible fitting scheme typically allows a significant improvement of the goodness of fit to experimental profiles in a very reasonable computational time. The NMA analysis also allows to automatically split macromolecules into rigid domains, or to be used together with the cross-linking data, as we demonstrated in the recent CASP13 challenge [7].

References:

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[4] https://team.inria.fr/nano-d/software/pepsi-sans/
[5] http://predictioncenter.org/casp13/zscores_final_assisted.cgi?target_flag=S
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Submitted by **GRUDININ, Sergei** on **Thursday 05 September 2019**

Abstract ID : **35**

STRUCTURAL INSIGHT INTO EUKARYOTIC STEROL TRANSPORT THROUGH NIEMANN-PICK TYPE C PROTEINS

Content

Niemann-Pick type C (NPC) proteins are essential for sterol homeostasis, believed to drive sterol integration into the vacuolar/lysosomal membrane before redistribution to other cellular membranes. Using a combination of crystallography, cryo-electronmicroscopy, biochemical and in vivo studies on the Saccharomyces cerevisiae NPC system, NCR1/NPC2, we recently generated a framework for sterol membrane integration (Winkler et al., (2019)). Sterols are transferred between hydrophobic pockets of vacuolar NPC2 and membrane-protein NCR1. NCR1 has its N terminal domain (NTD) positioned to deliver a sterol to a tunnel connecting NTD to the luminal membrane leaflet 50 Å away. A sterol is caught inside this tunnel during transport, and a proton-relay network of charged residues in the transmembrane region is linked to this tunnel supporting a proton-driven transport mechanism. We propose a model for sterol integration which clarifies the role of NPC proteins in this essential eukaryotic pathway and which rationalizes mutations in patients with Niemann-Pick disease Type C that I will present at the talk.

Winkler et al, Structural insight into eukaryotic sterol transport through Niemann-Pick Type C proteins, Cell (accepted, 2019)

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Submitted by **GRUNNING, Åsa** on **Thursday 05 September 2019**

Abstract ID : **37**

Serial crystallography at MAX IV

Content

The RÅC grant "Expanding the domain of protein serial crystallography: membrane protein and in cellulo crystallization" aims at developing serial crystallography methods in the area of crystallization, sample delivery and time-resolved applications. At MAX IV Laboratory we will further develop the serial crystallography methods being implemented at BioMAX and use these in experimental applications.

The beamline MicroMAX that is planned to be in the commissioning phase at the end of the RÅC project in 2022 has serial crystallography as one of its main applications. MicroMAX will be a microfocus beamline for macromolecular crystallography. MicroMAX will open up new opportunities to study life on a molecular level by producing X-rays in a very narrow but highly intense beam. The narrow beam size (down to 1 μm) will make it possible to work with micrometer-sized crystals and to optimize the data quality from larger inhomogeneous crystals. This will be important for difficult targets such as membrane proteins.

As there is rapid development of serial crystallography and in particular new sample delivery systems, MicroMAX will be made flexible to be able to accommodate new sample delivery methods such as chips, jet-driven sample delivery, micro-fluidics, acoustics and more. The setup will also include instrumentation for rotational data collection.

MicroMAX will with its performance give better opportunities for room-temperature data collection, with emphasis on serial approaches and development of time-resolved methods. This will provide knowledge of atomic structures that are more relevant for the functions of proteins in their natural environment. It will also enable a more sophisticated analysis of how proteins behave and interact with other molecules. This new insight can help in developing new medicines and in studying enzymes of interest in energy research and biotechnology.

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Comments:

poster

Status: SUBMITTED

Submitted by **BJELCIC, Monika** on **Thursday 05 September 2019**

Abstract ID : **38**

Crystallisation of inhibitor-bound OGG1 accelerates the generation of potent inhibitors

Content

The onset of inflammation is associated with reactive oxygen species and oxidative damage to macromolecules such as 7,8-dihydro-8-oxoguanine (8-oxoG) in DNA. The enzyme 8-oxoguanine DNA glycosylase 1 (OGG1) is primarily responsible for the excision of 8-oxoG during the repair of oxidative DNA damage. OGG1-deficient mice have been found to be resistant to acute and systemic inflammation, and it has been hypothesized that OGG1 inhibition may represent a strategy for the prevention and treatment of inflammation. To this end, a first-in-class inhibitor was developed by Visnes et al.1 which binds to human OGG1 in the place of 8-oxoG with high specificity, achieving the goal of reducing proinflammatory gene expression in mice. Co-crystallisation of human OGG1, using novel inhibitors developed by collaborators, has been a key part of the process. Structural data has uncovered the precise binding mode of this class of inhibitor and rapidly accelerated further inhibitor design. This has led to the production of new compounds displaying sub-nanomolar affinities.

1 Visnes et al. (2018) Small-molecule inhibitor of OGG1 suppresses proinflammatory gene expression and inflammation. Science. 362 (6414). pp. 834-839.

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Status: SUBMITTED

Submitted by **GRUNNING, Åsa** on **Thursday 05 September 2019**

Abstract ID : **39**

Two GH26 β-mannanases from Bacteroides ovatus: structure and role in galactomannan degradation

Content

Galactomannans are hemicelluloses composed of a β-1,4-linked mannose backbone with α-1,6-galactose substitutions. They are part of our diet as seed storage polysacchardies and food thickeners and are utilised by several human gut bacteria (1). One such bacteria, Bacteroides ovatus, contains a gene cluster encoding two glycoside hydrolase family 26 β-mannanases, BoMan26A and BoMan26B (2). BoMan26B generates a range of product lengths upon mannan hydrolysis, prefers longer substrates and is less restricted by galactose side-groups than BoMan26A, which mainly generates mannobiose (3,4). The results suggest that BoMan26B performs the initial attack on galactomannan, generating oligosaccharides that are further hydrolysed by BoMan26A. Crystal structures of these two enzymes reveal the structural basis for their biochemical differences. BoMan26B, with galactosyl-mannotetraose bound in subsites −5 to −2, has an open and long active site cleft with W112 in subsite −5 concluded to be involved in mannosyl interaction (4). Moreover, K149 in the −4 subsite interacted with the galactosyl side-group of the ligand, which may indicate a preference in for substituted manno-oligosaccharides (4). BoMan26A instead revealed a narrow active site cleft that is restricted in one end by a loop, explaining its preference for generating shorter products (6).

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Status: SUBMITTED

Submitted by **BÅGENHOLM, Viktoria** on **Friday 06 September 2019**

Abstract ID : **41**

Crystallization at LP3, case studies

Content

LP3 (Lund Protein Production Platform) is a cross-faculty expert center of Lund University for protein production, crystallization and structure determination. The staff at LP3 has gathered an extensive portfolio of knowledge from working with proteins from over 150 projects and a large range of protein families and types. The LP3 laboratory has an attractive selection of automated equipment for crystallization experiments, and a streamlined pipeline for regularly assessing of crystals for X-ray diffraction at BioMAX (MAXIV). As part of being a key infrastructure to researchers at Lund University, LP3 has a constantly increasing number of crystallization projects that are either fully coordinated at LP3 or in which the personnel is highly involved. On our poster we will present results from ongoing projects, among them for example branched-chain aminotransferase 2 (BCAT2) and human carbonic anhydrase IX (CA IX).
BCAT2 is the human mitochondrial enzyme that metabolizes branched chain amino acids (Val, Ile, Leu) into branched chain α-keto acids that fuel the TCA cycle (1). CA IX is an emerging cancer target and in focus for joined X-ray and neutron crystallography (2,3).

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Submitted by **GRUNNING, Åsa** on **Friday 06 September 2019**

Abstract ID : **42**

Structural insights into AQP2 targeting to multivesicular bodies

Content

The multivesicular sorting machinery is a crucial mechanism for targeting membrane proteins for recycling or degradation. The lysosomal trafficking regulator-interacting protein 5 (LIP5) which coordinates the action of this machinery is also known to bind directly to the membrane protein cargo. In case of aquaporin 2 (AQP2) the binding of LIP5 during the endocytic pathway in kidney collecting duct cells ensures an effective regulation of urine volume [1].
In our group, we have previously studied the role of AQP2 phosphorylation in AQP2-LIP5 interaction [2]. Currently we are focusing on elucidating the structural details of the complex in order to better understand how membrane proteins are delivered to the multivesicular bodies. We have constructed alanine mutants of single residues in the proposed binding sites of both AQP2 and LIP5. Studying the binding affinity of these mutants using fluorescence quenching helps us understand which residues are directly involved in the binding.
Further, AQP2 was successfully incorporated into MSP-based nanodiscs and negative stain electron microscopy confirmed homogeneous state of the particles. We have collected high resolution images on Titan Krios and are currently processing the data.

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Status: SUBMITTED

Submitted by **GRUNNING, Åsa** on **Friday 06 September 2019**

Abstract ID : **43**

Structural basis for glycerol conductance and selectivity of human aquaporin 7

Content

Lipid metabolism of triglycerides in adipocytes serves as our main source for storing and retrieving energy. The triglycerides are broken down into glycerol and free fatty acids in lipolysis, and glycerol exit the cell via the aquaglyceroporin aquaporin 7 (AQP7) to be available for gluconeogenesis. In this study we investigated the glycerol conductance and selectivity of AQP7 by solving the structure of human AQP7 using x-ray crystallography to 1.9 Å resolution in the presence of glycerol, and carrying out molecular dynamics simulations of glycerol and water passage. Glycerol molecules were observed in the AQP7 structure both with the hydroxyl groups facing the hydrophilic side of the pore, which is also seen in other aquaglyceroporin structures, and also facing the hydrophobic side, unique for AQP7. These observations support a conducting mechanism in which rotation of the glycerol molecule serves to break hydrogen bonds that releases glycerol from more tightly bound positions and thus facilitating its transition along the pore. In molecular dynamics simulations, the lack of complete unbinding and spontaneous binding of glycerol at the millisecond timescale and a 2-4 times lower water permeability in the presence of glycerol suggest a high glycerol affinity and that high water permeation is hindered by glycerol. AQP7 is an interesting target for drug development as AQP7-deficiency in mice is linked to obesity and diabetes, and increased adipocyte lipolysis and subsequent glycerol release is observed in tumor microenvironments. Our observations provide a framework for understanding the mechanism of glycerol transport over the plasma membrane in adipocytes.

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Status: SUBMITTED

Submitted by **GRUNNING, Åsa** on **Friday 06 September 2019**

Abstract ID : **44**

Structural characterization of plant Sugar Transport Protein STP6

Content

In plants, photosynthetically fixed carbon is used to synthesize sucrose which is distributed from mature leaves (sources) into areas of growth and storage (sinks) via the phloem. The sugar import from the apoplast in symplastically isolated sink tissues is mediated both by sucrose- and monosaccharide transporters. The monosaccharide transporters are sugar/H+ symporters known as the sugar transport proteins (STPs) that are members of the major facilitator superfamily (MFS) which are found in all kingdoms of life. The STPs are key regulators of plant development, essential for microbial defense and nutrient uptake in sink tissues throughout the plant. Recently, the structure of STP10 has been determined in our lab. This structure revealed distinctive new features compared to hitherto described members of the major facilitator superfamily (MFS). The exact roles of these new features in the molecular mechanism of transport remains to be elucidated. The perspective of this project will concern the structural determination of STP6 as well as direct functional characterization of the STP6 transport abilities with the aim of providing a model of the underlying molecular mechanism of transport. Elucidating the structure-function relationship of STP6 could provide molecularly directed solutions for crop improvement. Moreover, the possibility of providing valuable knowledge regarding sugar transporters in other organisms such as humans.

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Submitted by **GRUNNING, Åsa** on **Friday 06 September 2019**

Abstract ID : **45**

A structural look at the radical generating subunit of a unique ribonucleotide reductase using single particle cryoEM.

Content

Class I ribonucleotide reductases (RNR) contain a catalytic subunit (NrdA) and a radical generating subunit (NrdB), which together catalyse the reaction of reduction of ribonucleotides to deoxyribonucleotides, which in turn make up DNA. In this poster, I am studying the NrdB from the firmicute *Faklamia ignava*, which is a unique fusion protein as it has N-terminal add-ons of a glutaredoxine domain followed by an ATP cone binding domain, the ATP cone. The ATP cone acts as an allosteric on/off switch promoting enzyme activity in presence of ATP and brings about enzyme inhibition in presence of dATP. It has already been shown that this enzyme forms tetramers in presence of dATP. In this poster, I am trying to understand the structure of full length NrdB protein in the presence of dATP, using single particle cryo-electron microscopy. Single particle cryoEM is a structural biological technique which has been traditionally used to provide information about large protein complexes that resisted crystallization. However, recent advances in sample preparation, computation and instrumentation, have greatly increased its usability as a technique. In this poster, I will try to show how I have used single particle cryoEM as the dominant structural technique to look at my protein, and talk about sample preparation, data collection and processing.

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Status: SUBMITTED

Submitted by **BANERJEE, Ipsita** on **Friday 06 September 2019**

Abstract ID : **46**

Integrative Ensemble Modeling of a Large Membrane Protein Complex Using Diverse and Ambiguous Information

Content

Mitochondria contain approximately 1200 different proteins, 99% of which are synthesized on cytosolic ribosomes and need to be delivered into the right destination through the intermembrane space by transport machineries, such as the TIM chaperone. Currently, the mechanistic and structural details of how the TIM chaperone binds to these mitochondrial proteins remain elusive. To gain structural insight into the binding and chaperone mechanisms, we focused on the complex of the TIM9/10 chaperone and the mitochondrial GDP/GTP carrier membrane protein (Ggc1). Such complexes are difficult to study because they consist of a transiently formed, dynamic complex between two folded proteins and a membrane protein that should be solubilized and bound by the chaperone. X-ray crystallography has revealed the core structure of the free chaperone protein, but because of the dynamic nature and large size (˜1400 amino acids) of the complex its structural features have remained elusive. Using an integrative approach that combines biochemical assays, NMR spectroscopy and SAXS it was, however, able to obtain detailed but ambiguous information on the structures of the complex. In particular, the experiments showed that the complex consists of two wellstructured (TIM9)3/(TIM10) 3 hexamers bound to a mostlydisordered Ggc1. In this work, we developed a protocol to integrate such heterogeneous experimental data with a coarsegrained molecular model to provide a description of the conformational ensemble of the TIM9/10-Ggc1 complex. In particular, we used a hybrid structure-based model (to describe the intra-molecular interactions within the folded chaperone), an NMRderived contact potential for chaperone-client interactions and a knowledge-based potential (to describe the inter-molecular interactions between the chaperones and chaperone-client interactions). We used molecular dynamics (MD) simulations to sample the conformational landscape of the complex, and the resulting coarse-grained conformational ensemble was subsequently converted into all-atom resolution and refined using a Bayesian/Maximum Entropy reweighting approach using the SAXS data. This allows us to generate a weighted ensemble in agreement with experimental measurement. Such integrative structural modeling method is useful to generate a structural ensemble of large and dynamic proteins in a both efficient and reliable way.

Reference:
Katharina Weinhäupl, Caroline Lindau, Audrey Hessel, Yong Wang, Conny Schütze, Tobias Jores, Laura Melchionda, Birgit Schönfisch, Hubert Kalbacher, Beate Bersch, Doron Rapaport, Martha Brennich, Kresten Lindorff-Larsen, Nils Wiedemann*and Paul Schanda.* Structural Basis of Membrane Protein Chaperoning Through the Mitochondrial Intermembrane Space. Cell, 175, 1365-1379, (2018)

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Submitted by Submitted by GRUNNING, Åsa on Friday 06 September 201

Abstract ID : **47**

The Biological Laboratory at MAX IV

Content

The biological laboratory at MAX IV is the central laboratory facility for the support of life science related synchrotron experiments that are carried out at various beamlines and also for life science in-house research. The lab complements the various beamline-attached sample preparation facilities and are being developed to offer extended instrumentation access and support.

The Biolab is now available for users and in-house researchers with the function of basic protein preparation laboratory operation including limited protein analytics (UV-vis spectroscopy, gel electrophoresis, dynamic light scattering, HPLC etc.) as an entry check for protein samples before the experimental beamtime at MAX IV, a glovebox for sample preparation under anaerobic conditions, and storage of chemicals and protein samples. The biosafety level is now restricted to be Biosafety Level 1 for work with well-characterized agents and will be further developed to Biosafety Level 2 in the future.

Operation of new beamlines with life science users will increase the usage of the Biolab on a daily basis. The developmental direction of the beamlines would steer the development of the Biolab to cover the new needs. The latest status of the Biolab will be presented and input from our user community is appreciated.

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Status: SUBMITTED

Submitted by **GRUNNING, Åsa** on **Friday 06 September 2019**

Abstract ID : **48**

Structure and function studies of GLUT4 and ASPL complex in adipocytes

Content

Glucose transporters (GLUTs) are proteins responsible for glucose uptake into cells, recently, structures of GLUT1 and GLUT3 were determined. Different from GLUT1 and GLUT3, GLUT4 is sequestered intracellularly in the absence of insulin, and is distributed to the plasma membrane upon insulin stimulation where it can mediate glucose uptake. This trafficking is tightly controlled in several ways, for instance GLUT4 is retained at the Golgi by insulin sensitive tethering through TUG in the mouse. Hence, targeting the interface between TUG and GLUT4 is a potential therapeutic target to hinder the retention of GLUT4 at the intracellular compartments independently in the presence of insulin, and thus counteracting insulin resistance. However, whether GLUT4 and TUG (ASPL) interaction exists in human adipocytes, and how TUG (ASPL) regulates GLUT4 trafficking are unknown. In addition, complex structure study towards GLUT4 and ASPL would be more clearly and directly to clarify the mechanism of interaction and translocation.

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Status: SUBMITTED

Submitted by **HUANG, Peng** on **Saturday 07 September 2019**

Abstract ID : **49**

AdaptoCell – a microfluidic flow-cell for proteins for MAX IV users

Content

The contemporary challenges in structural biology seek deeper understanding of in vivo dynamics and functions of proteins and their interactions; the Swedish researchers need new tools and techniques for these competitive investigations. MAX IV Laboratory, the Swedish synchrotron facility for research, enables time-resolved in situ studies of proteins by several techniques. Balder, CoSAXS and MicroMAX beamlines designed to be state-of-the-art in their respective method: X-ray Absorption/Emission Spectroscopy (XAS/XES), Small Angle X-ray Scattering (SAXS), and Serial Synchrotron crystallography (SSX). The goal of the SSF ITM-17 granted project is to deliver adaptable microfluidic flow-cell platform, AdaptoCell for MAX IV users, to be integrated at beamlines and adapted to each method, deployed to investigate proteins in solution and to facilitate serial crystallography on micro crystals.

Year-one goal, the delivery of integrated microfluidic device for XAS, following up year two with add-on UV-vis spectroscopy lab-on-chip AdaptoCell-XAS and AdaptoCell-SAXS for time-resolved data collection, and year three AdaptoCell-SSX. We expect to release devices to Swedish academic and industry users for sample delivery after each finished development stage.

To optimize the AdaptoCell-XAS device, prototypes are currently designed and tested with respect to flow properties, X-ray resistance, data collection and protein health check. Present project status will be presented.

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Status: SUBMITTED

Submitted by **SIGFRIDSSON CLAUSS, Kajsa** on **Saturday 07 September 2019**

Abstract ID : **50**

Development of Escherichia coli Strains for Protein Perdeuteration

Content

Neutron macromolecular crystallography (NMX) offers an unique approach for locating individual atoms by leveraging the neutron scattering properties of the hydrogen isotope deuterium (D). However, production of deuterated proteins and growing large crystals of proteins is a bottleneck in neutron crystallography.

While recombinant production of perdeuterated proteins is relatively straightforward, improvements are needed. Bacteria in heavy water based growth medium experience severely reduced growth rate and biomass yield. We provide examples of improving protein perdeuteration by evolving *Escherichia coli* in deuterated conditions, pinpointing genetical adaptations and developing such strains for production of recombinant perdeuterated proteins. Developed strains have higher growth rate in deuterated conditions, with recombinant protein yields comparable to the parental strain. This is the first step in developing better strains for perdeuterated protein production as these strains are suitable for further genetical improvements.

The improved strains were used to perdeuterate Leishmania mexicana Triosephosphate Isomerase (TIM) for NMX. TIM is a key enzyme in glycolysis and it catalyzes the interconversion of glyceraldehyde-3-phosphate and dihydroxyacetone phosphate via proton transfer. Thus, NMX could provide additional information on protonation state of active site. We have crystalized perdeuterated TIM and collected high-resolution neutron diffraction data. In conclusion, we have developed faster growing E. coli strains for production of perdeuterated recombinant proteins and provided proof of usability by perdeuterating TIM and collecting neutron diffraction data.

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Status: SUBMITTED

Submitted by **KELPŠAS, Vinardas** on **Sunday 08 September 2019**

Abstract ID : **51**

Elucidating the function of membrane proteins with neutron crystallography

Content

Hydrogen (H) atoms play a crucial role for protein function and are involved in almost every mechanism. They are critical in understanding the function of various channels such as outer membrane porin F (OmpF). With the advancement in technology, neutron crystallography is used to determine hydrogen atoms as those are not visible by X-ray crystallography. Since it requires large volume crystals for good data, no neutron structures of membrane proteins have been determined yet. Thus, initially OmpF will be studied as a model system due to its high stability, yield and solubility in aqueous solutions. Necessary methods to improve crystal size and diffraction quality will be developed and implemented in this project. So far, sitting drop vapor diffusion method provided promising results with a crystal size of OmpF up to 200µm diffracting at a resolution of 2 A. In neutron scattering, H has a negative coherent scattering length (-3.74 fm) as compared to positive scattering length of its isotope deuterium (D) (6.67 fm). Therefore, all H atoms in the protein molecule are exchanged for D to obtain better electron density maps with reduced background. This can be done by different ways either by growing crystals under deuterated conditions or growing the recombinant protein in deuterated medium. Here, we chose the latter method where initial expression tests of OmpF were done in deuterated minimal medium with glycerol as the carbon source. Various parameters like IPTG concentration, temperature and incubation time were optimized to 0.5mM, 30 and 24hrs respectively to obtain maximum amount of protein. In future, these optimized conditions will be implemented for large scale production of OmpF in deuterated minimal medium. Vapor diffusion along with microseeding technique will be further explored to obtain large diffracting crystals of OmpF.

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Status: SUBMITTED

Submitted by **AGGARWAL, Swati** on **Sunday 08 September 2019**

Abstract ID : **52**

Integrative structural biology with complementary experimental methods to describe viral capsid protein self-assembly

Content

The genetic code of viruses, RNA or DNA, are typically protected in an icosahedral capsid, which is primarily assembled from over a hundred subunits of the same protein in a spontaneous self-assembly process. Similar highly efficient assembly processes are ubiquitous in biological systems; viral capsids present a unique platform to exploit for therapeutic advances in the targeted cellular delivery of cargo packaged within the capsid. Our research aims to provide a more detailed understanding of how this precise viral capsid protein assembly process occurs from a pool of single building blocks, and additionally the effect and organization of nucleic acid present during assembly. Here, we present results from small-angle neutron scattering experiments using contrast variation to reveal the final assembled structural organization of both the protein and nucleic acid components from recombinant Hepatitis B virus (HBV) capsid protein and a synthetically prepared RNA containing the capsid protein binding domain. These data revealed that RNA was localized along the inner capsid surface. Time-resolved small-angle x-ray scattering (SAXS) experiments were also used to determine the structure during HBV capsid assembly in the presence and absence of RNA. We employed Bayesian statistics-based computational methods to extract kinetic parameters of assembly and the overall size and shape of the dominant structural intermediates from the SAXS data. Additional single-particle cryoEM reconstructions are provided to assess the effect of RNA on the resulting assembled capsid structure. The combination of time-resolved scattering data, Bayesian statistics, and cryoEM structural analysis, provides a framework which not only describes the viral self-assembly process, but can be extended to other hierarchical assemblies in biology.

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Status: SUBMITTED

Submitted by **OLIVER, Ryan** on **Monday 09 September 2019**

Abstract ID : **53**

Solving the First Novel Protein Structure by Micro-Crystal Electron Diffraction

Content

Micro-crystal electron diffraction (MicroED) has shown in recent years to be a promising method for determining macromolecular structures (1–5). It enables structural biologists to study proteins from micron-sized 3D crystals that are too small to be studied by conventional X-ray crystallography. Furthermore, MicroED can be applied to biomolecules of low molecular weight that are beyond what can so far be resolved by single particle cryo-EM (6,7). However, up to now, all protein structures determined by MicroED had already been solved previously by X-ray crystallography. Here, we present for the first time an unknown protein structure – an R2lox metalloenzyme– solved using MicroED (8). MicroED data were collected from plate-like crystals with an average size of 2 μm × 2 μm × 0.5 μm. By overcoming challenges in sample handling, cryo-EM specimen preparation, limited data completeness and low signal-to-noise ratio, we are able to solve the structure by molecular replacement with a search model of less than 36% sequence identity. The resulting electrostatic scattering potential map at 3.0 Å resolution is of sufficient quality to allow accurate model building and refinement, providing biologically relevant information on the enzyme. Our results demonstrate MicroED can be used for solving novel protein structures, using only standard X-ray crystallography software. These findings illustrate that electron crystallography has the potential to become a widely applicable tool for revealing new insights into protein structure and function, opening up new opportunities for structural biologists.

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Status: SUBMITTED

Submitted by **XU, Hongyi** on **Monday 09 September 2019**

Abstract ID : **54**

Analysis of Lon quaternary structure status and its complexes as factors contributing to the protease activities.

Content

Lon protease belongs to the AAA + protein family (ATPases associated with various cellular activities) and is conserved among all domains of life. It degrades denatured proteins as well as proteins involved in key cellular metabolic processes. Tight regulation of Lon protease activity is essential for maintaining proteostasis in a cell. It has been shown that the Escherichia coli Lon protein (EcLon) can exist in two oligomeric states: hexamer and double hexamer (dodecamer). So far, the biological relevance of the formation of these two types of quaternary structures of Lon is still enigmatic. The main goal of my current work focus on analysis of how growth conditions or interactions with substrates and cofactors affect Lon structure. We would like to analyze the impact of specific phosphate-containing molecules as well as Lon substrates on the protease structure. Moreover, in our preliminary experiments, Lon mutant, which is predominantly dodecameric, interacts with polyphosphate less efficient. We consider that the residues responsible for PolyP binding are covered within the dodecameric structure of Lon, and therefore we expect that Lon quaternary structure transitions may serve as a mechanism of proteolysis regulation. The negative staining EM could directly estimate the number of Lon hexamers and dodecamers in the analyzed samples and/or in presence of cofactors. The wild-type Lon, as well as dodecameric Lon mutant can be used for cryo-EM analysis and 3D complex reconstruction. Based on my preliminary experiments I expect that obtaining high resolution 3D Lon complex reconstructions should allow for sequence docking and obtaining a bioinformatic model of such structures. Alternatively, docking of predicted structure into the experimentally obtained EM density maps could be done with using bioinformatic approach. Thorough structural analysis of Lon may not be a simple task but it is necessary for deeper understanding of mechanism of action of this protease.

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Status: SUBMITTED

Submitted by **ROPELEWSKA, Małgorzata** on **Tuesday 10 September 2019**

Abstract ID : **55**

Monitoring protein assemblies in vivo.

Content

Biomolecular assemblies play a central role in nearly all life processes, from the replication of the genetic material to the construction of macroscopic architecture as microtubules. There is great interest in the design of such self-assembling proteins, because it could lead to the development of innovative biomaterial for biomedical or nanotechnology applications. Nowadays, we are able to de novo design proteins to form complex assemblies, such as protein cage. But because the properties of elaborate protein assemblies are not sufficiently well understood, current generations of protein cages are highly porous and lack triggers system for reversible assembly/disassembly.

In my Ph.D. project, I aim to develop an in vivo essay for monitoring protein assembly expression and stability. This will help us to understand the behaviour of natural and designed protein assembly in a complex cellular environment. This method is based on a 3-color assembly essay, with a stress reporter, an expression reporter and an assembly reporter.

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Status: SUBMITTED

Submitted by **GRUNNING, Åsa** on **Tuesday 10 September 2019**

Abstract ID : **56**

Identification of Oligomeric States During Superoxide Dismutase Fibrillation by Parallel Factor Analysis of NMR Diffusion Data

Content

Protein aggregation and amyloid fibril formation is associated with a number of neurodegenerative diseases. Transient oligomeric protein states formed during the process of fibril formation coexist with mature fibrils and are known to cause cell death in disease model systems. Characterization of the early state oligomers of fibrillation reactions are challenging and progress in the field depends critically on the development of analytical methods that can provide information about the mechanisms and species involved in oligomerization and fibril formation. Here we show through a combination of NMR diffusion experiments and multi linear analysis the coexistence of four different soluble states during the fibrillation reaction of superoxide dismutase, which is associated with the neurodegenerative disease amyotrophic lateral sclerosis. The analysis efficiently unravel the number of involved species, their kinetic rates of formation, spectral contributions, and diffusion coefficients, without any prior knowledge of the time evolution of the process or chemical shift assignments of the various species. Over a time course of 6 weeks, during which SOD1 fibrils form, we detect the disappearance of the native monomeric species, formation of a partially unfolded intermediate in the dimer to tetramer size range, subsequent formation of a distinct similarly sized species that dominates the final spectrum detected by solution NMR, and parallel appearance of small peptide.

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Status: SUBMITTED

Submitted by **GRUNNING, Åsa** on **Tuesday 10 September 2019**

Abstract ID : **57**

Structural Studies of the Mitochondrial Pyruvate Carrier (MPC), A Common Target for Type 2 Diabetes and Cancer Treatment

Content

Mitochondria facilitate the synthesis of the universal free energy carrier ATP which is the final product of cellular metabolism in all living cells (1). In order to accomplish this, metabolites such as pyruvate need to reach the mitochondrial compartment. Whereas, the mitochondrial outer membrane is permeable to small molecules through the voltage-dependent anion channels and porins(2), the inner
membrane involves specific carriers for selective flow of metabolites in and out from the mitochondrial inner chamber (3). The carrier protein that brings pyruvate into the mitochondria was identified as the mitochondrial pyruvate carrier (MPC) in 2012 (4). Mitochondrial pyruvate carrier (MPC), a protein complex in the inner mitochondrial membrane, consists of two proteins, MPC1 and MPC2, both of which are conserved from yeast to mammals (5). Interestingly, a group of insulin-sensitizing drugs in type 2 diabetes treatment, known as thiazolidinediones (TZDs), have been recognized to inhibit the MPC in addition to activating their classical target, the transcription factor PPARγ (6). Importantly MPC has emerged as a potential target for cancer therapy, due to its involvement in the Warburg effect in cellular metabolism(7).
In this project we aim to solve the crystal structure of MPC and study the structure-based functional mechanism of pyruvate transfer into the mitochondria. We have selected seven different species as sources of MPCs genes (mpc1 and mpc2) in order to check expression and purification of the proteins for structural studies. All the genes have been cloned in pPICZ-B vector with cleavable C-terminal His tag and small scale protein expression in P. pastoris as expression host has been analyzed using western blot. Based on these results, the two best-expressing partners of the MPC complex from Saccharomyces cerevisiae was selected for large scale production in fermenters and purified using IMAC and size-exclusion chromatography. Whereas MPC1 was produced and purified in good quantity and quality for structural studies, a satisfactory yield of MPC2 has not yet been achieved. To
alleviate this, we are currently working on a new MPC2 construct, adding a different cleavable tag (His and Strep-II) at either the C and N terminus of the protein. We have also cloned both the partners and incorporated it in P. pastoris genome for co-expression and purification of the complex. Currently, crystallization trials of MPC1 is ongoing. Our ultimate goal is to reveal the first mechanistic models of how pyruvate being transported by the MPC, providing a framework for downstream drug development for type type 2 diabetes and cancer treatment.
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Submitted by **GRUNNING, Åsa** on **Tuesday 10 September 2019**

Abstract ID : **59**

Protein structural determination from disordered crystals

Content

Membrane proteins act as the communicators between the internal and external world of the cell, thus harbouring highly sought after drug targets amidst their ranks. Despite their importance, the current number of unique membrane protein structures in the Protein Data Bank comprise a minority of all solved structures[1]; a result of the key properties of a membrane protein - the location in the cellular membrane. In order to study a membrane protein, it is necessary to rst isolate the cellular membrane, and then to draw the protein out of the membrane, using detergents. Many dierent detergents can be used for this, and they all have dierent features. In the case of crystallography, these features may impact, or even hinder, crystal packing, resulting in a crystal of what we currently consider as poor quality, thus severely limiting the resolution of the data one may obtain from it.

In order to circumvent this issue, initial studies have been done to utilise continuous diraction; a feature of disordered crystals allowing de novo phasing, thus providing a novel method of structural determination introducing signicantly less bias than current methods, without the limitations imposed by crystal quality[2, 3].

We are currently planning to investigate a number of features determining the occurrence of continuous diraction, such as manipulation of crystal packing, diering crystal growth conditions, sample delivery systems, as well as investigating whether it is possible to attain the desired results “simply” using synchrotron radiation (as current ndings have required XFEL radiation). This will also be applied to other proteins of similar characteristics, in order to determine how to transfer these desired properties to a broader range of targets, thus hopefully allowing membrane proteins to become easier to study, and providing an alternative method for dealing with the always present phase problem of crystallography.

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Abstract ID : **60**

Exploring the structure and function of insect odorant receptor membrane proteins

Content

The insect olfactory system distinguishes a vast number of volatile compounds (odorants) using a relatively small number of olfactory sensory neurons. Drosophila melanogaster, for example, carries ˜60 odorant receptors (ORs), which are expressed in the membrane of olfactory sensory neurons.

The OR system itself is an odorant-gated ion channel, formed by a heteromultimer comprising a highly conserved odorant receptor co-receptor (Orco) subunit and a more divergent OR conferring odorant-specificity. Compared to G protein-coupled receptors, each subunit of the Orco-OR heteromultimer adopts an inverse heptahelical topology in the cell membrane.

A recent milestone towards resolving the OR architecture is a single-particle cryo-EM study of the Orco homotetramer at 3.5 Å resolution: Butterwick et al. 1 show that the Orco tetramer forms a Ca2+-permeable ion channel. They further propose that insect ORs show a similar quaternary structure as the Orco tetramer, where one or two Orco subunits are exchanged for an OR.

Our current research topics focuses on the Orco-OR structure across insect species. OR proteins are produced by infection of insect cells with viruses containing the respective OR gene. The extracted and purified transmembrane OR protein, stabilized with a detergent, will be crystallized and we aim to investigate the three-dimensional structure by single-crystal X-ray diffraction.

In addition, we have recently initiated an in silico study where the Orco and OR splice variant orthologues in several Drosophila species are compared using homology modelling. An important aim here is to explore the quaternary structures and to identify putative binding sites for odorants and the determinants governing the specificity of the receptors.

[1]. Butterwick, J. A. et al. Cryo-EM structure of the insect olfactory receptor Orco. Nature 560, 447–452 (2018).

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Abstract ID : **62**

Incorporation of guest proteins into the solvent channels of a host crystal (HOSTAL) for X-ray diffraction studies.

Content

X-ray crystallography is a powerful tool in structural biology as most protein structures today have been determined using this method. With the aim to allow structural determination from X-ray diffraction also of proteins excluded from conventional crystallography as they do not crystallize, we recently started to develop a crystal host (HOSTAL) method. This approach makes use of host crystal that are easy to obtain and possess large solvent channels into which small to mid-size guest proteins can be incorporated via soaking. Using brightfield and confocal microscopy we were able to study over time the diffusion of the small guest proteins cytochrome C and calmodulin into host crystals of domain swapped Tryptophan repressor from *Eschericia coli* .

We estimate that the guest protein can occupy ˜ 40 % of the solvent channels when soaked at high guest concentrations of > 100 mg/ml that motivated to pursue first X-ray diffraction studies. Although the analysis of the obtained diffraction data indicates differences for the host with guest compared to the host alone, the guest structures could not yet be solved in a simple way by e.g. molecular replacement. That suggest that the guest proteins may not be sufficiently ordered for structure determination using conventional methods. The current work focuses on ways to order the guest proteins by promoting specific host-guest interactions but also method development for the interpretation of weak signals from diffraction of semi-ordered systems and includes analysis of the diffuse scattering signal.

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Abstract ID : **63**

Assembly and symmetry of the fungal PX-containing core of the Pyruvate Dehydrogenase Complex

Content

The pyruvate dehydrogenase complex (PDC) is a central component of all aerobic respiration, connecting glycolysis to mitochondrial oxidation of pyruvate. Despite its central metabolic role, no comprehensive picture has detailed its structure, owing to flexibility and a pseudo-symmetrical assembly that appears to regulate stoichiometry by an unknown mechanism. We determined a cryo-EM reconstruction of the native and recombinant PDC from the filamentous fungus Neurospora crassa, for the first time showing how its X-component (PX) is incorporated. Surprisingly, we find that this component oligomerizes interior to the E2 core. This reduces the pseudo-icosahedral core to a strict tetrahedral symmetry. The separate oligomerization and volume occlusion limits binding stoichiometry, suggesting a possibly dynamic mechanism that reconciles the variety of binding stoichiometries found previously for S. cerevisiae. Through the current work, we overcome challenges inherent to classification and reconstruction of complexes with multiple inherent symmetries, and find broad implications regarding the interpretation of conventionally used classification methods. This supplements the knowledge of an important metabolic regulator, and expands the range of biomolecular structure analysis by cryo-EM.

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