21st Swedish Conference
on

Macromolecular Structure and Function

Tällberg, 16-19 June 2017

SBNet

SFBBM
Svenska föreningen för biokemi, biofysik och molekylärbiologi
Swedish society for biochemistry, biophysics and molecular biology

Sponsored by

Royal Swedish Academy of Sciences
Swedish Research Council
Wenner-Gren Foundations

AstraZeneca

BECKMAN COULTER

BIO-RAD

FORMULATRIX

GE Healthcare

Malvern

Molecular Dimensions

ThermoFisher SCIENTIFIC

Valegård Design

Programme

Friday June 16

16:30 – 18:00 Conference desk open
18:00 – 19:30 Dinner

20:00 – 21:00 Session I: Opening and The Svedberg award
Chair: Mats Hansson

20:00 – 20:15 Welcome and prize award
20:15 – 21:00 Presentation by the The Svedberg Awardee

21:00 – 21:40 PI meeting

Saturday June 17

07:00 – 08:45 Breakfast

09:00 – 12:00 Session II: Recent advances in cryo-EM
Chair: Stefan Knight
09:00 – 09:45 Eva Nogales (University of California Berkley):
Microtubule structure, dynamics, and regulatory interactions visualized by cryo-EM
09:50 – 10:10 Kenta Okamoto (Uppsala University):
Three-dimensional imaging of giant amoebal viruses

10:15 – 10:45 Coffee

10:45 – 11:30 Alexey Amunts (Stockholm University):
Protein synthesis in organelles at cryo-EM resolution
11:35 – 11:55 Hongyi Xu (Stockholm University):
Lysozyme structure solved from sub-micrometer needle shaped crystals by electron crystallography

12:00 – 13:30 Lunch

13:30 – 15:10 Session III: Folding and dynamics
Chair: Magnus Johansson
13:30 – 14:15 Patricia Clark (University of Notre Dame):
Cellular strategies to enhance protein folding
14:20 – 14:40 Björn M. Burmann (University of Gothenburg):
Chaperone–substrate complexes: Discerning their crucial role in the physiological function and toxicity of Parkinson's disease related α-Synuclein
14:45 – 15:05 Annika Söderholm (Uppsala University):
Functional and structural innovations in the real-time evolution of the $\beta\alpha_8$-barrel enzyme HisA

15:10 – 15:40 Coffee

15:40 – 17:50 Session IV: Nucleic acids and emerging methods
Chair: Daniel Larsson

15:40 – 16:25 Janosch Hennig (EMBL Heidelberg):
Integrating NMR and small-angle scattering to investigate protein-RNA complexes

16:30 – 16:50 Ivan Volkov (Uppsala University):
Single-molecule tRNA tracking in live bacterial cells

16:55 – 17:15 Oskar Berntsson (University of Gothenburg):
Signal transduction in drosophila melanogaster cryptochrome

17:20 – 17:40 Hugo Lebrette (Stockholm University):
Structural study of a metalloprotein using a drop-on demand sample delivery at a X-ray free-electron laser

18:00 – 19:30 Dinner

20:00 – 21:30 Poster Session I

21:45 – 22:45 Quiz

Sunday June 18

07:00 – 08:45 Breakfast

09:00 – 12:00 Session V: Protein complexes
Chair: Maria Selmer

09:00 – 09:45 Cynthia Wolberger (John Hopkins University):
Structural basis for histone H2B deubiquitination by the SAGA DUB module.

09:50 – 10:10 Michael Jarva (La Trobe University):
The X-ray structure of a defensin reveals a unique oligomeric membrane disruption complex upon binding phosphatidic acid

10:15 – 10:45 Coffee

10:45 – 11:05 Isha Raj (Karolinska Institutet):
Structural basis of egg coat-sperm recognition at fertilization

11:10 – 11:30 Geoffrey Masuyer (Stockholm University):
The structure of the tetanus toxin reveals pH-mediated domain dynamics

11:35-11:55 Laura H. Gunn (Uppsala University):
The novel Methanococcoides burtonii rubisco assembly domain facilitates protein oligomerisation

12:00 – 13:30 Lunch

13:30 – 15:10 Session VI: Structural biology & disease
Chair: Terese Bergfors

13:30 – 14:15 Tom Blundell (University of Cambridge):
Structural biology and drug discovery: combating the emergence of resistance in cancer and mycobacterial infections

14:20 – 14:40 Anna Munke (Uppsala University):
Phage display and kinetic selection of antibodies that selectively inhibit amyloid self-replication

14:45 – 15:05 Andreas Schmitt (Umeå University):
Structural and functional characterization of Gram+ T4SS adhesion components

15:10 – 15:40 Coffee

15:40 – 18:00 Annual football match and rowing on Lake Siljan

18:00 – 19:30 Dinner

20:00 – 21:30 Poster Session II

21:45 – 22:45 Sing along / Table tennis tournament

Monday 19th June

07:00 – 08:45 Breakfast

09:00 – 11:50 Session VII: Membrane proteins
Chair: Henrik Hansson

09:00 – 09:45 Changjiang Dong (University of East Anglia):
Structural insight into the Gram-negative bacterial outer membrane biogenesis and assembly

09:50 – 10:10 Rebecca J. Howard (Stockholm University):
Multi-site allosteric modulation by general anesthetics revealed by structural, functional, and computational studies in a model pentameric ligand-gated ion channel

10:15 – 10:45 Coffee

10:45 – 11:05 Florian Schmitz (University of Gothenburg):
Applying bimolecular fluorescence complementation to screen and purify aquaporin protein:protein complexes

11:10 – 11:30 Rachel North (University of Gothenburg):
Bacterial sialic acid transport

11:35 – 11:50 Closing of meeting and award of prizes for best presentation and poster

12:00 – 13:00 Lunch

Oral Presentations

Microtubule structure, dynamics, and regulatory interactions visualized by cryo-EM

Eva Nogales
University of California & Howard Hughes Medical Institute, Berkeley

Cell division is a complex, highly regulated process in which the microtubule (MT) cytoskeleton plays a central role, serving as energy source for dramatic chromosomal movements and acting as a scaffold that facilitates molecular encounters at the right time and place. Essential for MT function is dynamic instability, a property that can be both regulated and utilized for cellular work. The MT is built by the self-assembly of $\alpha\beta$-tubulin dimers and MT dynamics are due to the coupling of the assembly process to GTP hydrolysis in β-tubulin. Anticancer drugs like taxol stop cell division by interfering with MT dynamics, while many MT cellular partners modulate or utilize dynamic instability to carry out specific functions. We are using cryo-EM to visualize MTs and their ligands critical to the processes of chromosome segregation and cell division. By characterizing in atomic detail the conformational changes in MTs that accompany GTP hydrolysis, we have shed unique light into the structural basis of MT dynamic instability. We have also visualized the binding site and effect of anticancer drugs on MTs. We are now using the pipeline that we have optimized over the last three years to obtain high resolution structures of MTs bound to microtubule associated proteins (MAPs) in order to define the binding site of different MAPs, their effect of MT structure, and, if not yet known, the structure of the MAP itself in its functionally relevant state, on the MT.

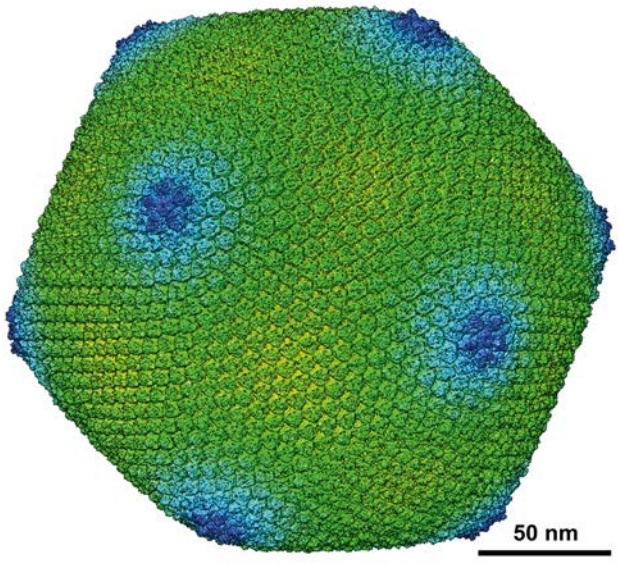
Three-dimensional imaging of giant amoebal viruses

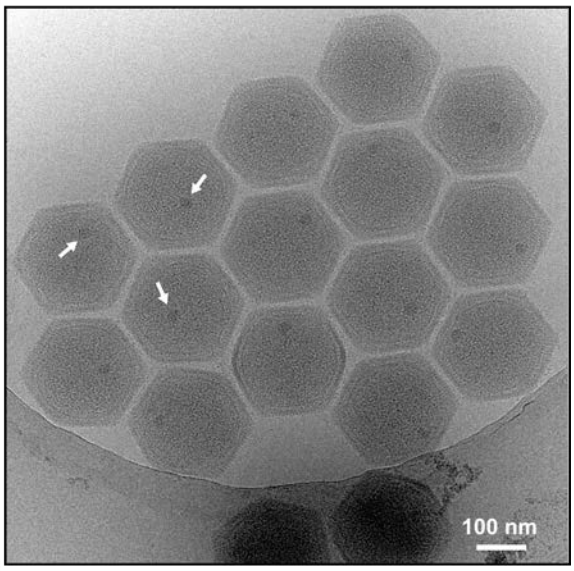
Kenta Okamoto[1], Naoyuki Miyazaki[2], Chuang Song, Daniel Larsson[1], Hemanth Kumer Narayana Reddy[1], Filipe Maia[1], Chantal Abergel[3], Jean-Michel Claverie[3], Janos Hajdu[1], Kazuyoshi Murata[2], Martin Svenda[1]

1. The laboratory of Molecular Biophysics, BMC, Uppsala University, Sweden, 2. The electron microscopy group, National Institute of Physiological Sciences (NIPS), Japan, 3. Structure and Genomics Information laboratory (IGS), CNRS, Aix-Marseille University

Giant (visible in optical light microscopes) amoebal viruses are a growing group of viruses that is raising research interests since the discovery of the bacterial sized Mimivirus. They possess several intriguing characteristics such as their large size and genome that do not fit the common definition of a virus. Their evolution supposedly originates from a common ancestor, dating back as far as to the early evolution of the dsDNA viruses. They are also believed to provide a link between viruses and eukaryotic cellular nucleus and therefore questions the very definition of life.

Since the discovery of Mimivirus, several other families of the giant viruses have been recently isolated from environmental water and permafrost soils. These viruses have quite large and unique pseudo-icosahedral or amphora-shaped morphologies. Our challenge lies in solving entire structures of these enormous viruses using electron cryo-microscopy (cryo-EM). Here, we present the first 3D structures of two amoebal viruses, Melbournevirus (*family Merseilleviridae*) and Pithovirus sibericum found in Siberian permafrost. We determined the ordered capsid lattice of the Melbournevirus particle to 9.1 Å resolution and it has the largest ever-determined T number to date with 9,240 major capsid proteins and putative minor proteins (Left figure). An unprecedented large and dense body (LDB) is consistently encapsulated in every Melbourenvirus particles (Right figure). The size of the LDB is around 30 nm, as the size range of a ribosome or a small virus. Secondary, a tomographic reconstruction was made of the entire 3D structure of the Pithovirus sibericum that is the largest virus known. An acceleration voltage of 1 MV was used to achieve sufficient penetration of the electrons to get a contrast of the micron-thick virus. The phase-contrast cryo-EM images reveal the potential subcompartmetalization of the virus.





Protein synthesis in organelles at cryo-EM resolution

Alexey Amunts
Department of Biochemistry and Biophysics, Stockholm University & Science for Life Laboratory

Mitochondria and chloroplasts have specialized ribosomes that have diverged from their bacterial and cytoplasmic counterparts. We have determined the structures of translating human mitoribosome, its assembly intermediates, as well as spinach chlororibosome in complex with its recycling factor and hibernating promoting factor at 3-3.5 Å resolution by single-particle electron cryo-microscopy. It reveals a distinct mechanism for mt-tRNA translocation, a remodeled architecture of the exit tunnel in different organellar ribosomes, and experimental evidence for the parallel evolution of ribosomes from chloroplast and mitochondrion. From the structural information we also identified new mitoribosomal assembly factors, which may link mitoribosomal biogenesis to other synthesis pathways in mitochondria.

Lysozyme Structure Solved from Sub-Micrometer Needle Shaped Crystals by Electron Crystallography

Hongyi Xu, Hugo Lebrette*, Wei Wan, Taimin Yang, Vivek Srinivas*, Sven Hovmöller, Martin Högbom* and Xiaodong Zou

Berzelii Center EXSELENT on Porous Materials and Inorganic and Structural Chemistry, Department of Materials and Environmental Chemistry, Stockholm University, SE-106 91 Stockholm, Sweden, *Department of Biochemistry and Biophysics, Stockholm University, SE-106 91 Stockholm, Sweden

Protein X-ray crystallography is a powerful method for solving 3D protein structures. However, a historical challenge is to obtain protein crystals of suitable solidity and size to be harvested and to allow X-ray data collection. In fact, the growth optimization of protein crystals from microcrystals is often intricate/troublesome and not always successful. This latter bottleneck is even narrower in the cases of membrane protein due to the difficulties in expressing, purifying and crystallizing such proteins. Electrons have unique properties and are complementary to X-rays and neutrons. They interact with matter 10^4 times stronger than do X-rays and neutrons and can be used for studying crystals with 10^8 times smaller volumes than with X-rays. Therefore, it is possible to collect electron diffraction from much smaller protein crystals. In this work, 3D electron diffraction patterns from needle-shaped lysozyme crystals as shown in Figure 1a. These needle-shaped crystals were broken into smaller fragments after the plunge freezing process (Figure 1a, Inset). 3D electron diffraction data was collected by continuously rotating the crystal in the electron beam while recording the ED patterns with the Timepix camera running in “movie” mode. A typical ED frame is shown as Figure 1b. The resolution at the corner of the frame is approximately 2Å. With an electron dose rate of 0.08 e-/Å^2/s and 2s exposure per frame, a 3D dataset typically contain 60 frames (about 54° coverage of the reciprocal lattice) can be collected from one single crystal, before the critical dose of 9 e-/Å^2 is reached. By processing the raw ED data using XDS and combining 12 datasets using XSCALE (82% completeness), we were able to solve the lysozyme structure to 2.2Å by molecular replacement (Figure 1c). Furthermore, it was also possible to solve the lysozyme structure to 2.5 Å by molecular replacement using only 1 single dataset with an overall completeness of 47%.

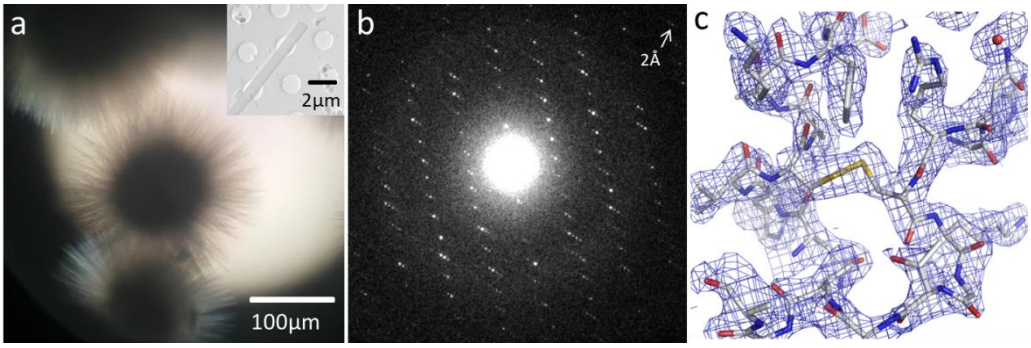


Figure 1 – a) As-grown needle-shaped lysozyme crystals viewed with an optical microscope. The inset is typical needle-shaped lysozyme crystals found on Quantifoil TEM grid prepared by plunge freezing. b) Typical electron diffraction frame collected from a needle-shaped lysozyme crystal. c) Electron density quality around a disulphide bridge. The $2F_o$-F_c electron density map is contoured to 1σ and coloured in blue. Carbon, oxygen and nitrogen atoms are coloured grey, red and blue, respectively.

Cellular strategies to enhance protein folding

Patricia L. Clark
Department of Chemistry & Biochemistry
University of Notre Dame, Notre Dame, IN 46556

Most of what we currently know about how the cell functions on a molecular level has been gleaned from detailed studies of the behavior of a pure solution of a biomolecule (or biomolecules) of interest. The specific biomolecules and conditions selected for these studies tend to be the simplest that lead to "good behavior" in the test tube, including thermodynamic equilibrium. But to what extent is we have learned from studies in the test tube predictive of the behavior of biomolecules *in vivo*? Protein folding is a great example of this conundrum: On the one hand, Christian Anfinsen received Nobel Prize in Chemistry for his demonstration that, for the protein ribonuclease, all of the information required for it to fold to its functional, native form is contained within the ribonuclease amino acid sequence at pH 8.2 and 24°C. On the other, much less well known hand is Anfinsen's subsequent discovery that ribonuclease fails to fold efficiently under physiological conditions (pH 7.4, 37°C) [1], a situation that he showed can be improved by adding to the refolding reaction the (at the time) newly characterized enzyme protein disulfide isomerase [2]. I will discuss the extent to which Anfinsen's principle can be extended to model the folding behavior of all proteins in the proteome and how spatiotemporal control of protein folding during polypeptide synthesis or translocation across a membrane can be used to manipulate protein production *in vivo*.

1. Canfield R.E. & Anfinsen C.B. (1963) *Biochemistry* **2**:1073.
2. Givol D., Goldberger R.F. & Anfinsen C.B (1964) *J. Biol. Chem.* **239**: 3114.

Chaperone–substrate-complexes: Discerning their crucial role in the physiological function and toxicity of Parkinson's disease related α-Synuclein.

Björn M. Burmann[#,*], Juan A. Gerez[**], Irena Burmann[*], Silvia Campioni[**], Pratibha Kumari[**], Magdalena Wawrzyniuk[***], Thomas Bock[*], Alexander Schmidt[*], Stefan G.D. Rüdiger[***], Roland Riek[**], Sebastian Hiller[*]

[#]Department of Chemistry and Molecualr Biology, Wallenberg Centre for Translational and Molecular Medicine, University of Gothenburg, Sweden; *Biozentrum Basel, University of Basel, Switzerland, ** Department of Chemistry and Applied Biosciences, ETH Zurich, Switzerland; [***]Bijvoet Center for Biomolecular Research Utrecht University, Netherlands

Parkinson's disease is one of the most common neurodegenerative disorders, pathologically manifested by the accumulation of aggregates of the intrinsically disordered protein α-Synuclein within amyloid deposits termed Lewy bodies. Based on our earlier studies how molecular chaperones stabilize clients, we investigate here the role of chaperone–α-Synuclein interactions in atomic detail, linking studies *in vitro* and *in cellulo*. Initially, an array of bacterial and mammalian chaperones is used to characterize the basis for the prevention of α-Synuclein aggregation at the atomic level by solution NMR spectroscopy. These experiments identify an interaction *via* the α-Synuclein amino-terminus as the general interaction motive.

We validate this motive in kinetic aggregation assays and by differential mass-spectrometry in cells. In addition, the effects of known post-translational modifications of α-Synuclein, as well as the role of lipid bilayers on the interaction of α-Synuclein with chaperones are characterized. Our data provide mechanistic insight how molecular chaperones control and modulate α-Synuclein function *in vivo* and how the disturbance of chaperone–α-Synuclein interactions leads to progress of pathological relevant species of α-Synuclein.

To address the cellular function of molecular chaperones on the physiological role of α-Synuclein we use high-resolution in-cell NMR-spectroscopy in living mammalian cells. Targeted knock-down of the combined function of the constitutively expressed Hsc70 and Hsp90 chaperones let to an enhanced membrane interaction of α-Synuclein coupled to aggregation of α-Synuclein. Our data thus reveals a new function of molecular chaperones by actively controlling the physiological function of α-Synuclein, providing a new angle on possibly exploiting these interactions to prevent the formation of α-synuclein toxic species.

Functional and structural innovations in the real-time evolution of the $\beta\alpha_8$-barrel enzyme HisA

Annika Söderholm, XIaohu Guo, Matilda S. Newton*, Joakim Näsvall**, Patrik Lundström***, Dan I. Andersson**, Wayne M. Patrick* and Maria Selmer

Department of Cell and Molecular Biology, Uppsala University, *Department of Biochemistry, University of Otago, New Zealand, **Department of Medical Biochemistry and Microbiology, Uppsala University, ***Department of Physics, Chemistry, and Biology, Linköping University

The $\beta\alpha_8$-barrel isomerase HisA catalyzes the fourth reaction in histidine biosynthesis. An equivalent isomerization reaction in tryptophan biosynthesis is catalyzed by TrpF. In a previous study, these enzymes were used in an experimental test of the duplication-divergence model of evolution. In that study, the evolution of *hisA* from *Salmonella enterica* was followed for 3,000 generations, under selection for both HisA and TrpF activity. *(1)* Here, we have gained an in-depth understanding of the innovations and evolutionary routes leading to TrpF activity and bifunctionality by characterizing eleven evolved mutants by crystallographic structure determination, NMR spectroscopy, enzyme kinetics, and relative protein expression level. The kinetics experiments revealed that selection acted on k_{cat} rather than K_M. Moreover, a biphasic relationship between enzyme performance and growth rate was observed where small improvements in low-performing enzymes was sufficient to provide close to wild type growth rate whereas, later in evolution, large improvements in activity had little effect on fitness. Crystallographic structure determination and NMR relaxation dispersion experiments showed that alteration of activity was established by changes in the conformations of active-site loops. The enzyme is dependent on mutually exclusive loop conformations for HisA respectively TrpF activity. TrpF-specializing mutations were epistatic and acted in two different manners, by stabilizing the TrpF-relevant loop conformation or by adaptation of the active site to the smaller TrpF substrate. Mutations promoting bi-functionality lead to increased dynamics, thereby allowing conformational sampling between loop conformations. This study provides a detailed analysis of the selection mechanisms in the evolution of new genes. *(2)*

1. Näsvall J, Sun L, Roth JR, Andersson DI (2012) "Real-time evolution of new genes by innovation, amplification, and divergence." Science 338(6105):384-7

2. Newton MS, Guo X, Söderholm A, Näsvall J, Lundström P, Andersson DI, Selmer M, Patrick WM (2017) "Structural and functional innovations in the real-time evolution of new (βα)8 barrel enzymes." PNAS (Epub ahead of print)

Integrating NMR and small-angle scattering to investigate protein-RNA complexes

Janosch Hennig
EMBL Heidelberg

RNA binding proteins (RBPs) are key factors for the regulation of gene expression. However, it is still poorly understood exactly how these trans-acting RBPs recognize their cognate RNA target to regulate gene expression. Thus, structural studies are crucial to obtain detailed mechanistic insights. This is especially important for ribonucleoprotein (RNP) complexes featuring more than one protein. Larger complexes consisting also of flexible regions complicate the utilization of available methods.

To overcome this problem, several different methods have to be combined. Here, it will be demonstrated how the combination of X-ray crystallography, nuclear magnetic resonance spectroscopy, small-angle X-ray and neutron scattering provides valuable insight into the assembly of RNP complexes, illustrated with three different systems: i) the Sxl-UNR translation repression complex, ii) the quaternary Brat-Pum-Nanos-*hb* mRNA complex, and iii) the TIA-1-RNA complex. The latter example shows for the first time how sortase-mediated segmental isotope labelling is utilized to enable subdomain-selective contrast matching in small-angle neutron scattering of multi-domain proteins.

One major difficulty encountered during structural investigations of protein-RNA complexes is the identification of cognate RNA motifs, RBPs specify for. This is paramount to prevent sliding or transient binding of the RBP, which makes crystallization impossible or results in severe line broadening of NMR signals. To this end, we developed SAXScreen, a small-angle X-ray scattering based method, to screen for RBP specific RNAs. This method will be presented and compared to other existing screening tools.

Single-molecule tRNA tracking in live bacterial cells

Ivan Volkov, Martin Lindén, Kaweng Ieong, Javier Aguirre, Johan Elf and Magnus Johansson

Uppsala University, Department of Cell and Molecular Biology, Uppsala, Sweden

Protein synthesis has been studied extensively over the years, and the combination of traditional biochemistry, structural approaches, and more recently single-molecule fluorescence based in vitro techniques, have led to a detailed picture of the molecular mechanisms of ribosome catalyzed protein synthesis. However, we have very sparse information about the dynamics of protein synthesis, in particular inside living cells. The sheer complexity of the translational system (do we know all the players yet?), and its interplay with other processes, make it very hard to connect the molecular details of protein synthesis with cell physiology and population biology, i.e. with the level at which selection pressure applies. To make these connections, we use in vitro dye-labeled and purified tRNA molecules to investigate detailed kinetics of protein synthesis inside living bacterial cells. Fluorescent initiator and elongator tRNAs are electroporated into E. coli cells and then followed using single-molecule tracking methods with high temporal and spatial resolution. A number of control experiments, including the use of antibiotic drugs, modified tRNAs, and genetically modified cells, all suggest that the tRNA molecules take active part in protein synthesis. Based on differences in diffusion rates if the tRNAs are freely diffusing, bound to proteins, or bound to ribosomes, we can estimate the dwell-time of the tRNAs on the ribosome, and hence measure the kinetics of translation initiation and elongation inside living cells.

Signal Transduction in Drosophila melanogaster Cryptochrome

Oskar Berntsson, Ryan Rodriguez*, Erik Schleicher* and Sebastian Westenhoff

University of Gothenburg, *University of Freiburg

Cryptochromes are blue light photoreceptors found in plants and animals. They are important for regulation of the circadian rhythm and have also been implicated in blue light depended sensing of magnetic fields. Within the protein matrix resides a Flavin adenine dinucleotide chromophore responsible for the blue light sensitivity. Upon illumination electron transfer along a series of conserved tryptophan residues is triggered. Eventually this leads to the undocking of the carboxy terminal tail of the protein, thereby enabling interaction with downstream proteins. The structural events that lead to this are poorly understood. We have applied time-resolved X-ray solution scattering on nanosecond to millisecond timescales along with molecular dynamics simulations to investigate the signal transduction in cryptochrome from the fruit fly *Drosophila melanogaster*. The data shows how the protein gets contracted within 300 nanoseconds before it extends after one millisecond. Together with the simulations the data indicates that a conserved histidine is the focal point for the relay of the signal from the chromophore to the carboxy terminal tail.

Structural study of a metalloprotein using a drop-on-demand sample delivery at a X-ray free-electron laser

Hugo Lebrette*, Vivek Srinivas*, Jan Kern** and Martin Högbom*

*Department of Biochemistry and Biophysics, Stockholm University, Stockholm, Sweden, **Molecular Biophysics and Integrated Bioimaging Division, Lawrence Berkeley National Laboratory, Berkeley, California, USA; and LCLS, SLAC National Accelerator Laboratory, Menlo Park, California, USA.

Synchrotron X-ray crystallography has proven to be a powerful method for studying the structure of metalloproteins. However, artifacts can be induced using this method, e.g. caused by the photoreduction of metal cofactors by extended exposure to X-rays, or induced by data collection at cryogenic temperatures.

A new method of X-ray crystallography at X-ray free-electron laser (XFEL) sources uses femtosecond pulses to produce diffraction signal at room temperature before radiation-induced changes occur. Consequently, XFELs provide new opportunities to study biologically relevant redox state of metalloproteins. Moreover, when combined with X-ray emission spectroscopy (XES), both global structures and the chemical properties at catalytic sites of metalloenzymes can be studied.

Using a newly developed drop-on-demand sample delivery, coupling an acoustic droplet ejection with a conveyor belt drive optimized for crystallography and spectroscopy measurements [1], we recently obtained X-ray crystal diffraction and XES data of a metalloprotein, simultaneously collected at a XFEL source (SLAC Linac Coherent Light Source, Stanford, USA). In our study, we compare this structure solved from a XFEL source with several structures of the same protein solved from synchrotron X-ray sources, focusing on the metal cofactor and its environment.

[1] Fuller FD *et al.* (2017) *Nat Methods* 14(4):443-449.

Structural basis for histone H2B deubiquitination by the SAGA DUB module

Cynthia Wolberger, Michael Morgan, Mahmood Haj-Yahya*, Muhammad Jbara**, Suman K. Maity**, Alison E. Ringel, Ashraf Brik**

Department of Biophysics and Biophysical Chemistry, Johns Hopkins University School of Medicine, Baltimore, MD 21205 USA, *Department of Chemistry, Ben-Gurion University of the Negev, Beer Sheva, Israel, 8410501, **Schulich Faculty of Chemistry, Technion-Israel Institute of Technology, Haifa, 3200008, Israel

Eukaryotic transcription is regulated by coactivator complexes that orchestrate the attachment and removal of specific histone modifications. Histone H2B monoubiquitinated at K123 (yeast) is a mark of actively transcribed chromatin that is required for histone H3 trimethylation, recruitment of H2A/H2B chaperones, and appropriate activation, elongation and mRNA export. H2B is deubiquitinated by the SAGA coactivator complex, which contains a four-protein deubiquitinating (DUB) module. The structure of the DUB module revealed a remarkably intertwined arrangement of subunits, which shed light on how the Sgf11, Sus1 and Sgf73 subunits are required in order for the catalytic subunit, Ubp8, to be active. To understand the molecular details by which the DUB module targets H2B-Ub, we determined the crystal structure of the yeast SAGA DUB module bound to a ubiquitinated nucleosome at 3.9 Å resolution. The structure reveals that the DUBm engages the nucleosome almost exclusively at the H2A/H2B acidic patch using an arginine cluster on the Sgf11 zinc finger domain, which lies adjacent to the active site of the Ubp8 catalytic domain. The docking of the DUB module on the nucleosome accounts for the role that phosphorylation of H2A-Y57/58 plays in regulating H2B deubiquitination by yeast and human SAGA. We tested the effect of H2A phosphorylation on DUB module activity by using a new method to generate synthetic H2A phosphorylated at Y57 and reconstituted the protein into nucleosomes containing native ubiquitinated H2B generated by intein biochemistry. We find that phosphorylation of H2A-Y57 directly interferes with cleavage of H2B-UB by the DUB module. Consistent with our observation that the DUB module engages the nucleosome almost exclusively through H2A/H2B, we find that the DUB module efficiently deubiquitinates H2B-Ub in either nucleosomes and on H2A/H2B-Ub dimers bound to FACT, which facilitates nucleosome disassembly and reassembly during the transcription cycle. These results indicate that SAGA could act target H2B at multiple stages during the transcription cycle, potentially deubiquitinating H2B when the nucleosome is disassembled to allow the passage of RNA polymerase.

The X-ray structure of a defensin reveals a unique oligomeric membrane disruption complex upon binding phosphatidic acid

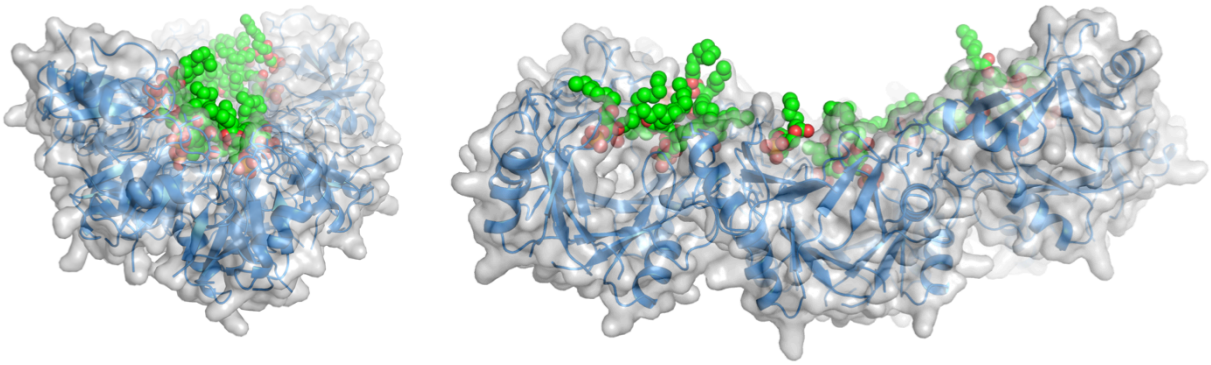
Michael Jarva, Fung Lay, Cassandra Humble, Mark Hulett, Mark Kvansakul

Department of Biochemistry and Genetics, La Trobe Institute for Molecular Science, La Trobe University, Melbourne, Australia.

Defensins belong to a diverse group of cationic antimicrobial peptides (CAPs) expressed in most plant and animal species as a first line of defense against invading microbes. The defensins dimerize and selectively bind phospholipid head groups such as phosphatidylinositol 4,5-bisphosphate (PIP_2) and phosphatidic acid (PA). Many of these defensins have been shown to permeabilize cell membranes, and once inside form oligomeric structures that rupture the membrane by a yet to be determined mechanism.

Our lab has previously determined the oligomeric structure of *Nicotiana alata* defensin 1 (NaD1) in complex with PIP_2[1] and *Nicotiana suaveolens* defensin 7 in complex with PA[2]. These two structures have demonstrated the defensins ability to form vastly different oligomeric structures upon binding specific lipids, but neither structure has fully described the membrane attack mechanism.

In a continuous effort to unravel the crucial underlying permeabilization mechanism of defensins we determined the structure of NaD1 in complex with PA. The complex crystallized as a 20-meric unit comprising of 10 NaD1-dimers and 14 PA molecules. The observed oligomer is flat and extends in two directions, which is a striking difference compared to previous structures that displayed long coil-like topologies. The overall topology of the NaD1-PA oligomer reveals what we hypothesize to be a putative membrane disruption configuration that ultimately destabilizes cell membranes through direct lipid sequestering by carpeting the target cell plasma membrane.



1. Poon, I. K. *et al.* Phosphoinositide-mediated oligomerization of a defensin induces cell lysis. *Elife* **3,** 1–27 (2014).

2. Kvansakul, M. *et al.* Binding of phosphatidic acid by NsD7 mediates formation of helical defensin-lipid oligomeric assemblies and membrane permeabilization. *Proc. Natl. Acad. Sci. U. S. A.* (2016).

Structural Basis of Egg Coat-Sperm Recognition at Fertilization

Isha Raj*, Hamed Sadat Al Hosseini*, Elisa Dioguardi*, Kaoru Nishimura*, Ling Han*, Alessandra Villa*, Daniele de Sanctis** and Luca Jovine*

*Department of Biosciences and Nutrition and Center for Innovative Medicine, Karolinska Institutet, Huddinge, SE-141 83, Sweden **ESRF - The European Synchrotron, Grenoble 38000, France

Species-restricted recognition between male and female gametes marks the beginning of life in all sexually reproducing organisms. The very first step of this fundamental biological process involves molecules present on the sperm surface and egg coat. Although the interaction between gametes has been studied for centuries, its molecular basis and evolutionary conservation remain unknown.

To explore a possible evolutionary connection, we determined crystal structures of a functionally essential N-terminal region of mammalian zona pellucida protein ZP2 and three N-terminal domain repeats of invertebrate egg coat protein VERL. The comparison of these structures demonstrates that, as we hypothesized earlier[1], sperm receptor proteins from vertebrates and invertebrates consist of repeated domains that share a common immunoglobulin-like "ZP-N" fold.

Biochemical and crystallographic studies of VERL domain repeats 1-3 and their interaction with cognate sperm protein lysin were performed to investigate how counterpart proteins on sperm and egg surface recognize each other at the molecular level. Of the first two VERL repeats evolving under positive selection[2], the more sequence divergent repeat 1 does not bind lysin. On the other hand repeat 2, which displays intermediate sequence divergence, interacts with lysin species-specifically but with a lower binding affinity than repeat 3, which is under concerted evolution and binds lysin tightly in a non-species-specific manner. Mutational analysis suggests that divergence of the N-terminal sequence of VERL inactivated repeat 1 and lowered the binding affinity of repeat 2, thus amplifying the effect of positive selection of lysin to generate species-specificity.

Our structures of gamete recognition complexes reveal that a conserved region of VERL repeats 2 and 3 establishes a largely hydrophobic interface with lysin. Since the latter is a highly amphipathic protein, this results in juxtaposition of highly positively charged lysin surfaces on adjacent VERL molecules. Thus, complex formation both disrupts the organization of egg coat filaments and triggers their electrostatic repulsion, thereby generating a hole for sperm penetration and fusion.

1. Swanson, W.J., Aagaard, J.E., Vacquier, V.D., Monné, M., Sadat Al Hosseini, H., and Jovine, L. (2011). The molecular basis of sex: linking yeast to human. Mol. Biol. Evol. 28, 1963–1966.

2. Galindo, B.E., Vacquier, V.D., and Swanson, W.J. (2003). Positive selection in the egg receptor for abalone sperm lysin. Proc. Natl. Acad. Sci. U. S. A. 100, 4639–4643.

The structure of the tetanus toxin reveals pH-mediated domain dynamics

Geoffrey Masuyer*, Julian Conrad**, and Pål Stenmark*

*Department of Biochemistry and Biophysics, Arrhenius Laboratories for Natural Sciences, Stockholm University, 10691 Stockholm, Sweden. ** Science for Life Laboratory, Stockholm University, 17121 Solna, Sweden

The tetanus neurotoxin (TeNT) is a highly potent toxin produced by *Clostridium tetani* that inhibits neurotransmission of inhibitory interneurons, causing spastic paralysis in the tetanus disease. TeNT differs from the other clostridial neurotoxins by its unique ability to target the central nervous system by retrograde axonal transport. The crystal structure of the tetanus toxin presents a 'closed' domain arrangement with two disulphide bridges. An integrative analysis combining X-ray crystallography, solution scattering and single particle electron cryo-microscopy reveals pH-mediated domain rearrangements that may confer TeNT the ability to adapt to the multiple environments encountered during intoxication, and facilitate binding to distinct receptors.

The novel *Methanococcoides burtonii* Rubisco Assembly Domain facilitates protein oligomerisation

Laura H. Gunn, Karin Valegård and Inger Andersson

From the department of Cell and Molecular Biology, Uppsala University. S-751 24 Uppsala, Sweden

The catalytic deficiencies of nature's CO_2-fixing enzyme, ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), often limit plant growth and resource-use-efficiency [1] and the efficiency of biofuel production [2]. Evolutionary constraints have hindered efforts to engineer more efficient plant Rubiscos [3], prompting interest in Rubisco isoforms from non-photosynthetic organisms, such as that found in the methanogenic archaeon *M. burtonii* [4].

Here we describe the first *M. burtonii* Rubisco (MbR) crystal structure to 2.6 Å [5]. A sequence insertion, unique to MbR, folds as a separate domain in the structure, and is named the Rubisco assembly domain (RAD) because of its role tethering together neighbouring large subunit (LSu) L_2 dimers into $(L_2)_5$ decamers (Fig. 1A). Biochemical approaches confirm that L_2 dimers are locked against each other by the coordination of negatively charged residues around a Mg^{2+} ion (Fig. 1B). The RAD is located between LSus of adjacent L_2 dimers. This position and locking function is similar to that of the small subunits (SSu) present in in L_8S_8 plant enzymes: the RAD is a SSu mimic. We also combine sequence, structure, function and evolution analyses to propose a re-organisation of the Rubisco classification system, introducing a new sub-group, called form IIIB. The RAD has potential utility in engineering strategies as a protein glue.

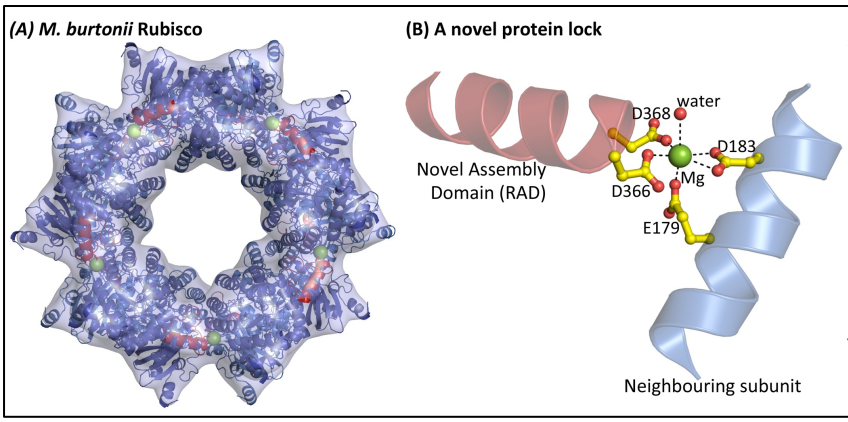


Figure 1. (A) Crystal structure of decameric *M. burtonii* Rubisco (pdb: 5MAC). Five L_2 dimers are locked together by the (B) coordination of four amino acids (yellow) around a Mg^{2+} ion (green). This lock-site involves the novel RAD (red).

References

1. Long, S.P. *et al*. Can improvement in photosynthesis increase crop yields? *Plant Cell Environ.* **29,** 315–330 (2006).
2. Stephenson, P.G. *et al*. Improving photosynthesis for algal biofuels: toward a green revolution. *Trends Biotechnol.* **29,** 615–623 (2011).
3. Durão, P. *et al.* Opposing effects of folding and assembly chaperones on evolvability of Rubisco. *Nat. Chem. Biol.* **11,** 148–155 (2015).
4. Wilson, R.H. *et al*. Evolving Methanococcoides burtonii archaeal Rubisco for improved photosynthesis and plant growth. *Sci. Rep.* **6,** (2016).
5. Gunn, L.H. *et al*. *et al*. Unique Structural Domain in Methanococcoides burtonii Rubisco Acts as a Small-subunit Mimic. *J. Biol. Chem.* jbc.M116.767145 (2017). doi:10.1074/jbc.M116.767145

Structural biology and drug discovery: combating the emergence of resistance in cancer and mycobacterial infections

Tom L Blundell
Department of Biochemistry, University of Cambridge,
Tennis Court Road, Cambridge CB2 1GA

The emergence of drug resistance is a challenge that is facing drug discovery in both cancer and infectious disease. Next generation sequencing has shown that mechanisms of resistance to the same drug in different tumours or pathogens can vary extensively. The challenge will be one in personalised or precision medicine. One approach is to exploit state-of-the-art methods to bring new drugs for different targets to the market, but this will be difficult to finance if patient populations are small. Structure-guided fragment-based screening techniques have proved effective in lead discovery not only for classical enzyme targets but also for less “druggable” targets such as protein-protein interfaces. Our approach involves a fast initial screening using biophysical techniques of a library of around 1000 compounds, followed by a validation step involving more rigorous use of related methods to define three-dimensional structure, kinetics and thermodynamics of fragment binding. The use of high throughput approaches does not end there, as it becomes a rapid technique to guide the elaboration of the fragments into larger molecular weight lead compounds. I will discuss progress in using these approaches for targets in cancer (protein-protein interfaces) and in Mycobacterium Tuberculosis and Mycobacterium Abscessus infections.
I will also review our new computational approaches using both statistical potentials and machine learning methods for understanding mechanisms of resistance. These have demonstrated that resistance does not only arise from direct interference of the resistance mutation to drug binding but can also result allosteric mechanisms, often modifying target interactions with other proteins. These lead to new ideas about repurposing and redesigning drugs and even in reclassifying patients in clinical trials.

Phage display and kinetic selection of antibodies that selectively inhibit amyloid self-replication

Anna Munke[1,2], Jonas Persson[1,3], Tanja Weiffert[1], Erwin De Genst[4,6], Georg Meisl[4], Paolo Arosio[4,5], Anna Carnerup[7], Christopher M Dobson[4], Michele Vendruscolo[4], Tuomas PJ Knowles[4,8] and Sara Linse[1]

[1]Dept. of Biochemistry and Structural Biology, Lund University, [2]Dept. of Cell and Molecular Biology, Uppsala University, [3]Dept. of Immunology, Genetics and Pathology, Uppsala University, [4]Dept. of Chemistry, University of Cambridge, [5]ETHZ Zürich, Dept. of Chemistry and Appl. Biosciences, [6]Astra Zeneca, Innovative Medicines, [7]Dept. of Physics, University of Cambridge, [8]Dept. of Physical Chemistry, Lund University

The aggregation of the amyloid β peptide (Aβ) into amyloid fibrils is a defining characteristic of Alzheimer's disease[1]. Because of the complexity of this aggregation process, effective therapeutic inhibitors will need to target the specific microscopic steps that lead to the production of the most highly neurotoxic species. We introduce a strategy for generating fibril-specific antibodies that selectively suppress fibril-dependent secondary nucleation of the 42-residue form of Aβ (Aβ42). We target this step because it has been shown to produce the majority of neurotoxic species during aggregation of Aβ42[2,3]. Starting from large phage display libraries of single-chain antibody fragments (scFvs), the three-stage approach that we describe includes: 1) selection of scFvs with high affinity for Aβ42 fibrils after removal of scFvs that bind Aβ42 in its monomeric form, 2) ranking, by surface plasmon resonance affinity measurements, of the resulting candidate scFvs that bind to the Aβ42 fibrils, and 3) kinetic screening and analysis to find the scFvs that inhibit selectively fibril-catalyzed secondary nucleation process in Aβ42 aggregation. By applying this approach, we have identified four scFvs that inhibit specifically the fibril-dependent secondary nucleation process. Our method also makes it possible to discard antibodies that inhibit elongation, an important factor because the suppression of elongation does not target directly the production of toxic oligomers and may even lead to its increase. On the basis of our results we suggest that the method described here could form the basis for rationally designed immunotherapeutic strategies to combat Alzheimer's disease and related neurodegenerative diseases.

1. Knowles T.P. *et al.* The amyloid state and its association with protein misfolding diseases. *Nat Rev Mol Cell Biol* **15**, 384-396 (2014)
2. Cohen S.I. *et al.* Proliferation of amyloid-β42 aggregates occurs through a secondary nucleation mechanism. *Proc Natl Acad Sci USA* **110**, 9758-9763 (2013)
3. Arosio P. *et al.* Quantification of the concentration of Aβ42 propagons during the lag phase by an amyloid chain reaction assay. *J Am Chem Soc* **136**, 219-225 (2014)

Structural and functional characterization of Gram+ T4SS adhesion components

Andreas Schmitt, Gary Dunny* and Ronnie Berntsson

Department for Medical Biochemistry and Biophysics, Umeå University, Umeå, Sweden *Department of Microbiology and Immunology, University of Minnesota, Minneapolis, USA

In the recent years, infections caused by multi-drug resistant bacteria have become an increasing problem in healthcare systems all over the world. Spread of antibiotic resistance and virulence factors within a population is promoted by the ability of many bacteria to transfer resistance-encoding genetic material to other cells. This transferable antibiotic resistance is mostly associated with mobile genetic elements such as plasmids. With conjugation, bacteria have evolved a highly effective system for the transfer of DNA from a donor to a recipient cell, mediated by proteins of the Type IV Secretion System (T4SS) [1]. T4SSs are therefore a major contributor to the spread of antibiotic resistance in many clinically relevant pathogens [2].

An important step in the process of conjugation is the formation of mating pairs between the donor and recipient cells. In Gram-positive (G+) T4SSs, adhesion of the cells is of particular interest, since there are no pili and thus adhesion proteins have to form mating cell aggregates and possibly also a pore in the cell wall of the receiving cell. We study a G+ T4SS from *Enterococcus faecalis,* where at least three proteins have been identified to be directly involved in the G+ T4SS adhesion process, named PrgA, PrgB and PrgC. It has been shown that the interaction of PrgA with PrgB is crucial for cell adhesion and that PrgB is a major contributor to *E. faecalis* virulence, while the molecular function of PrgC is not yet elucidated.

We were able to express, purify and crystallize different truncations of PrgA & PrgB and could solve the structure of the PrgA core domain as well as of two PrgB subconstructs, one of them in complex with DNA. The analysis of the structural data obtained for PrgA hint towards an unexpected function of that protein in the process of cell adhesion. Moreover, ITC and SPR based interaction studies provide insight in the molecular function of PrgB in cell adhesion and biofilm formation.

1 Christie PJ, Atmakuri K, Krishnamoorthy V, Jakubowski S & Cascales E (2005) Biogenesis, architecture, and function of bacterial type IV secretion systems. *Annu. Rev. Microbiol.* **59**, 451–485.

2 Hunt CP (1998) The emergence of enterococci as a cause of nosocomial infection. *Br. J. Biomed. Sci.* **55**, 149–156.

Structural insight into the Gram-negative bacterial outer membrane biogenesis and assembly

Changjiang Dong
Biomedical Research Centre, Norwich Medical School, University of East Anglia

Outer membrane is essential for all Gram-negative bacteria and plays critical roles in transporting nutrients, protecting bacteria from harsh environments, and avoiding host immune attacks. The outer membrane of Gram-negative bacteria consists of lipopolysaccharide, outer membrane proteins and phospholipids, which are essential for forming an integrate outer membrane and drug resistance. Outer membrane barrel proteins are synthesised in the cytoplasm in an unfolded form, and translocated across the inner membrane to the periplasm, where chaperon proteins escort the unfolded outer membrane proteins to the barrel assembly machine BAM machinery. The BAM machinery of E.coli contains five proteins, responsible for inserting and assembling the outer membrane proteins in the outer membrane. Mitochondria and chloroplasts also contain the outer membrane barrel proteins, and they are inserted into the outer membrane by Omp85 family proteins, suggesting that the outer membrane protein insertion into the outer membrane of mitochondria and chloroplasts may share the similar mechanism of that of the Gram-negative bacteria. Lipopolysaccharide is synthesised in the cytoplasm and transported and assembled by seven trans-envelop protein complex LptABCDEFG. The ABC transporter LptBFG extracts lipopolysaccharide from the periplasmic side of the inner membrane and pass it to LptC. The LptC, LptA and the N-terminal domain of LptD form a slide to transport lipopolysaccharide across the periplasm to the outer membrane protein LptD/E complex. LptD/E complex inserts lipopolysaccharide correctly in the outer leaflet of the outer membrane.

In this presentation, I will present our work in structural and functional studies of the outer membrane assembly machinery BamABCDE, as well as the the lipopolysaccharide transport and assembly in the outer membrane.

Multi-site allosteric modulation by general anesthetics revealed by structural, functional, and computational studies in a model pentameric ligand-gated ion channel

Rebecca J. Howard, Stephanie A. Heusser, Zaineb Fourati*, Marie Lycksell**, Göran Klement**, Haidai Hu*, Reinis R. Ruza*, Oliver Snow**, Özge Yoluk**, Changhun Yun**, Marc Delarue* and Erik Lindahl**

Stockholm University, Stockholm, Sweden, *Institut Pasteur, Paris, France, **KTH Royal Institute of Technology, Stockholm, Sweden

Allosteric modulation of pentameric ligand-gated ion channels, for example by alcohols and general anesthetics, has critical implications for receptor biophysics and drug development. Functional studies have revealed conserved sites of both positive and negative modulation in this receptor family, but a general mechanistic interpretation of these bimodal effects is lacking. The prokaryotic homolog GLIC functionally recapitulates modulation properties of human ion channels, and is accessible to structure determination in apparent open and closed states. Here we provide evidence from crystallography, electrophysiology, and molecular dynamics simulations showing that general anesthetics can allosterically favor closed channels by binding in a pore site, producing current inhibition (Figure, left); and can favor open channels by binding any of several subregions of a buried transmembrane cavity, producing potentiation (Figure, right). We further found that modifying the buried transmembrane pocket removed or even reversed effects of mutations in the ion channel pore, implicating tight coupling between allosteric and active sites. Our results support an integrated, multiple-site model for allosteric modulation, including specific details of gating rearrangements and domain interfaces influenced by anesthetizing agents.

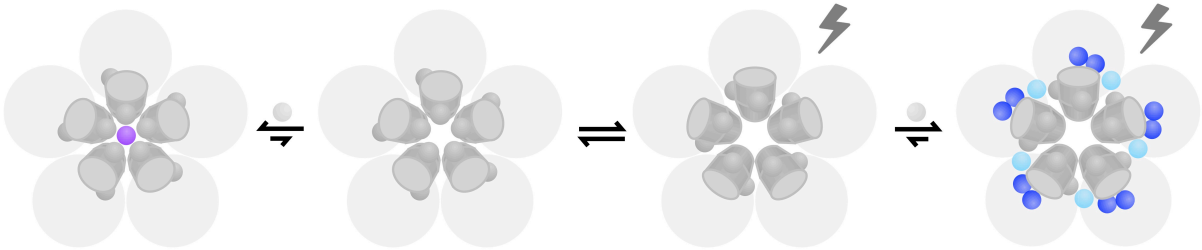


Figure: Simplified multi-site model, under partially activating conditions, for bimodal modulation via the transmembrane domain of pentameric ligand-gated ion channels (white), focusing on the pore-lining M2 helices (gray). *Left,* binding of general anesthetics in the pore site (purple) is favored in the closed state. *Right,* when the pore is capable of conducting current, anesthetic binding is favored in buried transmembrane sites, either within (blue) or between subunits (cyan).

Applying bimolecular fluorescence complementation to screen and purify aquaporin protein:protein complexes.

Florian Schmitz, Petra Båth, Richard Neutze and Kristina Hedfalk

From the Department of Chemistry/Biochemistry, University of Gothenburg, P.O. Box 462, SE-405 30 Göteborg, Sweden

Protein:protein interactions play key functional roles in the molecular machinery of the cell. A major challenge for structural biology is to gain high-resolution structural insight into how membrane protein function is regulated by protein:protein interactions. To this end we present a method to express, detect, and purify stable membrane protein complexes that are suitable for further structural characterization. Our approach utilizes bimolecular fluorescence complementation (BiFC), whereby each protein of an interaction pair is fused to nonfluorescent fragments of yellow fluorescent protein (YFP) that combine and mature as the complex is formed. YFP thus facilitates the visualization of protein:protein interactions in vivo, stabilizes the assembled complex, and provides a fluorescent marker during purification. This technique is validated by observing the formation of stable homotetramers of human aquaporin 0 (AQP0). The method's broader applicability is demonstrated by visualizing the interactions of AQP0 and human aquaporin 1 (AQP1) with the cytoplasmic regulatory protein calmodulin (CaM). The dependence of the AQP0-CaM complex on the AQP0 C-terminus is also demonstrated since the C-terminal truncated construct provides a negative control. This screening approach may therefore facilitate the production and purification of membrane protein:protein complexes for later structural studies by X-ray crystallography or single particle electron microscopy. Combining fluorescence activated cell sorting (FACS) with BiFC is an additional option to screen and include more membrane protein targets for structural insights.

Bacterial sialic acid transport

Rachel North[1,2], Weixiao Wahlgren[1], Elin Dunevall[1], Aviv Paz[3], Paola Bisignano[4], Parveen Goyal[1], Elin Claesson[1], Cesare Indiveri[5], Michael Grabe[4], Renwick Dobson[2], Jeff Abramson[3], S. Ramaswamy[6] and Rosmarie Friemann[1]

[1] University of Gothenburg, Gothenburg, Sweden, [2] University Of Canterbury, Christchurch, New Zealand, [3]University of California, Los Angeles, United States, [4]University of California, San Francisco, United States, [5]University of Calabria, Rende, Italy, [6]Institute for Stem Cell Biology and Regenerative Medicine, Bengaluru, India

Sialic acids comprise a varied group of nine-carbon amino sugars widely distributed among mammals and higher metazoans. Commensal and pathogenic bacteria that colonise heavily sialiated niches (e.g. the mammalian respiratory tract and gut) can scavenge sialic acids from their surrounding environment. Scavenged sialic acid is used as a carbon, nitrogen and energy source, or to evade the host immune response by decorating their outer surfaces in sialic acid.

Bacterial sialic acid membrane protein transport systems have been identified that belong to the tripartite ATP-independent periplasmic transporters, ATP-binding cassette, major facilitator superfamily and sodium solute symporter transport systems.

Here we report the unpublished 1.95 Å resolution crystal structure of a specific sialic acid sodium solute symporter, SiaT, in its outward-open conformation. The structure of SiaT was determined in complex with sodium and sialic acid bound, providing insight into how this transporter mediates the movement of sialic acid across the membrane. The overall structure contains 13 transmembrane helices and adopts a LeuT-like fold where the structural core is formed from two inverted repeats of five transmembrane helices each (Fig. 1). This type of fold is seen in other sodium symporters, including the leucine transporter (LeuT) and the galactose transporter (vSGLT).

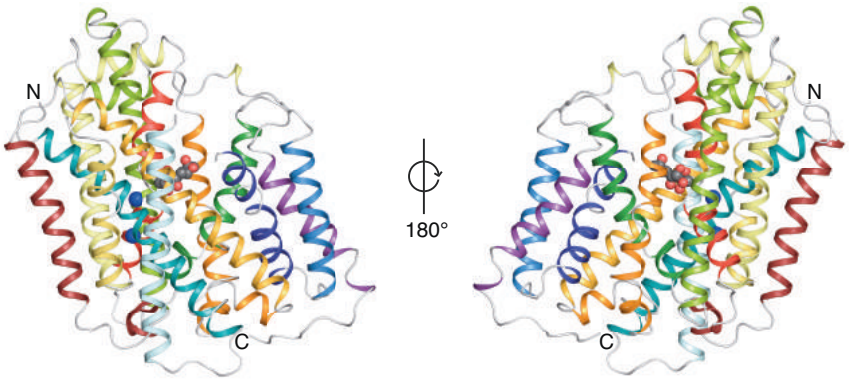


Fig. 1. Overall structure of sialic acid sodium solute symporter (SiaT)

A sodium molecule occupies a binding site equivalent to the Na2-site in other sodium solute symporters. A new and putative Na3-sodium binding site was also found at a previously proposed sodium escape pathway on the intracellular side. Structural and biochemical analyses elucidate essential transport residues, and for the first time a sialic acid transporter has been characterized. In addition, molecular modeling and molecular dynamics simulations provide insight into the transport mechanism employed by SiaT.

Poster list

Odd numbers – Presentation I, Saturday 20:00-21:30
Even numbers – Presentation II, Sunday 20:00-21:30

P 01 **Ribosomal standby sites characterized by single-molecule fluorescence microscopy**
Javier Aguirre Rivera, Uppsala University

P 02 **THz induced anisotropy in bovine trypsin**
Viktor Ahlberg Gagnér, The University of Gothenburg

P 03 **Allosteric regulation of Ubiquitination by the E2 UbcH6**
Alexandra Ahlner, Linköping University

P 04 **Interaction profiling through proteomic peptide phage display**
Muhammad Ali, Uppsala University

P 05 **A structural and functional investigation of ribonucleotide reductase class III in *Bacillus cereus***
Kristoffer K. Andersson, University of Oslo

P 06 **Structural characterization of two HisA mutants from *Salmonella enterica* showing TrpF activity**
Harald Bernhard, Uppsala University

P 07 **Galactomannan catabolism conferred by a polysaccharide utilisation locus of *Bacteroides ovatus***
Viktoria Bågenholm, Lund University

P 08 **Resolving the DNA interaction of the MexR antibiotics resistance regulatory protein**
Francesca Caporaletti, Linköping University

P 09 **Crystallization of Aquaporin 7 for future structure determination**
Sofia de Maré, Lund University

P 10 **Orphans find home: Metabolomics approach for activity and pathway profiling of orphan proteins**
Riccardo Diamanti, Stockholm University

P 11 **In silico design of a recombinant protein (CyDeg) with improved expression and Solubility**
Andrés Camilo Diaz Valencia, Universidad de los Andes / Corporación CorpoGen

P 12 **Structural studies of a metal-bound zinc-transporting P1B-type ATPase**
Annette Duelli, University of Copenhagen

P 13 **Purification and crystallization of Asparaginyl t-RNA synthetase (AsnRS) from *Brugia malayi*, with inhibitors**
Andreas Dunge, University of Gothenburg

P 14 **Taking the fish out of the can: Structural and biophysical characterization of Phoshoglucomutase 5 from Atlantic and Baltic herring**
Ulrich Eckhard, Uppsala University

P 15 **Approaching NMR studies of miR-34a in mammalian cells**
Hannes Feyrer, Karolinska Institutet

P 16 **Neutron crystallographic studies of cancer-related human carbonic anhydrase IX reveal details of water displacement and hydrogen bonding in inhibitor binding.**
Zoë Fisher, European Spallation Source ERIC

P 17 **Bayesian analysis of MicroScale Thermophoresis data to quantify affinity between human survivin and shugoshins**
Maria-Jose Garcia-Bonete, University of Gothenburg

P 18 **Interpreting the hydrolysis/transglycosylation partition in a β-glucosidase using constant pH molecular dynamics**
Inacrist Geronimo, Swedish University of Agricultural Sciences

P 19 **Towards the role of hydroxytriazole derivatives as potent and selective Aldo-Keto Reductase 1C3 inhibitors for CRPC treatment**
Christian Gindro, University of Gothenburg

P 20 **Structural biology of transition metal homeostasis in eukaryotes**
Kamil Górecki, Lund University

P 21 **High-throughput screening of *Mycobacterium tuberculosis* membrane proteins for structural and functional studies**
Kristine Grave, Stockholm University

P 22 **Towards the first crystal structure of a PIB-4-ATPase**
Christina Grønberg, University of Copenhagen

P 23 **Crystal structure of the emerging cancer target MTHFD2 in complex with a substrate-based inhibitor**
Robert Gustafsson, Stockholm University

P 24 **Can alternating fields affect the kinetics of tubulin polymerization?**
Rajiv Harimoorthy, University of Gothenburg

P 25 **Time-resolved X-ray solution scattering of the blue light photoreceptor: Phototropin**
Léocadie Henry, University of Gothenburg

P 26 **How spider silk makes ends meet: Structural insights from crystallography and small angle X-ray scattering (SAXS)**
Wangshu Jiang, Uppsala University

P 27 **Structural studies of Co(II)-transporting PIB-4 ATPases**
Md. Tamim-Al Jubair, University of Copenhagen

P 28 **Structural studies of aminoglycoside nucleotidyltransferases (ANTs)**
Sandesh Kanchugal P, Uppsala university

P 29 **LP3 and DEMAX**
Wolfgang Knecht, Lund University

P 30 **Entropy and water dynamics in enzymatic polycyclization reactions**
Charlotte Kürten, KTH Royal Institute of Technology

P 31 **The paralogous shell proteins CsoS4A and CsoS4B from the α–carboxysome assemble in a heteropentamer**
Anna Larsson, Uppsala University

P 32 **Investigating protein-detergent complexes through Small Angle X-Ray Scattering**
Michael Lerche, Stockholm University

P 33 **Structural studies of bacterial copper flux proteins**
Ping LI, Lund University

P 34 **Design, synthesis and *in vitro* biological evaluation of oligopeptides targeting *E. coli* type I signal peptidase (LepB)**
Lu Lu, Uppsala University

P 35 **Characterisation of the lipid-protein interactions of Fatty acyl CoA synthase from *M. tuberculosis***
Camilla Lundgren, Stockholm University

P 36 **Identification and characterization of a novel botulinum neurotoxin**
Markel Martínez-Carranza, Stockholm University

P 37 **Crystal structures rationalize properties of in vitro evolved ADH-A variants**
Dirk Maurer, Uppsala University

P 38 **Synthetic biology meets synthetic chemistry - *In vivo* activation of an apo-hydrogenase using synthetic complexes**
Livia S. Mészáros, Uppsala University, Department of Chemistry

P 39 **Structural characterization of Aquaporins**
Julie Winkel Missel, University of Copenhagen

P 40 **Coherent diffraction of single Rice dwarf virus particles**
Anna Munke, Uppsala University

P 41 **Current status and near future plans for BioMAX**
Jie Nan, MAX IV Laboratory

P 42 **Structural and functional studies of proteins linked to heavy metal ion transport**
Niloofar Nayeri, Lund University

P 43 **Comparative structure-function analysis of HydF scaffold proteins involved in [FeFe] hydrogenase maturation**
Brigitta Németh, Uppsala University

P 44 **Optimizing the crosslinking of AQP0 and CaM for structural studies**
Veronika Nesverova, Lund University

P 45 **Structure-based drug design for the regulatory protein PrfA to attenuate virulence of *Listeria monocytogenes***
Melanie Oelker, Umeå University

P 46 **Target-based drug discovery approaches on *Pseudomonas aeruginosa* UDP-diacylglucosamine pyrophosphohydrolase LpxH**
Lorenzo Picchianti, Uppsala University

P 47 **NUDT15 Hydrolyzes 6--Thio-DeoxyGTP to mediate the anticancer efficacy of 6--Thioguanine**
Daniel Rehling, Stockholm University

P 48 **Structural and functional insights into the energetic components of type IV secretion system**
Saima Rehman, Umeå University

P 49 **A cation-π interaction is the nucleus for an induced fit conformational transition in Adenylate kinase**
Per Rogne, Umeå University

P 50 **Unique allosteric activity regulation in ribonucleotide reductases**
Inna Rozman Grinberg, Stockholm University

P 51 **Chemical treatment of the C*v*-omega-transaminase for the stabilization of the Lys-PLP covalent bond**
Federica Ruggieri, SARomics Biostructures AB

P 52 **USP14 – A biophysical investigation of small-molecule binding to a deubiquitination enzyme**
Johannes Salomonsson, Linköping University

P 53 **Synthesis of Galectin-3 inhibitors**
Daniel Sarabi, University of Gothenburg

P 54 **Visualizing the inhibitory synapse: Structural studies of the Glycine receptor and gephyrin using electron microscopy**
Andreea Scacioc, KTH Royal Institute of Technology

P 55 **Fragment-screening studies on the ecto-5'-nucleotidase CD73**
Emma Rose Scaletti, University of Leipzig

P 56 **A fine-tuned composition of protein nanofibrils yields an upgraded functionality of displayed antibody binding domains**
Benjamin Schmuck, Swedish University of Agricultural Sciences

P 57 **Shedding light on miRNA targeting through structure**
Luis Silva, Karolinska Institutet

P 58 **Structural and functional characterization of PsaBCA, the manganese transporter in *Streptococcus pneumoniae***
Jennie Sjöhamn, La Trobe University

P 59 **Structural insights into the architecture of human Importin4_histone H3/H4_Asf1a complex and its histone H3 tail binding**
Ji-Joon Song, Korea Advanced Institute of Science and Technology

P 60 **Antimalarial propertied of β-Substituted Fosmidomycin Analogues: Discovery on target!**
Sanjeewani Sooriyaarachchi, Uppsala University

P 61 **Structural studies of epoxide hydrolase mechanism of LTA4H and its dynamic domain motion**
Alena Stsiapanava, Karolinska Institutet

P 62 **Structural studies on the Wag31 antigen from *Mycobacterium tuberculosis***
Adrian Suarez Covarrubias, Uppsala University

P 63 **Production and characterization of microcrystals for Serial Femtosecond Crystallography**
Martin Söderström, Göteborgs Universitet

P 64 **The role of metal co-factors in DNA polymerase ε**
Josy ter Beek, Umeå University

P 65 **Glucose transporters: Production, crystallization and inhibition**
Raminta Venskutonyte, Lund University

P 66 **Towards structural and functional characterization of zinc transporters**
Anders Wiuf, University of Copenhagen

P 67 **Understanding viral entry mechanisms Through Multi-Level Structural Analysis**
Eleonore von Castelmur, Linköpings universitet

P 68 **Identifying the TAILS complex component(s), a microtubule intraluminal interrupted helix found in human spermatozoa**
Davide Zabeo, University of Gothenburg

P 69 **Cryo-EM Imaging of the Packaged Genome in the Native and Swollen Tomato Bushy Stunt Virus Particles**

Daniel Larsson, Uppsala University

Poster Presentations

Ribosome Standby Site Characterized by Single-Molecule Fluorescence Microscopy

Javier Aguirre Rivera, Cedric Romilly, Gerhart Wagner, Magnus Johansson

Department of Cell and Molecular Biology, Uppsala University, Sweden.

Protein synthesis is a fundamental process for every living organism. Moreover, its complexity is affected by several factors. An important one among them is the secondary structure of the mRNA being translated. The mechanism of how ribosomes arrive at translation initiation regions located within highly structured mRNA has been previously studied using biochemical assays. These experiments suggest the existence of a standby unstructured region where the ribosome can bind nonspecifically to wait or even induce the availability of the ribosomal binding site (RBS).

Although the possible existence of standby ribosomes has been examined by traditional biochemical methods, the information obtained is constrained by the limitations of the methods, especially their unidimensionality, making a place for incomplete models. In order to overcome these limitations, single-molecule techniques can be used to observe the process in real-time, allowing the explanation of mechanistic questions in great detail. In this project, we use single-molecule Total Internal Reflection Fluorescence (TIRF) microscopy to observe the binding kinetics of fluorescently labeled 30S ribosomal subunits to surface-immobilized mRNAs with different secondary structure. Preliminary results seem to confirm the existence of ribosome standby sites. Therefore, further single-molecule studies are being carried out to characterize the dynamics of single ribosomes on standby: do they slide from the standby site to the RBS or does the process involve more ribosomes?

THz induced anisotropy in bovine trypsin

Viktor Ahlberg Gagnér, Hassan Bassereh, Ida Lundholm, Maria-José Garcia-Bonete, Vitali Zhaunerchyk[*], Gleb Bourenkov[*], Thomas Schneider[**], Gergely Katona

Department of Chemistry and Molecular Biology, The University of Gothenburg, Gothenburg, Sweden
[*]Departmet of Physics, The University of Gothenburg, Gothenburg, Sweden
[**]*EMBL c/o DESY, Hamburg*

The use of non-ionizing Terahertz (THz) radiation in technology is a new growing field. New medthods utilise THz radiation in for instance security screening at airports, and in medical treatment and diagnostics [1]. In contrast to already established diagnostics methods, the THz radiation can for instance screen patients with higher contrast compared to x-rays [2], and has higher resolution compared to for instance MRI and ultrasound [3]. Albeit non-ionizing, a study have shown that THz radiation might still induce changes biomolecules due to collective oscillations [4]. In addition, a new report from the National Toxicology program states that non-ionizing radio waves from cell phones might have a cancerous effect [5]. Therefore, it is imperative to study this protein interaction, not only for the sake of potential adverse effects biological systems, but also for the new insight this information provide to protein - light interactions.

In this study, the crystal structure of bovine trypsin was obtained via a so called time-resolved x-ray crystallography experiment. Here, the proteins were excited with 0.5 THz radiation, for 25 ms, and subsequently studied with 14 KeV x-rays for 3 ms, in a 50 % duty cycle (no THz radiation at half of the readout). This high resolution data (1.15 Å) show the structural anisotropy in individual atoms, represented by for instance the calculation of individual B-factors, and how the anisotropy might be altered by the THz radiation. This study also provides a good platform for developing statistical tools, to detect and validate structural differences.

References

[1] Wilmink GJ, Grundt JE. Current State of Research on Biological Effects of Terahertz Radiation. *J Infrared Milli Terahz Waves*. 2011;32:1074–1122
[2] Sun Y, Sy MY, Wang YX, Ahuja AT, Zhang YT, Pickwell-Macpherson E. A promising diagnostic method: Terahertz pulsed imaging and spectroscopy. *World J Radiol*. 2011 Mar 28;3(3):55-65.
[3] Hao Yu, Yang Shang. *Design of CMOS Millimeter-Wave and Terahertz Integrated Circuits with Metamaterials*. Boca Raton: CRC Press; 2016
[4] Lundholm, IV et al. Terahertz radiation induces non-thermal structural changes associated with Fröhlich condensation in a protein crystal. *Structural Dynamics*. 2015;2(5),054702
[5] National Toxicology Program. Cell Phones. Available from: https://ntp.niehs.nih.gov/results/areas/cellphones/ [Accessed 2017-03-08]

Allosteric Regulation of Ubiquitination by the E2 UbcH6

Alexandra Ahlner*, Veronika Csizmók*, Vivian Morad*, Madhanagopal Anandapadamanaban**, Francesca Caporaletti*, Björn Wallner* and Maria Sunnerhagen*

*Department of Physics Chemistry and Biology, Linköping University, **MRC Laboratory of Molecular Biology, Cambridge

Ubiquitination is a critical posttranslational regulator of proteostasis. Since dysregulation of proteostasis is diseases-related, understanding the molecular mechanisms underlining ubiquitination may help to design drugs for these diseases. The process of ubiquitination in human involves 2 E1 ubiquitin-activating enzymes, ~40 E2 ubiquitin-conjugating enzymes and ~620 E3 ubiquitin ligases, which jointly modify thousands of substrates. The E2 enzyme is central to this process, since it interacts with E1, E3 and substrates as well.

Interestingly, the structure of E2s do not change significantly upon E1 or E3 binding. However, by Nuclear Magnetic Resonance Spectroscopy (NMR), we can observe small but significant chemical shift perturbations (CSPs) on the E2 UbcH6 upon binding to the RING domain of one of its corresponding E3s, TRIM21. Since the CSPs occur in regions outside the direct interaction interface, and extend from the TRIM21 binding site to the E2 active site, we suggest that the CSPs represent an allosteric activation path[1].

We have recently started to investigate this proposed allosteric path in detail by NMR and mutational analysis. By analysis of the chemical shifts in “soft mutants” of residues lie in the allosteric path using chemical shift projection analysis (CHESPA)[2], we find that some of the mutations appear to mimic various on-path states in the RING ligand binding process. Our hypothesis is that the mutations shift the dynamic equilibrium of the enzyme along this path and we will proceed to investigate this by NMR relaxation and molecular dynamics simulations.

1. Anandapadamanaban M. et al, *Structural basis for bona fide capturing of an autoubiquitination-targeted lysine by the UBE2E1-TRIM21 E2-E3 complex*, Nature Communications, in review.
2. Gagné D. et al, *Network of long-range concerted chemical shift displacements upon ligand binding to human angiogenin.* Protein Sci. 2015 Apr;24(4)

Interaction profiling through proteomic peptide phage display

Muhammad Ali and Ylva Ivarsson
Department of Chemistry, Uppsala University, Sweden

A considerable part of the human proteome is intrinsically disordered. The disordered regions are enriched in short motifs serving as docking sites for peptide binding domains. Domain-motif interactions are crucial for the wiring of signaling pathways. These interactions are typically transient and difficult to capture through most conventional high-throughput methods. We therefore developed a novel approach for the large-scale profiling of domain-motifs interactions called Proteomic Peptide Phage Display (ProP-PD) (1). In ProP-PD we combine bioinformatics, oligonucleotide arrays, peptide phage display and next-generation sequencing. This allows the interrogation of domain-motif interactions on a proteome-wide scale and the *de novo* motif discovery. In our pilot experiment we generated two distinct phage libraries, one displaying all human C-terminal sequences and one displaying C-termini of known virus proteins. We used the ProP-PD libraries to identify interactions of human postsynaptic density 95/discs large/zonula occludens-1 (PDZ) domains. We successfully identified novel PDZ domain interactions of potential relevance to cellular signaling pathways and validated a subset of interactions with a high success rate. In a follow up study we created a ProP-PD library that displays peptides representing the disordered regions of the human proteome (2). We validated our disorderome library against a range of peptide binding domains, which provides novel insights into their binding preferences and suggest interactions of potential biological relevance as will be presented here. ProP-PD can be used to uncover protein-protein interactions of potential biological relevance in high-throughput experiments and provides information that is complementary to other methods. ProP-PD is scalable and can be developed to any target proteome of interest.

In an on-going study we are exploring the motif-mediated interactions of the PDZ domain of SHANK1. In particular, we are comparing its canonical interactions with C-terminal peptide ligands, with its unconventional interactions with internal peptide motifs, located at sites distinct from the C-terminal regions. SHANK1 is an adaptor protein in the postsynaptic density of excitatory synapses. Deregulation of SHANK1 has been linked to autism spectrum disorder. Through this study we uncover novel potentially biologically relevant interaction partners of SHANK1, explore how this ligands are bound through structural approaches, and provide novel insights into the function of this important adaptor protein.

1. Ivarsson, Y., Arnold, R., McLaughlin, M., Nim, S., Joshi, R., Ray, D., Liu, B., Teyra, J., Pawson, T., Moffat, J., Li, S. S., Sidhu, S. S., and Kim, P. M. (2014) Large-scale interaction profiling of PDZ domains through proteomic peptide-phage display using human and viral phage peptidomes. *Proceedings of the National Academy of Sciences of the United States of America* **111**, 2542-2547
2. Davey, N. E., Seo, M. H., Yadav, V. K., Jeon, J., Nim, S., Krystkowiak, I., Blikstad, C., Dong, D., Markova, N., Kim, P. M., and Ivarsson, Y. (2017) Discovery of short linear motif-mediated interactions through phage display of intrinsically disordered regions of the human proteome. *FEBS J* **284**, 485-498

A STRUCTURAL AND FUNCTIONAL INVESTIGATION OF RIBONUCLEOTIDE REDUCTASE CLASS III IN *BACILLUS CEREUS*

Hedda Johannesen[1]; Rohit Kumar[2], Hans-Petter Hersleth[1], Marta Hammerstad[1], Derek T. Logan[2], and K. Kristoffer Andersson[1]

[1]Department of Biosciences, University of Oslo, Pb 1066 Blindern, NO-0316 Oslo, Norway
[2]Department of Biochemistry & Structural Biology, Kemicentrum, Lund University, SE-221 00 Lund, Sweden

Ribonucleotide reductase (RNR) is responsible for the rate-limiting step of DNA synthesis and is therefore a target for anticancer, antibacterial and antiviral agents. The RNRs are divided into three classes based on their radical chemistry. The focus of this project is the strictly anaerobic Class III RNR from the pathogenic bacterium *Bacillus cereus.* It consists of a catalytic unit (NrdD) with a glycyl radical, and a radical initiator unit (NrdG) containing an iron-sulphur cluster. By combining structural and spectroscopic studies, in addition to binding and kinetic studies we hope to solve the X-ray structure of NrdG and understand the activation and re-activation of NrdD by NrdG. This knowledge should give new insight into class III RNR activation, and confirm or contribute to a reviewing of the current reaction mechanism. We are currently establishing purification procedures for NrdD and NrdG followed by crystallisation trial, that will partly be executed at Lund University.

Structural characterization of two HisA mutants from *Salmonella enterica* showing TrpF activity

Harald Bernhard[1], Ulrich Eckhard[1], Annika Söderholm[1], Sandesh Kanchugal P[1], Erik Lundin[2], Dan I Andersson[2] & Maria Selmer[1]

[1]Department of Cell and Molecular Biology, Uppsala University, Uppsala, Sweden
[2]Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden

HisA is a $(\beta\alpha)_8$ barrel enzyme from *Salmonella enterica (S. enterica)* of the histidine biosynthesis pathway, which catalyses a reaction called Amadori rearrangement, on the substrate ProFAR. The homologous protein TrpF catalyses an equivalent reaction on the related substrate PRA in the tryptophan biosynthesis pathway. These two proteins cannot replace each other, but in some members of gram-positive *Corynebacterium* genus a protein called PriA, a bifunctional HisA-like protein, catalyses both reactions. All this renders HisA a prime model to study the evolution of new enzymatic activities and the functional promiscuity of proteins.

The goal of this project is to get a better understanding how proteins acquire new functions. Therefore we solved crystal structures of two different mutants of HisA with a resolution of 1.5 Å. The first one, HisA A94S, has both HisA and TrpF activity, while the second mutant, HisA D129P, has lost HisA activity, but gained TrpF activity.

In wild type HisA D129, located at the catalytic face of the enzyme, is primarily responsible for positioning the non-reacting ribose of the substrate, which plays a role in transition from an open structure into a more rigid closed conformation. The absence of this residue in the D129P mutant explains why the mutant lacks HisA activity. We speculate that the D129P mutation also rigidifies the local structure and consequently abolishes the transition into the closed structure.

The HisA A94S mutation is located at the “back side” of the enzyme and we could identify an additional hydrogen bond between the sidechain oxygen of Ser94 and the backbone oxygen of Leu91, which is located at the end of an α-helix and seems to act as a capping residue. This could probably stabilize the α-helix and consequently rigidify the overall protein structure.

The open and closing mechanism of the structure could be virtually described as a breathing of the HisA protein. We speculate that the flexibility of HisA is preventing the TrpF substrate to bind the enzyme and thus Introducing rigidity could be a common mechanism of how TrpF activity is gained in the two mutants. We could probably find out more about this dynamic interplay by comparing the different structures with Molecular Dynamic or NMR experiments. This should shed some more light on the dynamics of the overall structure and the effect of individual point mutations.

Galactomannan catabolism conferred by a polysaccharide utilisation locus of *Bacteroides ovatus*

Viktoria Bågenholm[*], Sumitha K. Reddy[*], Hanene Bouraoui[*], Johan Morrill[*], Evelina Kulcinskaja[*,**], Constance M. Bahr[***], Oskar Aurelius[*,****], Theresa Rogers[***], Yao Xiao[***], Derek T. Logan[*], Eric C. Martens[***], Nicole M. Koropatkin[***] and Henrik Stålbrand[*]

[*]Department of Biochemistry and Structural Biology, Lund University P.O. Box 124, S-221 00, Lund, Sweden, [**]Present address: School of Chemistry, University of Nottingham, University Park, Nottingham, NG7 2RD, United Kingdom, [***]Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, Michigan 48109, USA, [****]Present address: Diamond Light Source, Harwell Science and Innovation Campus, Didcot OX11 0DE, United Kingdom

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 polysaccharides (1) and food thickeners (2, 3), and can be utilised by several different human gut bacteria (4). One of these bacteria, *Bacteroides ovatus*, contains a gene cluster, or polysaccharide utilisation locus (PUL) (5), encoding two glycoside hydrolase family 26 (GH26) β-mannanases, *Bo*Man26A and *Bo*Man26B, and a GH36 α-galactosidase, *Bo*Gal36A. We have characterised these enzymes, including a crystal structure of *Bo*Man26A, and shown that this PUL is essential for *B. ovatus* galactomannan utilisation. A model of the combined function of this galactomannan PUL (6) is presented: *Bo*Man26B is endo-acting, outer membrane associated and not restricted by galactose substitutions. It initially acts on galactomannan, producing comparably large oligosaccharide fragments. These are degalactoylated by the preiplasmic *Bo*Gal36A. *Bo*Man26A, which hydrolyses the subsequent fragments, is sensitive to galactose and primarily forms mannobiose. A crystal structure of *Bo*Man26A revealed a similar structure to the exo-mannobiohydrolase *Cj*Man26C from *Cellvibrio japonicus* (7), with a conserved loop closing one end of the active site cleft. In *Bo*Man26A this loop is likely subject to loop breathing or similar flexibility, since it would otherwise clash with longer substrates.

References

1. Scheller, H. V. and P. Ulvskov Annu Rev Plant Biol (2010). **61**: 263-289.
2. Barak, S. and D. Mudgil Int J Biol Macromol (2014). **66**: 74-80.
3. Mudgil, D., S. Barak and B. S. Khatkar J Food Sci Technol (2014). **51**(3): 409-418.
4. Kulcinskaja, E., A. Rosengren, R. Ibrahim, K. Kolenova and H. Stalbrand Appl Environ Microbiol (2013). **79**(1): 133-140.
5. Martens, E. C., E. C. Lowe, H. Chiang, N. A. Pudlo, M. Wu, N. P. McNulty, D. W. Abbott, B. Henrissat, H. J. Gilbert, D. N. Bolam and J. I. Gordon PLoS Biol (2011). **9**(12): e1001221.
6. Bagenholm, V., S. K. Reddy, H. Bouraoui, J. Morrill, E. Kulcinskaja, C. M. Bahr, O. Aurelius, T. Rogers, Y. Xiao, D. T. Logan, E. C. Martens, N. M. Koropatkin and H. Stalbrand J Biol Chem (2017). **292**(1): 229-243.
7. Cartmell, A., E. Topakas, V. M. A. Ducros, M. D. L. Suits, G. J. Davies and H. J. Gilbert Journal of Biological Chemistry (2008). **283**(49): 34403-34413.

Resolving the DNA interaction of the MexR antibiotics resistance regulatory protein

Francesca Caporaletti, Frank Gabel*, Anne Martel*, Maria Sunnerhagen**

Linköping Universitet, * Institut Laue-Langevin, ** Linköping Universitet

Acquired multidrug resistance (MDR) in pathogenic microbials is a world-wide threat to human health. Several MarR–like proteins regulate the expression of efflux pumps, which are multiprotein self-assembly complexes actively extruding chemical compounds with high toxicity to the host organism. Since incapacitating the repressor protein leads to continuously high production of the efflux proteins and thus increased survival for the bacteria, there is a high mutational pressure for acquired MDR. MexR is a key member of the MarR family, and is also mutated in MDR. Analyzing the structure–function relationships of MarR family proteins, both in their native and mutated forms, is fundamental to learn how to overcome innate and acquired MDR in future drug development.
Previously, the Sunnerhagen group has shown how MDR mutations of MexR retain their original fold but loose DNA binding due to conformational selection of a non-binding state (Andrésen C. et al. (2010), Anandapadamanaban M. et al. (2016)). To prove this hypothesis and to advance structural knowledge on the biologically active repressed state, we need to experimentally assay the DNA-bound conformation of MexR. Unfortunately however, there is yet no crystal structure of the DNA bound complex of MexR.

To resolve this, we are currently preparing to study this complex by neutron scattering by recording SANS small and large angle data. In particular, we will establish the respective inter-component distances and topology, exploiting the intrinsic DNA-protein scattering contrast by adjusting the D_2O content in an H_2O buffer to the protein (42%) or DNA (65% D_2O) matching points. A complex model at atomic level consistent with the scattering data will be refined by molecular dynamics simulations. We are currently preparing samples for a measuring time in early fall.

Crystallization of Aquaporin 7 for future structure determination

Sofia de Maré, Christian Krintel, Tatu J. K. Haataja and Karin Lindkvist-Petersson

Department of Experimental Medical Science, Lund University, BMC, 221 84 Lund, Sweden

Aquaglyceroporins are integral membrane proteins known to facilitate transport of glycerol. The aquaglyceroporin AQP7 is expressed in fat cells (adipocytes) where it regulates glycerol efflux as it translocates to the plasma membrane during lipolysis as a result of catecholamine stimulation. Deletion of AQP7 in mice leads to development of obesity and adipocyte hypertrophy, suggesting an important role in metabolism. We have discovered that AQP7 interacts with Perilipin 1 (PLIN1), an abundant protein in adipocytes that coats the lipid droplet, and that phosphorylation by protein kinase A of the cytosolic N-terminus of AQP7 diminishes the interaction, which allows AQP7 to move to the plasma membrane. To study the structural details of AQP7, we have produced large amounts of human AQP7 using the yeast *Picha pastoris*. Purification has been optimized through detergent screening resulting in highly pure and stable protein in various detergents. Diffracting crystals can be grown in several of these detergents and the current work is focusing on crystal optimization. By using different optimization strategies, such as additive and detergent screening, the diffraction has improved significantly.

Orphans find home: metabolomics approach for actvity and pathway profiling of orphan proteins

Riccardo Diamanti and Martin Högbom

Department of biochemisrty and biophysics, Stockholm University

Despite the advances in functional genomics has revolutionized the approach of researchers to protein characterization, it is clear that this task cannot be achieved solely by the comparison of genome sequences. Traditional enzymology, in contrast, requires purification and dilution of proteins which disrupts interaction possible partners and do not reproduce the reality in the cell milieu.

Several -omics approaches have been tried in the last decades to develop high-throughput functional studies with mixed results and metabolomics has been successfully used in plant research to identify protein substrates and pathways by comparing wild- type organisms with silenced mutants. In this work we explore the potential of metabolomics for protein characterization by looking at the change in the metabolome due to protein overexpression.

The promising prospective of metabolomics is combined with x-ray crystallography studies of the three membrane proteins used in the pool of metabolomics targets.

In Silico Design of a Recombinant Protein (CyDeg) with Improved Expression and Solubility.

AC. Diaz Valencia[*Δ+]; DA. Benitez Duarte*; D. Calderón Manrique*; A. Mongui Cruz*; M.L. Cepeda Hernández*; P. Del Portillo Obando*; W. Ocampo Moure*.

[Δ]Universidad de los Andes; [+]Corresponding Author: ac.diazv@uniandes.edu.co; *CorpoGen, Molecular Biotechnology Research Group.

Protein design is a very useful technique to create novel proteins to enhance or modify their functional properties. Two methods, computational and *in vitro* mutations of the specific gene are currently the pipeline used for these purposes.

In this study we report the bioinformatic design of a recombinant enzyme based on the human CYP2e1 protein, with the aim to increase its substrate affinity, expression and solubility.

Point mutations of the CyDeg protein sequence were carried out by evolutionary algorithms in order to increase its affinity. The evolutionary algorithm was also used to increase its solubility and expression levels which were tested with ESPRESSO (goo.gl/DBXzWA), ProtParam (goo.gl/Qy1rES) and SCRATCH (goo.gl/by0WtA). Codon usage for expression in *E. coli* was optimized via OPTIMIZER (goo.gl/pNLiLn) and secondary structures in the mRNA were analyzed with mfold (goo.gl/2nEzFM). Translational pause sites in the mRNA were analysed with RiboTempo software. Uncharged polar aminoacids were used to reduce the area of non-polar groups in the protein's surface. Models of the modified protein were obtained using I-tasser (goo.gl/FBSN1h), Phyre2 (goo.gl/szjCZn), 3D-Jiggsaw (goo.gl/m5HTJQ) and were superimposed with SuperPose (goo.gl/iNDG3n). The design of the novel protein predicted an increased probability of expression and solubility of 0,944 and 0,644 respectively compared to the originals. The modified protein structure showed a structural difference of 1,54 Å compared to the wild type.

Both proteins, wild type and modified were synthetized and cloned in a suitable expression vector. L-arabinose was used to induce protein expression. Protein in the soluble and insoluble fractions, for both the mutant and WT variants, was determined with anti-HisTag antibodies in Western blots.

Experimental results showed that the CyDeg protein solubility increased by 1,5X compared with the wild type and its expression increased by 1,6X. The fold increase was calculated according to band densitometry curves.

Structural studies of a metal-bound zinc-transporting P_{1B}-type ATPase

Annette Duelli[1], Elena Longhin[1], and Pontus Gourdon[1,2]

[1] Department of Biomedical Sciences, University of Copenhagen, Blegdamsvej 3, 2200 Copenhagen N, Denmark
[2] Department of Experimental Medical Science, Lund University, Sölvegatan 19, SE – 221 84 Lund, Sweden

P_{1B}-type ATPases play a central role in the regulation of the transition metal homeostasis in cells and are directly involved in the resistance against chemotherapeutic drugs. In addition, genetic variants of the human P_{1B}-type ATPases ATP7A and ATP7B are the cause for the severe Menke's and Wilson's disease resulting from copper deficiency and copper overload respectively. In spite of their biological significance the transport mechanism of P_{1B}-type ATPases and how metal specificity is achieved is still not well understood, since structural information in a metal-bound E1-state of any P_{1B}-type ATPase is lacking. Furthermore, the role of the class-specific so-called heavy metal binding domain in metal transport is debated.

By investigating the role of the heavy metal binding domain through small angle x-ray scattering, we will shed light on the intra- and inter-molecular dynamics during various reaction cycle intermediates. Moreover, we will identify conditions promoting metal-bound E1 conformations of the zinc transporting P_{1B}-type ATPase ZntA, with the aim to determine the first crystal structure of a metal-bound state and decipher the complete transport cycle of the P_{1B}-type ATPase subclass.

Purification and crystallization of Asparaginyl t-RNA synthetase (AsnRS) from *Brugia malayi*, with inhibitors

Andreas Dunge, Elin Dunevall, Morten Grøtli and Gisela Brändén

Department of Chemistry and Molecular Biology, University of Gothenburg

Brugia malayi (*B. malayi)* is one of three mosquito-borne nematodes that cause the tropical disease lymphatic filariasis which can develop into elephantiasis[1]. Asparaginyl t-RNA synthetase (AsnRS) has been recognized as a potential drug target in *B. malayi* since it is essential for protein production[2].

AsnRS, one of several aminoacyl t-RNA synthetase, recycles Asn-t-RNA in the cell[3]. The reaction is initiated by AsnRS catalyzing the activation of amino acid asparaginyl by adenylylation using ATP[3]. The AsnRS-Asn-AMP complex binds an empty Asn-t-RNA and fuses the amino acid and t-RNA which then releases an active Asn-t-RNA[4].

Potential inhibitors that lower the activity of AsnRS have been identified[4]. The aim of this project is to co-crystalize recombinant AsnRS from *B. malayi* with these inhibitors as well as newly discovered compounds and solve the structures using x-ray crystallography. Collected data could lead to additional modifications of the inhibitors to improve their potency and, long-term, a potential new anthelmintic drug.

Expression and purification of AsnRS using immobilized metal ion affinity chromatography and size exclusion chromatography is currently ongoing. An activity assay will be used to confirm that the protein is correctly folded and determine the potency of the inhibitors.

1. Organization, W. H. Investing to Overcome the Global Impact of Neglected Tropical Diseases: Third WHO Report on Neglected Tropical Diseases 2015. (World Health Organization, 2015).
2. Crepin, T. et al. A hybrid structural model of the complete Brugia malayi cytoplasmic asparaginyl-tRNA synthetase. J. Mol. Biol. **405,** 1056–1069 (2011).
3. Danel, F. et al. Asparaginyl-tRNA synthetase pre-transfer editing assay. Curr. Drug Discov. Technol. **8,** 66–75 (2011).
4. Sukuru, S. C. K. et al. Discovering New Classes of Brugia malayi Asparaginyl-tRNA Synthetase Inhibitors and Relating Specificity to Conformational Change. J. Comput. Aided Mol. Des. **20,** 159–178 (2006).

Taking the fish out of the can: Structural and biophysical characterization of Phoshoglucomutase 5 from Atlantic and Baltic herring

Ulrich Eckhard, Weihua Ye*, Sandesh Kanchugal, Leif Andersson*, Per Jemth*, and Maria Selmer

Department of Cell and Molecular Biology, Uppsala University, Sweden. * Department of Medical Biochemistry and Microbiology, Uppsala University.

Herring is emerging as a new vertebrate model to study the basis for genetic adaption due to its huge population sizes, eradicating the effects of random genetic drift. Furthermore, herring is one of few marine fish that reproduce throughout the brackish Baltic Sea where the salinity drops to 2–3‰ compared to 35‰ in the Atlantic Ocean, a rather recent ecological adaptation as the Baltic marine life is ≤10,000 years old. Adaptive mutations leading to amino acid substitutions in a number of proteins were identified in a previous whole genome population sequencing study [1], and we now aim to clarify the molecular details on the protein level.

One of the prime protein candidates for adaptation to the Baltic Sea is the cytosolic protein Phosphoglucomutase 5 (PGM5) [1], a 65 kDa enzyme belonging to a family of magnesium-dependent enzymes that catalyses the reversible transfer of a phosphate group between the 1'- and 6'-positions of a-D-glucose. The phosphoglucomutase family have a central role in cellular glucose homeostasis by mediating the switch between glycolysis and gluconeogenesis. Moreover, glucose-1-phosphate is an important intermediate also used for protein N-glycosylation. Nevertheless, the specific function of PGM5 is ill-defined, and its role in adaptation to different environmental conditions is elusive.

Here we report the first crystal structures of Atlantic and Baltic Phosphoglucomutase 5 at 2.25 and 2.0 Å resolution, solved by molecular replacement. Together with the complex structure with the reaction intermediate glucose-1,6-bisphosphate, we will present a first biochemical and biophysical characterization, and give first insights into the similarities and differences between the two proteins.

1 Barrio, A. M., Lamichhaney, S., Fan, G., Rafati, N., Pettersson, M., Zhang, H., Dainat, J., Ekman, D., Höppner, M., Jern, P., et al. (2016) The genetic basis for ecological adaptation of the Atlantic herring revealed by genome sequencing. eLife **5**, e12081.

Approaching NMR studies of miR-34a in mammalian cells

Hannes Feyrer, Ileana Guzzetti, Judith Schlagnitweit,

Emma Andersson* and Katja Petzold

Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, SE-171 77, * Department of Cell and Molecular Biology, Karolinska Institutet, Stockholm, SE-171 77

Micro-RNAs (miRNAs) are short non-coding RNAs (~22nt) that participate in the regulation of up to 60% of the human proteome. They perform their function by binding to a partially complementary mRNA sequence and recruiting the protein Argonaute, which then leads to degradation or stalling of translation [1]. The protagonist of this study is miR-34a, which is involved in many cellular processes including cell cycle control, differentiation or apoptosis [2]. Our group employs NMR spectroscopy to study the dynamics of the interaction between the miRNA and its target mRNA. However, it has been shown that the structure proteins adopt *in vitro* might differ from the one *in vivo* [3]. In-cell NMR provides insights into structure under conditions that represent the physiological environment, taking into account salt concentration, metabolite interaction and molecular crowding [4]. For our work, we use the human cell line HEK293T that should more closely mimic a biologically relevant system than bacterial or amphibian Oocytes, which are popular model systems for in-cell NMR. In contrast to proteins, RNAs have only rarely been studied using in-cell NMR [5] and protocols for transfection and measurement are not as well established. We show preliminary data including optimization of miRNA uptake, cell viability under NMR conditions and control experiments.

[1] M. Ostankovitch and A. M. Pyle, “Noncoding RNAs: A story of networks and long-distance relationships,” *J. Mol. Biol.*, vol. 425, no. 19, pp. 3577–3581, 2013.

[2] M. Rokavec, H. Li, L. Jiang, and H. Hermeking, “The p 53 / miR- 34 axis in development and disease,” *J. Mol. Cell Biol.*, pp. 214–230, 2014.

[3] F.-X. Theillet *et al.*, “Structural disorder of monomeric α-synuclein persists in mammalian cells.,” *Nature*, vol. 530, no. 7588, pp. 45–50, 2016.

[4] T. Ikeya *et al.*, “Improved in-cell structure determination of proteins at near-physiological concentration,” *Sci. Rep.*, vol. 6, no. November, p. 38312, 2016.

[5] R. Hänsel *et al.*, “Evaluation of parameters critical for observing nucleic acids inside living Xenopus laevis oocytes by in-cell NMR spectroscopy,” *J. Am. Chem. Soc.*, 2009.

Neutron crystallographic studies of cancer-related human carbonic anhydrase IX reveal details of water displacement and hydrogen bonding in inhibitor binding.

Zoë Fisher[1,2], Katarina Koruza[2,] Brian Mahon[3], Cynthia Okoh[3], Wolfgang Knecht[2], Robert McKenna[3]

[1] Scientific Activities, Division, European Spallation Source ERIC, 22100 Lund, Sweden[2]Molecular Cell Biology Unit, Biology Department, Lund University, 22362 Lund, Sweden[3]Biochemistry and Molecular Biology Department, University of Florida, 32610 Gainesville FL, USA

Human carbonic anhydrase IX (HCA IX) expression in aggressive tumors, under hypoxic conditions, is an indicator of metastasis and poor cancer patient prognosis. As such, HCA IX has emerged as an important cancer target, but efforts to develop specific inhibitors for HCA IX are complicated by the presence of 14 other HCA isoforms that share a common structural fold and high sequence similarity. It has been well established that ligand binding to a target protein is mediated through numerous interactions that may include: H-bonding directly and/or through intervening waters, electrostatic interactions with charged or polar amino acid side chains, metal coordination, energetic changes through water displacement, aromatic stacking, or other hydrophobic interactions. Using X-ray crystallography for structure based drug design is a common and powerful approach, but does not always give complete insight into binding mechanisms. This is because the magnitude of X-ray scattering from an atom depends on the atomic number (Z) for that element, i.e. light atoms in proteins contribute very little to X-ray scattering data. This makes the observation of hydrogen atoms almost impossible. Neutrons offer a complementary approach in that they are scattered from atomic nuclei from different elements to a similar extent. In practice this means that the nuclear density maps for C, N, D (the isotope of H), and O atoms all appear to a similar extent, even at medium (~2 Å) resolution. In this way neutron crystallography offers a powerful approach to observe the details of ligand binding that involves H atoms. Our goal is to determine the atomic details of a promising drug lead (saccharin) and a saccharin-sugar conjugated compound (#674), by neutron protein crystallography. Comparing neutron crystal structures of unbound and inhibitor-bound HCA IX provides a unique opportunity to directly investigate how saccharin binds through H-bonding, the role of water displacement, and how the making/breaking of H-bonds modulates binding and isoform specificity. We are now extending our studies to complexes with the larger inhibitor and it is expected that fine structural detail, unique to neutron crystallography, can be applied for rational drug design.

Bayesian analysis of MicroScale Thermophoresis data to quantify affinity between human survivin and shugoshins.

Maria-Jose Garcia-Bonete[1], Maja Jensen[1], Christian V. Recktenwald[2], Volker Stadler[3], Maria Bokarewa[4] and Gergely Katona[1]

1. Dept. Chemistry and Molecular Biology - University of Gothenburg, Sweden. 2. Dept. Medical Biochemistry - University of Gothenburg, Sweden. 3. GmbH, Rischerstrasse 12, 69123 Heidelberg, Germany. 4. Dept. Rheumatology and Inflammation Research - The Sahlgrenska Academy at University of Gothenburg, Sweden.

Survivin is a small human protein of the Inhibitor Apoptosis Protein (IAP) family. It is involved in cell division and apoptosis inhibition[1,5,7]. In cancer, it is related with chemotherapy resistance, recurrence and bad outcome and in patients with rheumatoid arthritis, high levels of this protein are present in extracellular plasma and synovial fluid[1,2,5].

Shugoshin proteins protect the cohesion protein complex to prevent premature loss of centromeric cohesion, missegregation and mitotic arrected[6]. Their function in the spindle associated complex and kinetochores protection are regulated by the Chromosomal Passenger Complex (CPC) which consists of Survivin, Borealin, Incept and Aurora B kinase[4,6] .

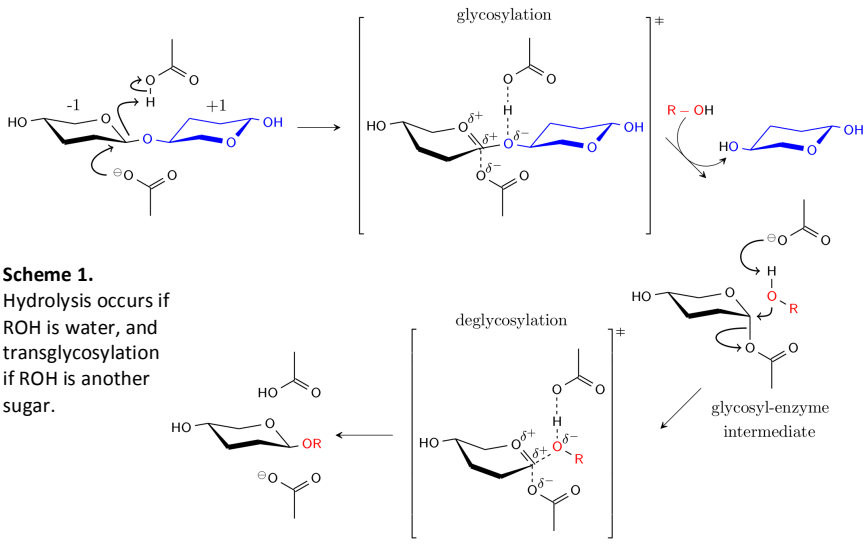
Interaction between Survivin and hSgo1 and hSgo2 have been identified using protein microarray binding assay and validated using fluorescence size exclusion chromatography and MicroScale Thermophoresis. Bayesian inference has been used to analyse thermophoresis binding[3]. This approach can be very useful to autonomously analyse binding curves without manual intervention and without introducing bias by subjective outlier rejection.

1. Altieri D. C. (2003) Survivin, versatile modulation of cell division and apoptosis in cancer. *Oncogene*, 22:8581–8589.
2. Baran M. *et al* (2009) Survivin is an essential mediator of arthritits interacting with urokinase signalling. *Journal of Cellular and Molecular Medicine*, 13:3797-3808.
3. Hines K. E. *et al* (2014) Determination of parameter identifiability in nonlinear biophysical models: A Bayesian approach. *J Gen Physiol* 143, 401-416.
4. Jeyaprakash A. *et al* (2007) Structure of a Survivin-Borealin-INCENP core complex reveals how chromosomal passengers travel together. *Cell* 131, 271-285.
5. Mita A. C. *et al* (2008) Survivin: key regulator of mitosis and apoptosis and novel target for cancer therapeutics. *Clinical Cancer Research* 15:5000-5005.
6.Tsukahara T. *et al* (2010) Phosphorylation of the CPC by Cdk1 promotes chromosome bi-orientation. *Nature* 467, 719-723.
7. Verdecia M. A. *et al* (2000) Structure of the human anti-apoptotic protein survivin reveals a dimeric arrangement. *Nature Structural Biology,* 7:602-608.

Interpreting the hydrolysis/transglycosylation partition in a β-glucosidase using constant pH molecular dynamics

Inacrist Geronimo*, Christina M. Payne** and Mats Sandgren*

*Swedish University of Agricultural Sciences, **University of Kentucky



Scheme 1. Hydrolysis occurs if ROH is water, and transglycosylation if ROH is another sugar.

β-glucosidases play an important role in cellulose degradation by relieving endoglucanase and cellobiohydrolase product inhibition through hydrolysis of cellobiose to glucose. Among those used in commercial enzyme cocktails is Cel3A from *Hypocrea jecorina* (*Hj*Cel3A), which was found to enhance the conversion of various cellulosic substrates by nearly 10%.[1] However, the catalytic activity of *Hj*Cel3A and other β-glucosidases is significantly reduced at high cellobiose or glucose concentration due to transglycosylation, a competing pathway to hydrolysis, wherein another sugar, instead of water, is transferred to the glycosyl-enzyme intermediate (Scheme 1). Previous studies, such as that by Turunen et al.,[2] suggest that the partition between the two reactions depends on the pK_a of the acid/base residue and/or nucleophile. The impact of the pK_a of ionizable residues on the pH-activity profile and hydrolysis/transglycosylation partition of *Hj*Cel3A is investigated using constant pH molecular dynamics. In this method, ionizable residues are switched between the protonated and deprotonated states through the introduction of a titration coordinate, λ, which continuously changes between 0 (protonated) and 1 (deprotonated).[3] pK_a changes during the catalytic reaction are monitored and factors that modulate this property, including hydrogen bonding, charge coupling with other residues, and solvent accessibility, are identified. The findings of this study could serve as guide to protein engineering of β-glucosidase variants with improved hydrolytic activity.

References

(1) Karkehabadi, S.; Helmich, K. E.; Kaper, T.; Hansson, H.; Mikkelsen, N.-E.; Gudmundsson, M.; Piens, K.; Fujdala, M.; Banerjee, G.; Scott-Craig, J. S.; Walton, J. D.; Phillips, G. N.; Sandgren, M. *J. Biol. Chem.* **2014**, *289*, 31624-31637.

(2) Anbarasan, S.; Timoharju, T.; Barthomeuf, J.; Pastinen, O.; Rouvinen, J.; Leisola, M.; Turunen, O. *J. Mol. Catal. B: Enzym.* **2015**, *118*, 62-69.

(3) Wallace, J. A.; Shen, J. K. *J. Chem. Theory Comput.* **2011**, *7*, 2617-2629.

Towards the role of hydroxytriazole derivatives as potent and selective Aldo-Keto Reductase 1C3 inhibitors for CRPC treatment

Christian Gindro[1,2], Donatella Boschi[2], Weixiao Yuan Wahlgren[1], Agnese Chiara Pippione[2], Marco Lolli[2], Rosmarie Friemann[1], and Klaus Pors[3], Salam Al-Karadaghi[4]

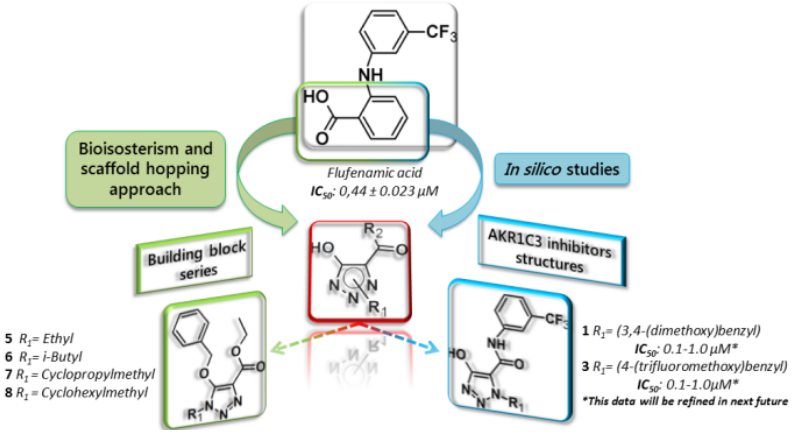
[1]Department of Chemisty and Molecular Biology, University of Gothenburg, Gothenburg, Sweden; [2]Department of Science and Drug Technology, University of Torino, Turin, Italy; [3]Institute of Cancer Therapeutics, University of Bradford, Bradford, UK; [3]Department of Biochemistry and Structural Biology, Lund University, Sweden.

Prostate cancer (PC) represents the most widespread cancer in men in the western countries. In some instances, normal therapy is yet followed by a relapse into a more aggressive prostate cancer, referred to as Castrate Resistant Prostate Cancer (CRPC). This form of the disease goes along with an increase of the male sexual hormones level, due to a greater androgen biosynthesis in the neoplastic tissue and to an abnormal activation of the androgen receptor (AR) signaling pathway, which is brought about by the amplification and the aberrant mutation of AR gene. Nowadays, several drugs are being used in pharmacological therapies, but all of them lead to drug resistance. A new interesting drug target is the enzyme Aldo-Keto Reductase 1C3 isoform (AKR1C3). AKR1C3 is overexpressed in CRPC and it manages to catalyze a weak androgen in a potent androgen conversion by a NADPH-dependent reduction. Consequently, AKR1C3 inhibition leads to a dramatic decrease in the synthesis of the main male hormones in CRPC disease cases.

The NSAIDs indomethacin (IC_{50}= 0.90 µM) and flufenamic acid (IC_{50}= 0.44 µM) have recently been characterized as potent inhibitors of AKR1C3. In order to improve potency and selectivity towards AKR1C3, yet avoiding COX-1 and COX-2 inhibition, we have modulated flufenamic acid structure by replacing the key role benzoic acid moiety with a 5-hydroxy-1,2,3-triazole carbonyl core, through an innovative bioisosteric and scaffold hopping approach.

We aim to design new AKR1C3 inhibitors based on an N-substituted hydroxytriazole core.

Different modulated hydroxytriazole compounds have been synthesized: N-substituted building blocks 5 to 8 and AKR1C3 inhibitors 1-3. Finally, these last derivatives have been assayed *in vitro*, for their inhibiting activity on AKR1C3 affording to interesting results: both of them have demonstrated a preliminary IC_{50} value of 0.1-1.0 µM. We are currently working on the structure determination of AKR1C3 co-crystallized with the above described compunds for further rounds of structure based drug design.



Structural biology of transition metal homeostasis in eukaryotes

Kamil Górecki, Ping Li, Niloofar Nayeri, Karin Lindkvist and Pontus Gourdon*

Department of Experimental Medical Science, Lund University, Sölvegatan 19, SE - 221 84 Lund, Sweden,
*Department of Biomedical Sciences, University of Copenhagen, Blegdamsvej 3, 2200 Copenhagen N, Denmark

Transition metals are essential as cofactors in every living cell. Examples include copper with redox properties suitable for assisting catalysing numerous reactions, and zinc which binds to about 15 % of the human proteome. However, transition metals are also toxic at elevated concentrations; copper generates reactive oxygen species, detrimental to proteins, nucleic acids and lipids. Therefore, the intracellular levels of these metals are strictly controlled, and a range of membrane proteins that facilitate import and export plays a crucial role in this homeostasis.

Structures of many metal transporters remain largely unknown. This project is focused on elucidating molecular details of such membrane proteins exploiting X-ray crystallography. Several targets have been successfully overexpressed in *Saccharomyces cerevisiae* and *Pichia pastoris*. The proteins were fused to green fluorescent protein in order to facilitate purification and quantification. Samples are extracted from the cell membranes and purified using affinity and size-exclusion chromatography displaying promising monodispersity and stability, and the GFP tag cleaved off. Crystallization experiments are currently on-going employing different detergent and lipid-based approaches, and initial crystals are being tested for their diffraction properties.

High-throughput screening of *Mycobacterium tuberculosis* membrane proteins for structural and functional studies

K. Grave, M. D. Bennett, M. Högbom

Department of Biochemistry and Biophysics, Stockholm University, Stockholm, Sweden

Despite a long history of study, tuberculosis remains a prevalent disease in many parts of the world. *Mycobacterium tuberculosis* (*Mt*) is developing resistance to most available anti-tuberculosis drugs. Globally the number of annual multidrug resistant *Mt* cases is rising, urging the scientific community to look for new alternatives to fight the disease. Membrane proteins are prominent drug targets, however production of these proteins in large amounts for structural analysis, an essential component in modern drug development, is a bottleneck.

In order to identify promising protein candidates for structural analysis, we have applied high-throughput cloning and detergent screening techniques on a large library of essential *Mt* transmembrane proteins fused with C-terminal folding reporter GFP. The GFP reporter is primarily used to simultaneously estimate protein expression levels in different bacterial systems (*E. coli*, *Mycobacterium smegmatis* and *Lactococcus lactis*), to analyse protein stability in different types of detergents and compare solubilisation efficiencies using fluorescence size-exclusion chromatography (FSEC).

This primary analysis allowed a fast selection of a substantial number of protein targets and has already led to the determination of a high-resolution crystal structure of one protein target involved in a central lipid biosynthesis pathway.

Towards the first crystal structure of a P_{IB-4}-ATPase

Christina Grønberg[1], Qiaoxia Hu[1], Tamim Al Jubair[1] and Pontus Gourdon[1,2]

[1] Department of Biomedical Sciences, University of Copenhagen, Blegdamsvej 3, 2200 Copenhagen N, Denmark
[2] Department of Experimental Medical Science, Lund University, Sölvegatan 19, SE – 221 84 Lund, Sweden

Transition metals are essential micronutrients in probably all organisms, required as cofactors for several proteins involved in a range of vital cellular processes. However, as excessive amounts of heavy metals are highly toxic to cells, the intracellular levels are tightly controlled. This makes the heavy-metal transporting P-type ATPases (P_{IB}-ATPases) very interesting targets, being key proteins for the regulation and maintenance of heavy metal homeostasis. The P_{IB}-ATPases are divided into seven subgroups based on conserved residues predicted to confer metal selectivity. The first two subgroups (P_{IB-1} and P_{IB-2}) are the most well-studied and crystal structures are available for both of them. Less is known about the P_{IB-4} ATPases, which are proposed to transport Co(II), Zn(II), and/or Ni(II). Furthermore, the stoichiometry (i.e. number of ions transported per ATP) is obscure, and their overall architecture remains to be elucidated; sequence analyses suggest that they lack the so-called heavy metal binding domain, which is common in most other P_{IB}-ATPases.

Here we describe our initial efforts to functionally and structurally characterize a P_{IB-4} ATPases, based on purification procedure from which we retain significant quantities of pure and active protein. *E. coli* is exploited as an overproduction host, and the construct contains a C-terminal his-tag. The protein is extracted from the cell membranes, and purified using affinity and size-exclusion chromatography resulting in a monodisperse peak profile, and a yield of approximately 20 mg purified protein per litre cell culture is reproducibly achieved. Furthermore, a simple colorimetric ATPase assay, measuring the amount of generated free phosphate due to ATP hydrolysis, has been used to demonstrate that the purified protein is functional. Structural studies using X-ray crystallography is currently on-going, and initial crystals have been achieved.

Crystal structure of the emerging cancer target MTHFD2 in complex with a substrate-based inhibitor

Robert Gustafsson, Ann-Sofie Jemth*, Nina M.S. Gustafsson*, Katarina Färnegårdh**, Olga Loseva*, Elisée Wiita*, Nadilly Bonagas*, Leif Dahllund***, Sabin Llona-Minguez*, Maria Häggblad****, Martin Henriksson*,Yasmin Andersson***, Evert Homan*, Thomas Helleday*, and Pål Stenmark

Department of Biochemistry and Biophysics, Stockholm University, Stockholm, Sweden.
*Science for Life Laboratory, Division of Translational Medicine and Chemical Biology, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden.
**Drug Discovery and Development Platform, Science for Life Laboratory, Department of Organic Chemistry, Stockholm University, Solna, Sweden.
***Drug Discovery and Development Platform, Science for Life Laboratory, School of Biotechnology, Royal Institute of Technology, Solna, Sweden.
****Biochemical and Cellular Screening, Science for Life Laboratory, Department of Biochemistry and Biophysics, Stockholm University, Stockholm, Sweden.

To sustain their proliferation, cancer cells become dependent on one-carbon metabolism to support purine and thymidylate synthesis. Indeed, one of the most highly upregulated enzymes during neoplastic transformation is MTHFD2, a mitochondrial methylenetetrahydrofolate dehydrogenase and cyclohydrolase involved in one-carbon metabolism. Because MTHFD2 is expressed normally only during embryonic development, it offers a disease-selective therapeutic target for eradicating cancer cells while sparing healthy cells. Here we report the synthesis and preclinical characterization of the first inhibitor of human MTHFD2. We also disclose the first crystal structure of MTHFD2 in complex with a substrate-based inhibitor and the enzyme cofactors NAD^+ and inorganic phosphate. Our work provides a rationale for continued development of a structural framework for the generation of potent and selective MTHFD2 inhibitors for cancer treatment.

Reference: Gustafsson, R., et al. (2017) Cancer Research; 77(4); 937-948.

Can alternating electric fields affect the kinetics of microtubule polymerization?

Rajiv Harimoorthy[a], Guo Chen[a], Greger Hammarin[a], Gisela Brändén[a], Peter Berntsen[b], Richard Neutze[a]

Affiliations;
[a]Department of Chemistry and Molecular Biology, University of Gothenburg, Box 462, Gothenburg, Sweden
[b]Center for Advanced Molecular Imaging, La Trobe University, Melbourne, Victoria 3086, Australia

Abstract:
Microtubules are extended tubular polymers of tubulin that are a component of the cytoskeleton present throughout the cytoplasm. These microtubules are highly dynamic and provide a platform for intracellular transport and are also involved in a variety of cellular processes. They are formed by the non-covalent polymerization of α and β tubulin dimers that require energy input in the form of GTP. Microtubules have a distinct polarity with one end having the α subunits exposed and the other end having the β subunits exposed, and these are termed the (-) and (+) ends, respectively. Elongation of a microtubule typically occurs at the (+) end. The (+) end of a microtubule is the region where assembly and disassembly of dimers take place which results in dynamic instability. During polymerization both the subunits of the dimer are bound to a molecule of GTP which is the stable state.

We are developing tools to investigate if alternating electric fields in the kHz to GHz domain can influence the kinetics of microtubule formation. We have developed a setup which applies an alternating electric field across a sample containing tubulin, and an ultraviolet light scattering probe monitors the formation of microtubules. The kinetics of microtubule polymerization is followed as a function of the frequency and energy of the applied electric field. We speculate that it may be possible to observe an increase or decrease in the rate of polymerization when an electric field is applied, and preliminary results towards this goal are presented.

Time-resolved X-ray solution scattering of the blue light photoreceptor: Phototropin

Léocadie Henry, Oskar Berntsson, Sebastian Westenhoff

University of Gothenburg, department of Chemistry and Molecular Biology

Light is one of the most important physical signals from the environment which influences plant growth and development. Through evolution, plants have acquired light response systems triggered by photosensitive proteins. Phototropins are photoreceptors sensitive to blue-light (BL). They respond to the fluctuating environmental light conditions to control various biological processes such as phototropism, stomatal opening or chloroplast movement[1]. They are composed of two photosensory modules (light oxygen-voltage sensing (LOV) domains) and a serine/threonine kinase (STK) domain. Each LOV module contains a flavin bound chromophore, which upon photon absorption will undergo structural and electronic rearrangements and lead downstream to auto-phosphorylation of the kinase domain. Even though BL receptors have been investigated for a long time, the molecular mechanism of phototransduction is still under debate. The studies to date have been focusing on phototropin lacking the C-term kinase. The role of the different LOV domains, though quite similar in sequence is still not completely understood. By combining X-ray crystallography and time-resolved Small Angle X-ray Scattering (SAXS), we wish to probe the structural rearrangements occurring after photon absorption in phototropins and figure out the signal transduction mechanism from the chromophore to the STK. So far, time-resolved SAXS data have been recorded on various phototropin constructs from the algae *Chlamydomonas reinhardtii*. The photocycle comprising a triplet-state LOV-715 and a second-intermediate LOV-390 in LOV1 has been identified as well as phototransduction kinetics of different domain construct. By the mean of X-ray crystallography, the next step in this project is to determine the three dimensional structure of the full-length and the LOV2-STK phototropin. Since LOV-domain containing proteins are conserved throughout evolution, understanding the mechanism of this functional light sensor would lead to major advances into protein signalling and phototransduction not only in plants but among various kingdom.

1. Christie, J. M. Phototropin blue-light receptors. *Annu Rev Plant Biol* **58,** 21–45 (2007).

Time-resolved X-ray solution scattering of the blue light photoreceptor: Phototropin

Léocadie Henry, Oskar Berntsson, Sebastian Westenhoff

University of Gothenburg, department of Chemistry and Molecular Biology

Light is one of the most important physical signals from the environment which influences plant growth and development. Through evolution, plants have acquired light response systems triggered by photosensitive proteins. Phototropins are photoreceptors sensitive to blue-light (BL). They respond to the fluctuating environmental light conditions to control various biological processes such as phototropism, stomatal opening or chloroplast movement[1]. They are composed of two photosensory modules (light oxygen-voltage sensing (LOV) domains) and a serine/threonine kinase (STK) domain. Each LOV module contains a flavin bound chromophore, which upon photon absorption will undergo structural and electronic rearrangements and lead downstream to auto-phosphorylation of the kinase domain. Even though BL receptors have been investigated for a long time, the molecular mechanism of phototransduction is still under debate. The studies to date have been focusing on phototropin lacking the C-term kinase. The role of the different LOV domains, though quite similar in sequence is still not completely understood. By combining X-ray crystallography and time-resolved Small Angle X-ray Scattering (SAXS), we wish to probe the structural rearrangements occurring after photon absorption in phototropins and figure out the signal transduction mechanism from the chromophore to the STK. So far, time-resolved SAXS data have been recorded on various phototropin constructs from the algae *Chlamydomonas reinhardtii*. The photocycle comprising a triplet-state LOV-715 and a second-intermediate LOV-390 in LOV1 has been identified as well as phototransduction kinetics of different domain construct. By the mean of X-ray crystallography, the next step in this project is to determine the three dimensional structure of the full-length and the LOV2-STK phototropin. Since LOV-domain containing proteins are conserved throughout evolution, understanding the mechanism of this functional light sensor would lead to major advances into protein signalling and phototransduction not only in plants but among various kingdom.

1. Christie, J. M. Phototropin blue-light receptors. *Annu Rev Plant Biol* **58,** 21–45 (2007).

How spider silk makes ends meet: structural insights from crystallography and small angle X-ray scattering (SAXS)

Wangshu Jiang[1], Alexander Shkumatov[2], My Hedhammar[3], Stefan D Knight[1]

[1]Department of Cell and Molecular Biology, Uppsala University, [2]VIB-VUB Center for Structural Biology, Vrije Universiteit Brussel, [3]Div. of Protein Technology, KTH Royal Institute of Technology

Spider silks have extraordinary extensibility, tensile strength and biodegradability. The mechanism of how spider silk is produced and converted from liquid to solid form has fascinated mankind for ages.

Spider silks are polymers of spidroins, large proteins (~4000 amino acid residues) with repetitive poly-Ala/Gly-rich segments flanked by conserved regulatory α-helical N-terminal (NT; ~130 residues) and C-terminal (CT; ~110 residues) domains. During polymerization, the repetitive regions undergo a structural conversion from soluble random coil and α-helical conformations to a β-sheet rich amyloid-like structure.

Spider silk glands consist of three parts: tail, sac and duct. Spidroins are produce in the tail, stored in the sac as a concentrated solution ('dope'). As the spidroin dope passes through the tapered S-shaped duct, chemical changes (including decreased pH (from neutral to below 5.7) and reduced NaCl concentration) and mechanical sheer forces that align the spidroin molecules trigger the conversion of dope into silk. The structure determination of both NT and CT brought great advances in understanding their regulatory roles. NT is folded as a five-helix bundle with a conserved distinct dipolar charge distribution. While NT is monomeric and extremely soluble at neutral pH and above, NT rapidly dimerizes as pH decreases. NT dimerization depends on the two-step protonation of three conserved glutamic acid side chains and is thought to proceed via the formation of an ensemble of intermediate, relatively unstable dimer species, to a fully formed "locked" dimer conformation.

We have now discovered an alternative dimer conformation of NT, distinct from the previously described locked NT dimer. Based on the crystal structure together with SAXS measurements under physiologically relevant conditions (pH and NaCl concentration combinations), we propose a series of possible structural transitions of NT during the formation of spider silk.

Structural studies of Co(II)-transporting PIB-4 ATPases

Tamim Al-Jubair*, Qiaoxia Hu*, Christina Grønberg* and Pontus Gourdon*

* Department of Biomedical Sciences, University of Copenhagen, Blegdamsvej 3B, DK-2200 Copenhagen, Denmark

Trace metals such as cobalt are critical components for a plethora of functions required for cell survival (1). At the same time, elevated concentrations of cobalt are toxic and hence homeostasis has to be maintained. The PIB-4 ATPases tightly regulate this metal through active transport across cellular membranes (2, 3), allowing efflux of Co^{2+}, Zn^{2+} and Cd^{2+} (4, 5). Furthermore, PIB-ATPases confer survival of pathogenic bacteria during infection (6). Nevertheless, our understanding of the structure and the determinants of these proteins is limited.

In this study we aim to determine the first crystal structure of a PIB-4 ATPase. We have targeted nine proteins from different sources. All the genes have been cloned in pET52(b) vector with C-terminal His tag. We analyzed the protein expression and selected one target, which provided the necessary yields for structural and functional studies. To generate untagged protein, the gene was re-cloned in the pET22(b) vector and the expression and purification of untagged protein optimized. The protein was subjected to crytallization process and at the same time optimization of the expression and purification process for other targets were continued. A colorimetric functional assay has also been exploited to characterize the metal ion sensitivity of the protein.

Our ultimate goal is to reveal the first mechanistic models of the entire protein class, providing a framework for downstream more detailed studies. Furthermore, since homologous proteins are not available in human and because they are crucial for the infection of pathogenic bacteria, these proteins represent potential drug targets for developing novel antibiotics to combat the rising threat of multidrug resistant bacteria.

1. Waldron KJ, Rutherford JC, Ford D, & Robinson NJ (2009) Metalloproteins and metal sensing. *Nature* 460(7257):823-830.
2. Zielazinski EL, Cutsail GE, 3rd, Hoffman BM, Stemmler TL, & Rosenzweig AC (2012) Characterization of a cobalt-specific P(1B)-ATPase. *Biochemistry* 51(40):7891-7900.
3. Raimunda D, Long JE, Sassetti CM, & Arguello JM (2012) Role in metal homeostasis of CtpD, a Co(2)(+) transporting P(1B4)-ATPase of Mycobacterium smegmatis. *Molecular microbiology* 84(6):1139-1149.
4. Arguello JM (2003) Identification of ion-selectivity determinants in heavy-metal transport P1B-type ATPases. *The Journal of membrane biology* 195(2):93-108.
5. Traverso ME, *et al.* (2010) Identification of a hemerythrin-like domain in a P1B-type transport ATPase. *Biochemistry* 49(33):7060-7068.
6. Botella H, *et al.* (2011) Mycobacterial p(1)-type ATPases mediate resistance to zinc poisoning in human macrophages. *Cell host & microbe* 10(3):248-259.

Structural studies of aminoglycoside nucleotidyltransferases (ANTs)

Sandesh Kanchugal P, Ana Laura Stern, Sander Van der Verren, Hugo Gutierrez de Teran and Maria Selmer
Department of Cell and Molecular Biology, Uppsala University, Uppsala, Sweden

Aminoglycosides are broad-spectrum antibiotics that target the bacterial protein synthesis. Common resistance mechanisms involve chemical modification of the drugs. One type of resistance-mediating enzymes are aminoglycoside nucleotidyltransferases (ANTs). AadA is an ANT (3") 9' enzyme that confers resistance against the two aminoglycosides streptomycin and spectinomycin by adenylation of the 3'' and 9' hydroxyl positions of the two drugs. Streptomycin is a flexible molecule containing three ring structures, while spectinomycin is a more rigid tricyclic compound.

After solving crystal structures of AadA from *Salmonella enterica* in apo state [1] and in complex with ATP and streptomycin[2], we now set out to clarify how this family of enzymes bind to spectinomycin. Manual docking of spectinomycin to AadA was followed by molecular dynamics simulations, supporting a model where the modified ring in the two substrates bind similarly to the enzyme, while other parts of the two drugs interact with different residues of the enzyme.

Bioinformatic characterization of the AadA family showed distinct patterns of conservation for adenyl transferase enzymes active on both substrates and enzymes only active on spectinomycin.

We expressed and purified three different ANT9 enzymes, annotated as only being active on spectinomycin. Binding assays and structural studies are underway to explain their mechanism of spectinomycin recognition and modification.

1. Chen, Y., Näsvall, J., Wu, S., Andersson, D. I. & Selmer, M. Structure of AadA from Salmonella enterica: a monomeric aminoglycoside (3'')(9) adenyltransferase. *Acta Crystallogr Sect D Biological Crystallogr* **71,** 2267–2277 (2015).

2. Stern, A. L., Van der Verren S., Gutierrez de Teran, H. & Selmer, M. Structural mechanism of the aminoglycoside (3")(9) adenyl transferase AadA from *Salmonella enterica. submitted for publication*

LP3 and DEMAX

Wolfgang Knecht and Zoe Fisher*

Lund Protein Production Platform (LP3), Lund University, Sölvegatan 35, 22362 Lund, Sweden and *Scientific Activities Division, European Spallation Source ERIC (ESS), Tunavägen 24, 22100 Lund, Sweden.

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 unsurprising 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) High-throughput crystallization, and 3) Stable isotope labelling and bio-deuteration of biological macromolecules (proteins and lipids).

In July 2016, the DEuteration and MAcromolecular Xtallization (DEMAX) platform of the European Spallation Source ERIC (ESS) moved into Biology House A at Lund University and co-localized with LP3. DEMAX and LP3 are coordinating in their efforts to develop cost-effective production of deuterated proteins for macromolecular crystallography, enable crystallization of interesting proteins for neutron work, and for the production of labeled proteins/lipids for neutron reflectometry.

For more information and access see: www.lu.se/lp3

Entropy and water dynamics in enzymatic polycyclization reactions

Charlotte Kürten[a], Per-Olof Syrén[a,b]

[a] Science for Life Laboratory, Stockholm – School of Biotechnology, Protein Engineering and Enzyme Design, KTH, Sweden

[b] KTH Royal Institute of Technology – School of Chemical Science and Engineering, Sweden

Polycyclic molecules have potent chemical and biological properties and are widely used in various industrial applications e.g. as flavoring compounds in the food industry or bioactive compounds in pharmaceutical industries. They are generated in complex polycyclization reactions that follow sophisticated and concerted reaction pathways associated with very high entropic costs originating from the loss of rotational freedom of the pre-folded substrate.
Enzymatic polycyclization of linear isoprene molecules by terpene cyclases generates a vast divergence in the chemical class of terpene molecules leading to new functionalities and bioactivities in all domains of life. Triterpene cyclases introduce two- to six ring structures into linear 30-carbon molecules, forming versatile scaffolds with potent activities or as substrates for further functionalization and modification reactions leading to the miscellaneous "terpenosome" of higher organisms[1].

Herein, computational design and biophysical analyses of the membrane-bound human oxidosqualene cyclase[2,3] showed that enzymatic lanosterol production is driven by a favorable entropy of activation. *In silico* analyses revealed a tunnel network in hOSC that allows for water passage between the active site and the surrounding bulk solvent. By introducing rationally designed, tunnel perturbating, single point amino acid substitutions we could show that the thermodynamic profile of the enzymatic reaction is dependent of the tunnel and water network and thus can be altered by changing the tunnel pattern.
The importance of water dynamics in sophisticated biosynthetic machineries intrigued us to study the interplay of solvent and protein dynamics in triterpene cyclases. Differences in hOSC variants with obscured tunnel networks and disturbed thermodynamic signatures are studied with an LC-MS/MS based approach[4] together with crystallographic structural determination of the membrane protein variants.

1 Miettinen, K. *et al.* The ancient CYP716 family is a major contributor to the diversification of eudicot triterpenoid biosynthesis. *Nature Communications* **8**, 14153, doi:10.1038/ncomms14153 (2017).

2 Kurten, C. & Syren, P. O. Unraveling Entropic Rate Acceleration Induced by Solvent Dynamics in Membrane Enzymes. *J Vis Exp*, e53168, doi:10.3791/53168 (2016).

3 Kurten, C., Uhlen, M. & Syren, P. O. Overexpression of functional human oxidosqualene cyclase in Escherichia coli. *Protein Expr Purif* **115**, 46-53, doi:10.1016/j.pep.2015.04.015 (2015).

4 Feng, Y. *et al.* Global analysis of protein structural changes in complex proteomes. *Nat Biotechnol* **32**, 1036-1044, doi:10.1038/nbt.2999 (2014).

The paralogous shell proteins CsoS4A and CsoS4B from the α–carboxysome assemble in a heteropentamer

Anna M. Larsson, Dirk Hasse, Huma Shakoor* and Inger Andersson

Laboratory of Molecular Biophysics, Department of Cell and Molecular Biology, Uppsala University, Husargatan 3, Box 596, SE-75124 Uppsala, Sweden, *National Centre of Excellence in Molecular Biology, University of the Punjab, Lahore, Pakistan

Carboxysomes are bacterial microcompartments that sequester the CO_2-fixing machinery of the cell by encapsulating Rubisco together with carbonic anhydrase and other auxiliary enzymes, thereby increasing the CO_2 concentration at the site of Rubisco (Shively *et al*., 1973). Carboxysomes are found in the cytoplasm of nearly all cyanobacteria and some chemoautotrophic bacteria. Depending on genomic organisation and sequence, two types of carboxysomes have been defined: the α–carboxysome, occurring mainly in chemoautotrophic bacteria and oceanic picocyanobacteria, and the β–carboxysome occurring in other types of cyanobacteria.

Halothiobacillus neapolitanus is an obligate chemolithoautotrophic gram negative bacterium that obtains energy from reduced inorganic sulfur compounds (Kelly and Wood, 2000) and serves as a model organism for structural and functional studies of carboxysomal CO_2–fixation. Genes coding for two paralogous pentameric shell proteins are conserved in nearly all cyanobacteria and chemoautotrophic bacteria with α–carboxysomes, whereas the β–carboxysomes contain only one pentameric shell protein (CcmL; Abdul-Rahman *et al*., 2013). The pentameric shell proteins of *H. neapolitanus*, CsoS4A and CsoS4B, are believed to form the vertices of the α–carboxysome and are important for the retention of CO_2 within the protein shell (Cai *et al*., 2009).

The genes *csoS4A* and *csoS4B* from *H. neapolitanus* DSM151475 were co-expressed in *E. coli* and co-purified using a histidine affinity tag on the C-terminal end of the CsoS4B protein. The co-purified proteins eluted as pentamers from analytical size exclusion chromatography at a ratio 3 CsoS4A: 2 CsoS4B as estimated by denaturing gel electrophoresis. Crystal structures of CsoS4A (Tanaka *et al.*, 2008) and CsoS4B (sequence identity of 40%) as well as the mixture of the two proteins show a similar overall fold.

Abdul-Rahman F., Petit E., and Blanchard J. L. (2013) J Phylogen Evolution Biol 1:118. doi: 10.4172/2329-9002.1000118

Cai, F., Menon, B. B., Cannon, G. C., Curry, K. J., Shively, J. M., Heinhorst, S. (2009) *PLoS One*, 4(10), 7521.

Kelly, D. P and Wood, A. P. (2000) *Int. J. Syst. Evol. Microbiol*, 50, 511-516.

Shively, J. M., Ball, F., Brown, D. H., and Saunders, R. E. (1973) *Science,* 182, 584-586.

Tanaka, S., Kerfeld, C. A., Sawaya, M. R., Cai, F., Heinhorst, S., Cannon, G. C., and Yeates, T. O. (2008) *Science*, 319, 1083-1086.

Investigating protein- detergent complexes through Small Angle X-Ray Scattering

Michael Lerche, Hena Sandhu, Mikaella Rapp and Martin Högbom

Stockholm University, Dept of Biochemistry and Biophysics

BioSaxs has within the recent decade become a powerful complimentary technique in structural biology. Recent advances in microfluidics and the installation of online size exclusion chromatography at most 3rd generation synchrotrons allows fast screening and dynamic structural studies not feasible in protein crystallography. However, the complications arising from the modelling of properties of detergent coronas have hindered the applicability on membrane protein systems

Herein we attempt to model the micelles of both the very mild detergent digitonin and the more commonly used DDM and use the information obtained to model the in-solution protein-detergent complex structure of mitochondrial supercomplexes and the magnesium transporter CorA in different states.

Structural studies of bacterial copper flux proteins

Ping Li, Kamil Gorecki, Niloofar Nayeri, Karin Lindkvist, Pontus Gourdon*

Department of Experimental Medical Science, Lund University, Sölvegatan 19, SE - 221 84 Lund, Sweden,
*Department of Biomedical Sciences, University of Copenhagen, Blegdamsvej 3, 2200 Copenhagen N, Denmark

Metals such as zinc, manganese, copper and iron are critical component of proteins involved in variety of cellular processes. Copper is an essential trace element for bacterial growth, serving in e.g. electron transfer and dioxygen transport. However, too much Cu is toxic for bacteria, and therefore the copper levels inside bacterial cells have to be tightly regulated. The mechanisms involved in copper transport and homeostasis in bacteria are only partially understood. To elucidate details of copper uptake and resistance, this project focuses on structural and functional studies of proteins associated with prokaryotic copper flux across cellular membranes. We cloned the full length and N-terminally truncated bacterial Cu transporter into an expression vector and expressed the proteins in E. coli. High purity protein samples were obtained through Immobilized Metal Affinity Chromatography (IMAC), and subsequent size exclusion chromatography. Initial crystallization conditions were found using broad screening. Currently, crystal optimization is executed in order to obtain high quality diffraction data.

Design, Synthesis and *In Vitro* Biological Evaluation of Oligopeptides Targeting *E. coli* Type I Signal Peptidase (LepB)

Lu Lu[a], Maria De Rosa[b], Anders Karlén[b], Sherry Mowbray[a], T. Alwyn Jones[a]

[a] Uppsala University, Department of Cell and Molecular Biology, BMC, Box 596, SE-751 24 Uppsala, Sweden

[b] Uppsala University, Department of Organic Pharmaceutical Chemistry, BMC, SE-751 23 Uppsala, Sweden

Type I signal peptidases are potential targets for the development of new antibacterial agents. In this study, we explored the possibility of finding potent *E. coli* LepB inhibitors by optimizing a previously reported hit compound, decanoyl-PTANA-CHO. In particular, we wished to investigate the influence of modifications at the N- and C-termini. In general, good improvement of inhibitory potency was obtained, with IC_{50}s in the low nanomolar range. Two of the best inhibitors also showed promising antimicrobial activity, with MICs in the low μg/mL range for several bacterial strains. Furthermore, the selection of resistant mutants provided strong support that LepB is the target for these compounds. Although more optimization will be required to obtain inhibitors that also have favorable cytotoxicity and hemolytic profiles, we have shown that minor changes in the inhibitor structures often produce large effects on these properties that can be exploited in future studies.

Characterisation of the lipid-protein interactions of Fatty acyl CoA synthase from *M. tuberculosis*

Camilla AK. Lundgren*, Charlotta S. Andersson, Audur Magnusdottir, Changrong GE, Åke Weislander, Daniel Martines Molina, Martin Högbom*

*Stockholm University

Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis*. It is estimated that a third of the world's population is infected and increasing numbers of multi-drug-resistant strains has put this disease on the World Health Organization (WHO) global emergency list.

The bacteria have a unique cell envelope, with a high content of very long and complex lipids. The lipid rich cell envelope is essential for both virulence and drug resistance; hence enzymes involved in its metabolism are attractive drug targets. Our group has solved the structure of a fatty acyl-CoA synthetase, FadD13, located in an operon essential for virulence and intracellular growth of the pathogen.

The structure revealed a putative membrane-binding surface on the protein and we have shown that it does bind to a model membrane. We propose that FadD13 is functionally a monomer, which binds to the cytoplasmic side of the membrane. However, in solution FadD13 is a dimer. We have created a set of mutant variants with changes to the proposed membrane-binding patch. These changes seem to affect FadD13's affinity for certain lipids and it monomeric/dimeric state in solution.

Identification and characterization of a novel botulinum neurotoxin

Sicai Zhang[1], Geoffrey Masuyer[2], Jie Zhang[1], Yi Shen[1], Daniel Lundin[2], Linda Henriksson[2], Shin-Ichiro Miyashita[1], Markel Martínez-Carranza[2], Min Dong[1], Pål Stenmark[2]

[1] Department of Urology, Boston Children's Hospital, Department of Microbiology and Immunobiology and Department of Surgery, Harvard Medical School, Boston, Massachusetts 02115, USA. [2] Department of Biochemistry and Biophysics, Stockholm University, SE-106 91 Stockholm, Sweden.

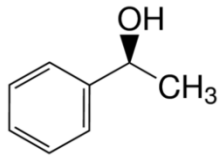
Botulinum neurotoxins are known to have seven serotypes (BoNT/A–G). We have identified and characterized a new BoNT serotype, tentatively named BoNT/X, which has the lowest sequence identity with other BoNTs and is not recognized by antisera against known BoNTs. Similar to BoNT/B/D/F/G, BoNT/X cleaves vesicle-associated membrane proteins (VAMP) 1, 2 and 3, but at a novel site. Remarkably, BoNT/X is the only toxin that also cleaves non-canonical substrates VAMP4, VAMP5 and Ykt6. To validate its activity, a small amount of full-length BoNT/X was assembled by linking two non-toxic fragments using a transpeptidase (sortase). Assembled BoNT/X cleaves VAMP2 and VAMP4 in cultured neurons and causes flaccid paralysis in mice. Thus, BoNT/X is a novel BoNT with a unique substrate profile. Furthermore, we have determined the crystal structure of the catalytic domain of BoNT/X. The discovery of BoNT/X posts a challenge to develop effective countermeasures, provides a novel tool for studying intracellular membrane trafficking, and presents a new potential therapeutic toxin for modulating secretions in cells.

Crystal structures rationalize properties of in vitro evolved ADH-A variants

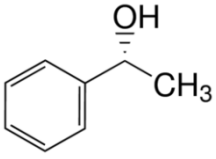
Dirk Maurer, Emil Hamnevik, Thilak R. Enugala, Mikael Widersten, Doreen Dobritzsch

Department of Chemistry, Uppsala University

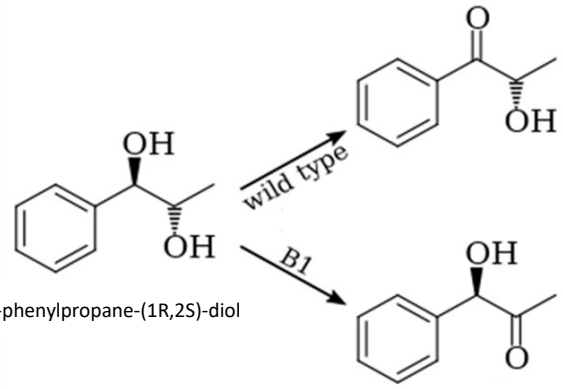
The ability to alter the redox state of alcohols and the related aldehydes and ketones is central in synthetic chemistry and has traditionally been catalyzed by different heavy metal-containing reagents. The questionable atom economy and also efficiency of such systems has triggered an interest in utilizing enzymes as biocatalysts for such transformations. Alcohol dehydrogenase A (ADH-A) from *Rhodococcus ruber* DSM 44541 is a promising biocatalyst for redox transformations of arylsubstituted secondary alcohols and ketones [1]. The enzyme is stereoselective in the oxidation of 1-phenylethanol with a 270-fold preference for the (S)-enantiomer [2]. It also displays a strong regiopreference for secondary alcohols and ketones; primary alcohols are very poor substrates in comparison.
In a collaborative project, the Widersten group generated different enzyme variants by iterative saturation mutagenesis. The four mutated positions, divided into two libraries, are all located in the active-site cavity. The generated variants of library A and B were selected for improved catalytic activity with (R)-1-phenylethanol and 1-phenylpropane-(1R,2S)-diol, respectively. The A2 variant and its offspring showed a change in enantiopreference, from (S)- to (R)-1-phenylethanol, whereas one variant from the B library, B1, aquired an altered regioselectivity. It preferentially oxidizes the hydroxyl group at position 2 rather than position 1 of 1-phenylpropane-(1R,2S)-diol. We determined the crystal structures of these variants, revealing changes in active site architecture that give rise to these effects.



S-1-phenylethanol



R-1-phenylethanol



1-phenylpropane-(1R,2S)-diol

[1] Kosjek, B.; Stampfer, W.; Pogorevc, M.; Goessler, W.; Faber, K.; Kroutil, W. Biotechnol. Bioengineer. 2004, 86, 55-62.

[2] Hamnevik, E.; Blikstad, C.; Norrehed, S.; Widersten, M. J. Mol. Catal. B Enzym. 2014, 99, 68-78.

Synthetic biology meets synthetic chemistry - *In vivo* activation of an apo-hydrogenase using synthetic complexes

L. S. Mészáros*, N. Khanna**, C. Esmieu*, P. Lindblad** and G. Berggren*

** Molecular Biomimetics, Department of Chemistry – Ångström Laboratory, Uppsala University, 75120 Uppsala, Sweden, ** Microbial Chemistry, Department of Chemistry – Ångström Laboratory, Uppsala University, 75120 Uppsala, Sweden*

[FeFe] hydrogenases (HydA) are fascinating enzymes that catalyse the interconversion between H_2 and protons with remarkable efficiency. The reaction occurs at the H-cluster featuring a, in biology unique, dinuclear [2Fe] subsite (Fig. 1).[1] Synthetic chemistry has long been a powerful tool in studies of this cluster, via the preparation of biomimetic model compounds.[2] In 2013 some of us could show how such synthetic complexes can be introduced into the enzyme itself under *in vitro* conditions, thus providing a direct link between biomimetic chemistry and biology, and allowing the manipulation of the enzyme using synthetic chemistry.[3, 4]

Recently we discovered how this concept can be extended to *in vivo* conditions, and the apo-enzyme activated using synthetic compounds inside living cells.[5] Here I will present how this can be used to generate both active "native" hydrogenases, as well as "artificial" hydrogenases incorporating non-native cofactors resulting in enzymes with new spectroscopic and catalytic properties.

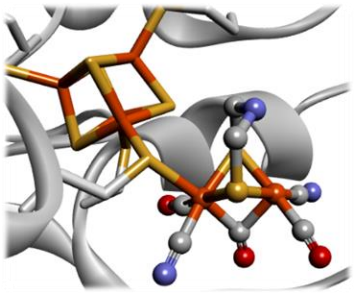


Figure 1: Cartoon representation of the H-cluster (S = yellow; Fe = orange; N = blue; O = red; C = white)

References:

1. Lubitz, W., Ogata, H., Rüdiger, O. & Reijerse, E. Hydrogenases. *Chem. Rev.* (2014) **114**, 4081-4148.

2. Simmons, T.R., Berggren, G., Bacchi, M., Fontecave, M. & Artero, V. Mimicking hydrogenases: From biomimetics to artificial enzymes. *Coord. Chem. Rev.* (2014) **270–271**, 127-150.

3. Berggren, G. et al. Biomimetic assembly and activation of [FeFe]-hydrogenases. *Nature* (2013) **499**, 66-69.

4. Esselborn, J. et al. Spontaneous activation of [FeFe]-hydrogenases by an inorganic [2Fe] active site mimic. *Nat. Chem. Biol.* (2013) **9**, 607-609.

5. Khanna, N.; Esmieu, C.; Mészáros, S.L.; Lindblad, P. and Berggren, G. *In vivo* activation of an [FeFe] hydrogenase using synthetic cofactors. *Energy & Environmental Science* (2017) DOI: 10.1039/C7EE00135E.

Structural Characterization of Aquaporins

Julie Winkel Missel[1], Kamil Gotfryd[1], Per Amstrup Pedersen[2] & Pontus Gourdon[1,3]

[1]Membrane Protein Structural Biology Group, Faculty of Medicine, University of Copenhagen, Denmark
[2]Section for Molecular Integrative Physiology, Faculty of Science, University of Copenhagen, Denmark
[3] Department of Experimental Medical Science, Lund University, Sweden

Aquaporins (AQP) are channels responsible for flux of water and other small solutes across cellular membranes. They are distributed in all cell types in the body, and therefore mutations and dysfunctions in human AQPs are related to a plethora of diseases. This includes cancer, brain oedema and Alzheimer's. Structural characterization of human AQPs has the potential to trigger structure-based drug-design efforts with the ambition to cure the above-mentioned diseases. AQPs can be sub-divided into three groups: orthodox AQPs, aquaglyceroporins and unorthodox AQPs. The orthodox AQPs are responsible for strict water transport, while the aquaglyceroporins are allow flux of water as well as other small solutes like glycerol. The selectivity of the unorthodox AQPs' remains elusive, and they have a low sequence similarity to other AQPs. Currently AQP0, 1, 2, 4 and 5 have been structurally determined along several bacterial and plant homologs. The aim of this project is to shed further light on the structures and transport mechanism of the AQPs, and to map their physiological importance. Hitherto, we have established procedures for overproduction and purification, and conducted crystallization experiments of different AQPs. For the functional characterization, we exploit methods like intact yeast cell stop-flow assay, proteoliposomes and polymersomes.

Coherent diffraction of single Rice dwarf virus particles

Anna Munke, Gijs van der Schot, M. Marvin Seibert, Andrew Aquila* *et al.***

Uppsala University, *SLAC National Accelerator Laboratory, ** The SPI initiative[1]

X-ray Free Electron Lasers (XFELs) have enabled "diffraction before destruction" experiments in structural biology, both in single particle imaging (SPI) and crystallography. The added value of XFELs as opposed to conventional crystallography at synchrotrons is their high brilliance, short femtosecond pulses and the ability to collect data at room temperature. Consequently, diffraction from micro- and nanocrystals[1] and even single particles can be detected before radiation damage occurs[2], in a time resolved fashion under physiological conditions.

Single particle imaging using X-ray Free Electron Lasers has made major advancements recently, which has facilitated experiments on smaller samples compared to the earliest reported works on giant viruses[3] and cells[4]. Here the technique was used to image the c. 70 nm icosahedral Rice dwarf virus, a virus that causes rice dwarf disease and severe economic damage in Asian countries.

In a single particle imaging experiment, virus particles are aerosolized and delivered via an aerodynamic lens injector into the pulsed X-ray beam and diffraction images are recorded on two area detectors. Here, we present two datasets using the Linac Coherent Light Source – one recorded using hard X-rays at the Coherent X-ray Imaging beamline[5] and one recorded using soft X-rays at the Atomic Molecular Optics beamline (unpublished data).

1. Aquila, A. *et al.* The linac coherent light source single particle imaging road map. *Struct. Dyn.* ***2***, 041701 (2015)
2. Chapman, H.N., *et al.* Femtosecond X-ray protein nanocrystallography. *Nature*, **470**, 73-77 (2011)
3. Neutze, R., *et al.* Potential for biomolecular imaging with femtosecond X-ray pulses. *Nature*, **406**, 752-757 (2000)
4. Seibert, M.M., *et al.* Single mimivirus particles intercepted and imaged with an X-ray laser. *Nature*, **470** (2011)
5. Seibert, M.M. *et al.* Femtosecond diffractive imaging of biological cells. *J. Phys. B At. Mol. Opt. Phys.* **43**, 194015 (2010)
6. Munke, A. *et al.* Coherent diffraction of single Rice Dwarf Virus particles using hard X-rays at the Linac Coherent Light Source, *Sci. Data*, **3**, 160064 (2016)

Current status and near future plans for BioMAX

Jie Nan, Thomas Ursby, Roberto Appio, Johan Unge, Mikel Eguiraun, Fredrik Bolmsten, Ross Friel, Robert Lizatovic & Uwe Mueller

MAX IV Laboratory, Fotongatan 2, 225 92 Lund, Sweden

BioMAX is the first X-ray macromolecular crystallography beamline at MAX IV Laboratory, and is designed to be a stable and reliable installation as well as user friendly [1,2]. Since its first diffraction experiment in July 2016, a lot of progress has been achieved, mainly including: 1) *experiment set-up*: after the successful startup of the hybrid pixel-detector Eiger 16M, a new sample environment HClab was recently put into operation. The ISARA sample changer and beam conditioning unit (BCU) were installed and are being commissioned; 2) *software*: the new version of beamline control software MXCuBE3 is continuously being developed in collaboration with the ESRF [3]. EDNA and several processing pipelines have been set up on the MAX IV HPC; 3) *user operation*: the first user experiment was carried out in 2016 December before the winter shutdown, and recently BioMAX has begun routine user operations for 50% of the delivered high current beam. The remaining beamtime is used for commissioning of BioMAX. In additional to finishing up the on-going projects, our near future plan includes, but not limited to, the introduction of raster scans, enabling serial crystallography at BioMAX, *in-situ* diffraction and other experimental possibilities.

References

[1] M.M.G.M. Thunnissen et al. BioMAX: The Future Macromolecular Crystallography Beamline at MAX IV. *J. Phys. Conf. Ser.* 425, 072012 (2013)

[2] M.M.G.M. Thunnissen et al. The macromolecular crystallography beamlines BioMAX and MicroMAX at the MAX IV laboratory. *Acta Cryst. A* 71, s190-s190 (2015)

[3] U. Mueller et al. MXCuBE3: A New Era of MX-Beamline Control Begins. *Synchrotron Radiat News* 30, 22-27 (2017)

Structural and functional studies of proteins linked to heavy metal ion transport

Niloofar Nayeri, Kamil Górecki, Ping Li, Karin Lindkvist, Pontus Gourdon*

Department of Experimental Medical Science, Lund University, Sölvegatan 19, SE - 221 84 Lund, Sweden,
*Department of Biomedical Sciences, University of Copenhagen, Blegdamsvej 3, 2200 Copenhagen N, Denmark

Transition metal ions, eg. Iron, copper and zinc, are essential elements required for survival in all organisms from bacteria to humans, nevertheless, elevated levels are highly toxic for cells. Through different regulatory mechanisms, cells ensure that ions are provided in the required amounts, and ion channels and transporters are critical in this process. Precise understanding of such ion transport events requires insight into the protein structure of such proteins, which will also provide information important for applied sciences.

Here, our efforts to obtain crystals of a family of proteins linked to heavy metal transport will be summarized. Members from both and yeast have been studied. Proteins were overproduced and extracted in various detergent and lipid mixtures. Solubilized proteins were then purified using affinity and size-exclusion chromatography, and subjected to crystallisation yielding initial crystals. Positive results from this work have the potential to reveal imperative aspects of the structure and function of these proteins, and to pave the way for subsequent drug discovery work.

Comparative structure-function analysis of HydF scaffold proteins involved in [FeFe] hydrogenase maturation

Németh Brigitta , Esmieu Charlène, Berggren Gustav

Department of Chemistry Ångström,
Uppsala University, Sweden

The [FeFe] hydrogenases are well-known metalloproteins that play a key role in the microbial redox homeostasis via the reversible reduction of protons and oxidation of hydrogen gas. The [FeFe] hydrogenase proteins have a wide structural and functional diversity – including different subunits, domains, organic and inorganic cofactors – still, the structure of the active site is highly conserved [1]. The active site – the H-cluster – is composed by a "classical" [4Fe4S] cluster coupled to a, in biology unique, dinuclear Fe complex called the [2Fe] subsite. The biosynthesis of the [2Fe] subsite requires at least 3 hydrogenase specific maturation proteins, HydE and HydG (radical SAM enzymes) and HydF (a scaffold protein). In short, the HydE and HydG proteins construct a precursor of the [2Fe] subsite on HydF, from where it is transferred to apo-hydrogenase [2].
Recently, it was shown how the natural Hyd-maturation machinery can be circumvented, and the hydrogenase enzyme activated *in vitro* by synthetic [2Fe] subsite mimics [3, 4]. A series of studies have now underscored that these semi-synthetic hydrogenases indeed have spectroscopic and catalytic properties indistinguishable from the native enzyme [3-6].
It has previously been shown that HydF from *Thermotoga maritima* also can be loaded with synthetic [2Fe] subsite mimics, and that this semi-synthetic HydF can mimic the reactivity of native HydF and transfer the synthetic cargo to the hydrogenase [3]. Here I will present how we are currently using this technique to explore the HydF protein and the mechanism of hydrogenase maturation in detail. I currently study two HydF proteins with different origin (*Thermotoga maritima, Clostridium acetobotulyticum*), which display differences in properties like isoelectric point, heat stability, GTPase enzyme activity and most importantly in their coordination of their respective [4Fe4S] clusters. This work includes a comparative study of the quaternary structure and [FeS] cluster coordination as a function of co-factor integration level of the selected model proteins.

References

[1] Vignais et al. (2001) FEMS Microbiology reviews
[2] Lubitz et al. (2014) Chemical reviews
[3] Berggren et al. (2013) Nature
[4] Esselborn et al. (2013) Nature Chemical Biology
[5] Birrell et al. (2016) Israel Journal of Chemistry
[6] Caserta et al. (2016) Biochimica et Biophysica Acta (BBA) - Bioenergetics

Optimizing the crosslinking of AQP0 and CaM for structural studies

Veronika Nesverova*, Rawya Antar*, Susanna Horsefield*

*Department of Biochemistry and Structural Biology, Lund University, Sweden,

Human aquaporin 0 (AQP0) is a membrane-bound water channel that is found exclusively in mammalian eye lens cells. It functions as a water channel but also as an adhesive protein mediating cell-cell junctions. Gating of AQP0 has been proposed to be allosterically regulated by binding of calmodulin in response to cytoplasmic Ca^{2+} concentrations.[1] Calmodulin (CaM) is a small soluble protein functioning as a secondary messenger in many signalling pathways. It is known to regulate the permeability of many membrane-bound channels and transporters.

A pseudo-atomic model of the AQP0-CaM complex was constructed based on data from electron microscopy. For this the native AQP0 was cross-linked with recombinant CaM. ITC analysis of the binding of AQP0 binding site mutants to CaM revealed the potential mechanism and stoichiometry. Molecular dynamics simulations suggest that upon CaM binding the AQP0 is stabilized in a closed conformation where Tyr149 almost completely blocks the channel. [1]

In this study we purified AQP0 from *Pichia pastoris* and CaM from *E.Coli* in order to further elucidate the mechanism of the interaction and water passage regulation. The cross-linking procedure using zero-length cross-linking reagent 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) will be optimized in order to obtain yield of the complex that is high enough for crystallography studies. The results of the cross-linking procedure will be evaluated using SDS-PAGE and western blotting. The complex will be purified and crystallography trials will be set up. The binding site will also be investigated using mass spectrometry.

[1] Reichow SL, Clemens DM, Freites JA, et al. Allosteric mechanism of water channel gating by Ca^{2+}–calmodulin. *Nature structural & molecular biology*. 2013;20(9):1085-1092. doi:10.1038/nsmb.2630.

Structure-based drug design for the regulatory protein PrfA to attenuate virulence of *Listeria monocytogenes*

Melanie Oelker, Martina Kuhlén, Marie Lindgren*, Christin Grundström, Jörgen Johansson*, Fredrik Almqvist and Elisabeth Sauer-Eriksson

Department of Chemistry and *Molecular Biology, Umeå University, 90187 Umeå, Sweden

To overcome the problem of increasing antibiotic resistances not only the development of new antibiotics but also the search for novel strategies is important for future treatments of bacterial infections. One possible strategy is to develop drugs which affect the virulence instead of the survival of the bacteria. An advantage of this strategy is that it lowers the pressure for the bacteria to develop resistances against the drug.

The positive regulatory factor of listeriolysin production (PrfA) from *Listeria monocytogenes* is a member of the large bacterial Crp/Fnr superfamily. The members of this protein family are transcription factors, which can respond to a broad spectrum of intracellular and exogenous signals. They share a common structure with an N-terminal nucleotide-binding domain, which for many family members constitutes the binding site for the activator molecule, and a C-terminal DNA-binding domain containing a helix-turn-helix (HTH) motif, which binds to the corresponding response element in the DNA. The HTH motif is not folded in the inactive form of the protein, but becomes folded upon activation.

PrfA is an activator of important virulence factors in *L. monocytogenes* and its inactivation leads to a decreased virulence of the bacteria. This renders PrfA to a target for the development of novel drugs, which affect the virulence of this pathogen. Furthermore, as PrfA and the regulation of the corresponding genes in *L. monocytogenes* are well investigated, this system can also be used as a model system for other important pathogens.

In a previous study glutathione (GSH) was identified to bind and activate PrfA [1]. In a second study PrfA was screened against ring-fused 2-pyridone compounds and a hit, C10, was found to bind to PrfA and reduce the expression of the virulence factors [2]. The structural studies showed that GSH and C10 are binding to the same site of the protein, called AI or tunnel site, however C10 also binds a second binding site, BII. In a follow-up study (unpublished data) new compounds, based on C10, were tested and the results suggest that binding site AI is more important for PrfA inactivation than binding site BII.

In this project, new lead compounds will be designed based on binding site AI. These compounds will be tested both in vitro and in vivo. We aim to obtain compounds with high specificity for binding site AI, leading to increased binding affinity in vivo, and increased ability to inactivate PrfA.

1. Hall M, et al. (2016) Structural basis for glutathione-mediated activation of the virulence regulatory protein PrfA in *Listeria*. Proc Natl Acad Sci USA. 113:14733–14738.
2. Good JA, et al. (2016) Attenuating Listeria monocytogenes virulence by targeting the regulatory protein PrfA. Cell Chem Biol 23(3):404–414.

Target-based drug discovery approaches on *Pseudomonas aeruginosa* UDP-diacylglucosamine pyrophosphohydrolase LpxH

Lorenzo Picchianti,[1] Sanjeewani Sooriyaarachchi,[1] Vivek Konda,[2], Terese Bergfors,[1] Anders Karlén,[2] T. Alwyn Jones,[1,] and Sherry L. Mowbray,[1*,]

[1] Science for Life Laboratory, Department of Cell and Molecular Biology, Uppsala University, Biomedical Center, Box 574, SE-751 23 Uppsala, Sweden;*

[2]Department of Medicinal Chemistry, Uppsala University, Biomedical Center, Box 596, SE-751 24 Uppsala, Sweden

Drug resistance is named among other series human health hazardous problems and required rapid and coordinated methods to deal with. This makes the need of novel antibiotic compounds critical. A high-throughput phenotypic screening studies on Gram-negative bacteria suggested that interfering biogenesis of the bacterial cell envelope has significant potential for therapeutic intervention, which would lead the path to approach novel drug targets (1)

One pathway validated as essential for the survival of many Gram-negative pathogens is that needed for the biosynthesis of Lipid A, the hydrophobic component of the lipopolysaccharides that dominate the outer membrane. The fourth step in the pathway is carried out by the enzyme LpxH, which cleaves the pyrophosphate bond of UDP-2,3-diacylglucosamine to generate 2,3-diacylglucosamine 1-phosphate (lipid X) and UMP. The pathogen *Pseudomonas aeruginosa* is one of the major sources of human disease that is not properly addressed by current antibiotics. We work on LpxH enzyme from *P. aeruginosa* in the present study.

The project is at an earlier stage and *P. aeruginosa* LpxH was overproduced in *E.coli,* purified and crystallized using the hanging drop vapor diffusion method in the presence of two substrate analogues. X-ray diffraction data to 2.6Å resolution were collected from an orthorhombic crystal form belonging to the space group $C222_1$. Structural analysis by molecular replacement in in progress.

(1). Nayar, A. S., Dougherty, T. J., Ferguson, K. E., Granger, B. A., McWilliams, L., Stacey, C., Leach, L. J., Narita, S., Tokuda, H., Miller, A., Brown, D. G.,

Target-based drug discovery approaches on *Pseudomonas aeruginosa* UDP-diacylglucosamine pyrophosphohydrolase LpxH

Lorenzo Picchianti,[1] Sanjeewani Sooriyaarachchi,[1] Vivek Konda,[2], Terese Bergfors,[1] Anders Karlén,[2] T. Alwyn Jones,[1,] and Sherry L. Mowbray,[1*,]

[1] Science for Life Laboratory, Department of Cell and Molecular Biology, Uppsala University, Biomedical Center, Box 574, SE-751 23 Uppsala, Sweden;*

[2]Department of Medicinal Chemistry, Uppsala University, Biomedical Center, Box 596, SE-751 24 Uppsala, Sweden

Drug resistance is named among other series human health hazardous problems and required rapid and coordinated methods to deal with. This makes the need of novel antibiotic compounds critical. A high-throughput phenotypic screening studies on Gram-negative bacteria suggested that interfering biogenesis of the bacterial cell envelope has significant potential for therapeutic intervention, which would lead the path to approach novel drug targets (1)

One pathway validated as essential for the survival of many Gram-negative pathogens is that needed for the biosynthesis of Lipid A, the hydrophobic component of the lipopolysaccharides that dominate the outer membrane. The fourth step in the pathway is carried out by the enzyme LpxH, which cleaves the pyrophosphate bond of UDP-2,3-diacylglucosamine to generate 2,3-diacylglucosamine 1-phosphate (lipid X) and UMP. The pathogen *Pseudomonas aeruginosa* is one of the major sources of human disease that is not properly addressed by current antibiotics. We work on LpxH enzyme from *P. aeruginosa* in the present study.

The project is at an earlier stage and *P. aeruginosa* LpxH was overproduced in *E.coli,* purified and crystallized using the hanging drop vapor diffusion method in the presence of two substrate analogues. X-ray diffraction data to 2.6Å resolution were collected from an orthorhombic crystal form belonging to the space group $C222_1$. Structural analysis by molecular replacement in in progress.

(1). Nayar, A. S., Dougherty, T. J., Ferguson, K. E., Granger, B. A., McWilliams, L., Stacey, C., Leach, L. J., Narita, S., Tokuda, H., Miller, A., Brown, D. G.,

NUDT15 Hydrolyzes 6-Thio-DeoxyGTP to Mediate the Anticancer Efficacy of 6-Thioguanine

Nicholas C.K.Valerie, Anna Hagenkort, Brent D.G. Page, Geoffrey Masuyer*, Daniel Rehling*, Megan Carter*, Luka Bevc, Patrick Herr, Evert Homan, Nina G. Sheppard, Pål Stenmark*, Ann-Sofie Jemth, and Thomas Helleday

Science for Life Laboratory, Division of Translational Medicine and Chemical Biology, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden, * Department of Biochemistry and Biophysics, Stockholm University, Stockholm,Sweden.

Thiopurines are a standard treatment for childhood leukemia, but like all chemotherapeutics, their use is limited by inherent or acquired resistance in patients. Recently, the nucleoside diphosphate hydrolase NUDT15 has received attention on the basis of its ability to hydrolyze the thiopurine effector metabolites 6-thio-deoxyGTP (6-thio-dGTP) and 6-thio-GTP, thereby limiting the efficacy of thiopurines. In particular, increasing evidence suggests an association between the NUDT15 missense variant, R139C, and thiopurine sensitivity. In this study, we elucidated the role of NUDT15 and NUDT15 R139C in thiopurine metabolism. In vitro and cellular results argued that 6-thio-dGTP and 6-thio-GTP are favored substrates for NUDT15, a finding supported by a crystallographic determination of NUDT15 in complex with 6-thio-GMP. We found that NUDT15 R139C mutation did not affect enzymatic activity but instead negatively influenced protein stability, likely due to a loss of supportive intramolecular bonds that caused rapid proteasomal degradation in cells. Mechanistic investigations in cells indicated that NUDT15 ablation potentiated induction of the DNA damage checkpoint and cancer cell death by 6-thioguanine. Taken together, our results defined how NUDT15 limits thiopurine efficacy and how genetic ablation via the R139C missense mutation confers sensitivity to thiopurine treatment in patients. *Cancer Res 2016; 76(18); 5501–11.*

Structural and functional insights into the energetic components of type IV secretion system

Saima Rehman[1] and Ronnie Berntsson[1]

[1]Department of Medical Biochemistry and Biophysics, Umeå University, Sweden

The type IV secretion system (T4SS) is a sophisticated nano-machinery that is responsible for pathogenicity and also for the transmission of genetic material via conjugation, during which large DNA-protein complexes are transported. As a consequence, genes encoding for, among others, antibiotic resistance are being horizontally transferred from donor to the recipient bacteria (1-3). The antibiotic resistance is an alarming issue in this era, especially in the health care system, resulting in nosocomial infections. So far, only a few model systems of T4SSs have been studied in any detail and there is no structural knowledge available for gram positive T4SS.

Our current project will decipher the structural and functional mechanism of the energetic proteins of a T4SS from *Enterococcus faecali*. The whole process of substrate transfer is powered by a couple of putative ATPases and initial DNA processing and transmission is completed with the help of a relaxase and accessory protein. The significant differences in gram positive and gram negative bacteria avert us to rely on the information obtained exclusively by gram negative bacteria. For instance, the recruitment of the substrate DNA in conjunction with other proteins and involvement of these ATPases during conjugation remain to be clarified. We are keen to obtain the atomic resolution structures of these energetic proteins, in apo forms and/or in cargo with DNA substrate and other proteins (coupling protein, relaxase, ATPase and piloting protein), by exploiting X-ray crystallography appended with biochemical analyses. Furthermore, these heterogeneous assemblies (ca 200kDa) will be structurally explored at near-atomic resolution with the help of single particle cryo-EM. Up to now, we have successfully cloned and expressed these proteins and have obtained initial crystals for a couple of them. Interaction studies on these proteins are being investigated with the help of ITC and SPR.

The findings will allow us to understand in depth, the molecular architecture and mechanism of conjugation system, especially the crucial part played by the energetic proteins, which in turn can lead to potential targets for robust antibiotics.

[1] Christie, P. J., *et al*. *J Annu Rev Microbiol* 59, 451–485 (2005).
[2] Li, F., et al. *J Bacteriol* 194.15: 4041–4051 (2012).
[3] Whitaker N., *et al*. *J Bacteriol* 197:2335–2349 (2015).

A cation-π interaction is the nucleus for an induced fit conformational transition in Adenylate kinase

Per Rogne and Magnus Wolf-Watz

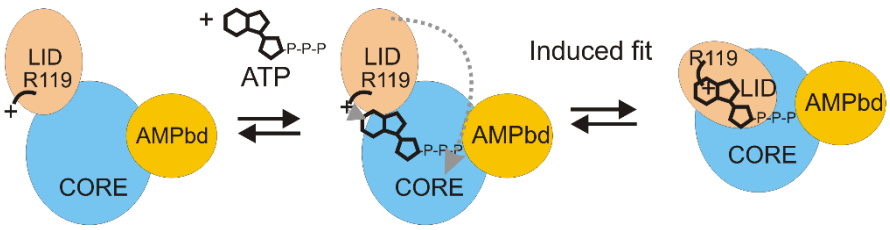
Department of Chemistry, Umeå University

Adenylate kinase populates predominantly two distinct conformations, an open inactive conformation, highly populated during substrate free conditions, and a closed and active conformation, highly populated when bound to its substrates ATP and AMP or two ADPs. During the catalytic cycle the enzyme exchanges between these two conformations. The opening of the product bound enzyme in order to release the products has been shown to be rate limiting[1].

In this study we have shown that closing of the enzyme is induced by the binding of the substrate. We found that the conformational transition is triggered by one single cation-π interaction between the π-electrons of the aromatic adenosine moiety of ATP and the positively charge of amino acid residue arginine 119.

By removing the positive side-chain of arginine 119, replacing the arginine with an alanine (R119A), we created an adenylate kinase variant that was almost completely inactive while retaining a considerable binding affinity towards ATP.

An in depth study to compare the chemical shifts of the R119A to those of the wild-type enzyme, both in the substrate bound and substrate bound forms, showed that the R119A variant was predominantly in the open state while bound to ATP. This shows that the arginine side chain is required for closing of the domain. Taken together we have discovered that an induced fit transition required for Adk activity is nucleated by a single cation-π interaction.



Schematic picture of the events leading to closure of the ATP binding domain upon substrate binding in Adenylate Kinase

[1] Wolf-Watz, M., Thai, V., Henzler-Wildman, K., Hadjipavlou, G., Eisenmesser, E.Z., and Kern, D. (2004). Nature Structural & Molecular Biology 11, 945-949.

Unique allosteric activity regulation in ribonucleotide reductases

Inna Rozman Grinberg[1], Daniel Lundin[1], Mikael Crona[2,], Mahmudul Hasan[1,3,], Venkateswara Rao Jonna[4,], Christoph Loderer[1], Margareta Sahlin[1], Natalia Markova[5], Ilya Borovok[6], Anders Hofer[4], Derek T Logan[3] and Britt-Marie Sjöberg[1]

[1]Department of Biochemistry and Biophysics, Stockholm University, Sweden, [2]Swedish Orphan Biovitrum AB, Stockholm, Sweden, [3]Department of Biochemistry and Structural Biology, Lund University, Sweden, [4]Department of Medical Biochemistry and Biophysics, Umeå University, Sweden, [5]Malvern Instruments Nordic AB, Solna, Sweden and [6]Department of Molecular Microbiology and Biotechnology, Tel-Aviv University, Israel.

Ribonucleotide reductases (RNRs) are essential enzymes in all free-living cells, providing the only known de novo pathway for the biosynthesis of deoxyribonucleotides (dNTPs), the immediate precursors for DNA synthesis and repair (Hofer *et al*, 2012). RNRs are important targets in anti-microbial and anti-cancer therapeutic research and therefore have been extensively studied.

RNRs catalyze the controlled reduction of all four ribonucleotides using radical chemistry and are classified into three major classes based on their mechanism of radical generation and quaternary structural differences (Reichard, 2010). Class I RNRs consist of a large, catalytic subunit and a smaller, radical-generating subunit, which together form the active complex.

To avoid misbalanced levels of dNTPs, and as a consequence increased mutation rates, RNRs are tightly controlled. Allosteric regulation of RNRs affects both substrate specificity and overall activity. The specificity-site binds dNTPs and determines which nucleotide will be reduced at the active site to ensure balanced levels of the four dNTPs in the cell. Additionally, most members of class I RNRs, many class III RNRs, and some class II RNRs, possess an overall activity regulation site of ~100 amino acid residues called the ATP-cone (Aravind *et al*, 2000). Acting as a regulatory master switch, the allosteric activity site senses intracellular nucleotide concentrations by competitive binding of ATP and dATP. When ATP is bound, the enzyme is active, and when concentrations of deoxyribonucleotides rise, binding of dATP switches the enzyme off. This mechanism ensures sufficient but not excessive amounts of nucleotides. The overall enzyme activity is regulated via subunit oligomerization. Interestingly, the structure and organization of subunits in active and inactive complexes varies dramatically between species (Hofer *et al*, 2012).

Here we present a novel mechanism of activity regulation in ribonucleotide reductases. Structural and functional characterization of a selected ribonucleotide reductase is shown.

References:

Aravind L, Wolf YI, Koonin EV, (2000). "The ATP-cone: an evolutionarily mobile, ATP-binding regulatory domain." J Mol Microbiol Biotechnol 2(2): 191-194

Hofer, A., et al. (2012). "DNA building blocks: keeping control of manufacture." Crit Rev Biochem Mol Biol 47(1): 50-63.

Reichard P. (2010). "Ribonucleotide reductases: substrate specificity by allostery." Biochem Biophys Res Commun 21; 396(1):19-23.

Chemical treatment of the *Cv*-omega-transaminase for the stabilization of the Lys-PLP covalent bond

Federica Ruggieri[1,2], Derek T. Logan[1], Björn Walse[1], Per Berglund[2]

[1] SARomics Biostructures AB, Lund, Sweden and [2]Royal Institute of Technology, Stockholm, Sweden.

Structures of enzyme-cofactor complexes are of great interest for characterization of the active-site pocket and clarification of the catalytic mechanism based on the cofactor's chemical environment.

Transaminases are pyridoxal-5'-phosphate-dependent enzymes that catalyse the reversible reductive amination of ketones in a stereoselective fashion following a bi-bi ping-pong mechanism. These industrially important enzymes are particularly interesting for application in the synthesis of chiral amines with high enantiopurity. Both R- and S-selective transaminases are known, and some successful examples of enzyme engineering have been reported for both classes, mostly achieved by consecutive cycles of random mutagenesis and screening[1]. The rationalisation of the role of each mutation would aid the development of rationally designed catalysts. To do so structures of the holo-forms of transaminases are needed.

Transaminases are usually active as homodimers, and in their resting state the cofactor PLP is covalently bound to the side-chain nitrogen of a catalytic lysine to form a Schiff base. This linkage is alternatively formed and broken throughout the catalytic cycle, and it appears to be prone to breakage in the presence of light (unpublished data). Previously published structures of the *Chromobacterium violaceum* (S)-amine-transaminase revealed significant rearrangement of an active-site loop upon cofactor binding[2], and preliminary data suggest that the phosphate group of the cofactor might play a role in stabilizing the active homodimer (unpublished data).

In the course of the present project, a methodology to stabilise the covalent Lys-PLP bond has been developed, thus reducing light sensitivity and preventing the cofactor leakage responsible for active site conformational changes and possibly loss of dimerization. The methodology, based on chemical reduction of the Schiff-base bond, did not cause any unwanted chemical alteration of the protein when compared to non-reduced structures and resulted in a 10 °C increase in the T_m values.

UV measurements support the effectiveness of the approach, the spectroscopical properties of the treated protein being more similar to those of the PMP-enzyme (aminated free cofactor intermediate), in that neither enzyme form shows the characteristic absorption peak due to the Schiff base (λ = 400 nm).

Sub-optimal reduction performed prior to co-crystallization with a substrate allowed us to obtain mixed PMP/PLP complexes. The cofactor ring tilt observed in this structure is in agreement with data independently published by others[3].

[1] Savile C.K. et al., *Science* **329**, 305-309 (2010).
[2] Humble M.S et al., *FEBS J.* **279**, 779-792 (2012)
[3] Ngo H.P.T. et al, Acta Cryst. D**70**, 596-606 (2014)

USP14 – a biophysical investigation of small-molecule binding to a deubiquitination enzyme

Johannes Salomonsson, Vivian Morad, Alexandra Ahlner, Eleonore von Castelmur, Maria Sunnerhagen, Padraig D'Arcy* and Stig Linder*

Division of Chemistry, Department of Physics, Chemistry and Biology, Linköping University, *Cancer Center Karolinska, Department of Oncology and Pathology, Karolinska Institute

The role of ubiquitin-proteasome pathway (UPS) is essential to regulate most cellular processes through degradation of involved proteins. In cancer cells are proteasomal activity increased and related to cell proliferation and survival. Studies have shown proteasome inhibitors successfully inducing apoptosis of multiple myeloma (MM) cells, but due to resistance a new target for therapy may be appropriate.

USP14, ubiquitin-specific protease-14, is a deubiquitinating enzyme responsible for cleaving the isopeptide bond between ubiquitin and its substrate protein, making it available for degradation by the proteasome. USP14 are more overexpressed in MM cells compared to regular cells and inhibition of its activity may decrease proliferation.

USP14 consists of two domains, the C-terminal catalytic domain responsible for cleaving the isopeptide bond between ubiquitin and its substrate protein, and the N-terminal ubiquitin-like domain which is involved in recruitment of the proteasome. Three constructs of USP14, the full-length protein, the C-terminal domain and the N-terminal domain are expressed, purified and screened towards ten different small molecules potential to inhibit USP14s function to determine if and where binding occurs.

In this presentation, we will show our results on binding and stability for our USP14 constructs, indicating a pronounced role for the C-terminal domain in ligand binding. We have used circular dichroism spectroscopy, fluorescence spectroscopy and light scattering to study thermal stability, secondary structure composition and ligand binding

References:
Min Shen et al. *Targeting the ubiquitin–proteasome system for cancer therapy. Expert Opinion on Therapeutic Target*, Volume 17, 1091-1108 (2013)
Ze Tian et al. *A novel small molecule inhibitor of deubiquitylating enzyme USP14 and UCHL5 induces apoptosis in multiple myeloma and overcomes bortezomib resistance*. BLOOD, Volume 123, 706-716 (2014)
Min Hu et al*. Structure and mechanisms of the proteasome-associated deubiquitinating enzyme USP14*. The EMBO Journal, Volume 24, 3747–3756 (2005)

Synthesis of Galectin-3 inhibitors

Daniel Sarabi, Priya Verma*, Ulf Nilsson**

Lund University, *Lund University, ** Lund University

Galectins are a family of animal lectins and are characterized by their binding affinity to β-galactosides. Inhibition of this protein has shown anti-cancer effects in a mouse model. The aim of this project was to synthesize carbohydrate based galectin-3 inhibitors, amide derivatives, and coumarin derivatives from anomeric propargyl amide of galactose. Amide derivatives with butylamine, 3-amino-1-propanol, propargylamine were successfully synthesized as well as a 7-chloro-coumarin derivative. K_d-values of these 4 compounds were evaluated against Galectin-3 using fluorescence polarization assay. Amide derivatives with butylamine, 3-amino-1-propanol showed binding to Galectin-3 with a K_d-value of 1400 µM and 1000 µM respectively. Both compounds show indeed a better binding affinity than that of the natural substrate, β-D-Galactose with K_d-value of 4400 µM against Galectin-3. Compounds with propargylamine and chloro coumarin amide derivatives were non-binding at low concentrations. However, future work may include various reactions on propargylamide alkyne, such as synthesis of 2-Bromoimidazoles and substituted pyrroles bearing alkyl, aryl, and heteroaryl substituents in order to extend further towards subsite E of galectin-3.

Visualizing the inhibitory synapse: Structural studies of the Glycine receptor and gephyrin using electron microscopy

Andreea Scacioc[1,2]*, Tales Rocha de Moura[1,2]*, Carsten Mim[1,2]

Royal Istitute of Technology (KTH), Karolinska Institutet

*shared authorship

1. *Background*

Our understanding how signals are transmitted and integrated in the nervous system, depends on our knowledge about the regulation of inhibition and excitation. The glycinergic synapse is a great model system to gain insights into inhibition because of its simple anatomy. Moreover, studying the glycinergic synapse is synonymous to gaining knowledge about the mechanism of pain to control it.

Glycine receptors (GlyR) are the main components of the glycinergic synapse. They are ligand gated ion channels conducting chloride as soon as glycine binds to their extracellular domain. On their cytosolic side, GlyRs bind gephyrin – a scaffold protein. Gephyrin is important for anchoring and trafficking GlyRs, but also to recruit other components of the glycinergic synapse.

Recently, two structures of the GlyR were determined. However, these structures miss functionally important parts of the GlyR, i.e., the gephyrin binding loop. Moreover, these structures are determined in detergent rather than in a lipid membrane. Similarly, no full-length gephyrin structures are available neither of free gephyrin nor when assembled in membrane-associated scaffoldings.

2. *Aims, approaches and preliminary results*

The aim of this project is to express and purify components of the glycinergic synapse, in their physiologically relevant state, so we can assemble the glycinergic synapse *in vitro*. Next, we aim to study both individual components and the reconstituted inhibitory synapse through electron microscopy (EM).

Aim 1 – Visualizing the full-length Glycine receptor in a lipidic membrane (or stabilized by amphipols):
We hypothesize that the GlyR interactions with lipids are important for its conformation. This is why, we aim to visualize GlyR in 2D crystals, nanodiscs and amphipols, using electron microscopy and electron crystallography. The GlyR purification was modified in towards reconstitution into a lipidic environment. Currently, 2D crystals are being optimized while nanodiscs and amphipols samples are being visualized using cryo-EM.

Aim 2 – Atomistic studies of full-length gephyrin in solution and as a membrane scaffold:
Once the heteropentameric GlyR is purified and reconstituted in a lipid membrane, the next building block towards the synthetic inhibitory synapse is the addition of its scaffolding protein, gephyrin. We aim at understanding the scaffolding mechanism of gephyrin and, in particular, the transition from the solution conformation of gephyrin to its scaffold conformation. Towards this end, the full-length gephyrin purification was optimized for cryo-EM. Next, GraFix was used to further improve the sample. Finally, we collected data for gephyrin in solution using cryo-EM. A preliminary low resolution model for its full-length conformation was obtained.

Fragment-screening studies on the ecto-5'-nucleotidase CD73

Emma Rose Scaletti*, Franziska Huschmann**, Uwe Mueller**, Manfred S. Weiss** and Norbert Straeter*

* Univeristy of Leipzig, Germany ** Helmholtz-Zentrum Berlin, Germany

The purinergic signalling cascade involves the hydrolysis of ATP to ADP, AMP and finally adenosine and free phosphate [1]. These molecules act on P1, P2X and P2Y receptors, which influence a wide range of physiological process such as cell proliferation, differentiation and cell death [2]. The pathway is regulated by a family of cell surface located ecto-nucleotidase enzymes including the nucleotide triphosphate diphosphohydrolases (NTPDases) and ecto-5'-nucleotidase (e5NT, also known as CD73) [3].

E5NT catalyses the hydrolysis of AMP and thus represents a key control point for the levels of extracellular adenosine [1,4]. E5NT has been shown to be overexpressed in a multitude of cancer cell types, where the increased levels of adenosine impair adaptive antitumor immune responses, thereby enhancing tumour growth and metastasis [5-6]. Structural studies of e5NT indicate that the enzyme is a homodimer, in which each individual monomer consists of distinct two domains. The enzyme exists in both open and closed conformations, where a large domain rotation of 114° is required for substrate binding and product release [7]. Interestingly, ADP and ATP are competitive inhibitors of e5NT, with the best characterized inhibitor being the ADP analogue adenosine 5'-(α,β-methylene)diphosphate (AMPCP) [8]. Unfortunately, while these types compounds are potent inhibitors of e5NT they have disadvantages. They are not orally bioavailable and may indiscriminately target other enzymes that use nucleotides as substrates, limiting their usefulness as potential drugs [3,7].

To identify inhibitors of e5NT which display higher specificity for the enzyme, a fragment-based screening approach was utilised. E5NT was expressed in *E. coli* as inclusion bodies, following which the protein was refolded and purified. E5NT crystals were grown and soaked with individual compounds from the selected fragment library (containing 100 fragments). Diffraction data was collected, following which 100 structures were solved, refined and inspected for fragment binding. Of the 100 fragments tested, 10 structures contained electron density consistent with ligand binding. Four fragments were located in the substrate-binding domain, where molecules such as adenosine and AMPCP have previously been shown to interact. Six fragments were located in areas of crystal-mediated binding. Two fragments displayed binding modes distinct from the substrate-binding site and represent potential allosteric inhibitors of e5NT.

References:

1. Zimmermann, H. (1992). *Biochem. J.* **285**, 345–365.
2. Krissinel, E., & Henrick, K. (2004). *Acta Crystallogr. D Biol. Crystallogr.* **60**, 2256–2268.
3. Zebisch, M., & Straeter, N. (2008). *Proc. Natl. Acad. Sci. USA.* **105**, 6882–6887.
4. Braun, N., Brendel, P., & Zimmermann, H. (1995). *Brain Res. Dev. Brain Res.* **88**, 79–86.
5. Stagg, J., & Smyth, M.J. (2010). *Oncogene.* **29**, 5346–5358.
6. Zhang, B. (2012). *Onco. Immunology.* **1**, 67–70.
7. Knapp, K., Zebisch, M., Pippel, J., El-Tayeb, A., & Straeter, N. (2012). *Structure.* **20**, 2161-2173.
8. Garcıa-Ayllon, M.S., Vidal, C.J., & Munoz-Delgado, E. (2001). *J. Neurosci. Res.* **66**, 656–665.

A Fine-tuned Composition of Protein Nanofibrils Yields an Upgraded Functionality of Displayed Antibody Binding Domains

Benjamin Schmuck, Mats Sandgren, Torleif Härd

Department of Molecular Sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden

Elevated performance of instruments and electronic devices is frequently attained through miniaturization of the involved components, which increases the number of functional units in a given volume. Analogously, to conquer the limitations of materials used for the purification of monoclonal antibodies and for the sensitivity of immunoassays, the support for capturing antibodies requires miniaturization. A suitable scaffold for this purpose are cross–β structured protein nanofibrils, as they offer a superior surface area over volume ratio and because manipulation can be implemented genetically. To display the antibody binding Z-domain dimers (ZZ)[1] along the surface of the fibrils and grant maximal accessibility to the functional units, the N-terminal fragments of the fibrillating translation release factor Sup35[2] or ureidosuccinate transporter Ure2[3], both from *Saccharomyces cerevisae,* are simultaneously fibrillated with the chimeric-proteins Sup35-ZZ and ZZ-Ure2, respectively. Optimization of the fibril composition yields a binding capacity of 1.8 mg antibody per mg fibril, which is a binding capacity that is almost 20-fold higher, compared to the commercially available affinity medium gold standard, protein A sepharose. This study lifts the craft of nanofibril functionalization to the next level, and offers a universal framework to improve biomaterials that rely on the display of functional proteins or enzymes[4].

1. Nilsson, J., et al., *Competitive elution of protein A fusion proteins allows specific recovery under mild conditions.* European journal of biochemistry / FEBS, 1994. **224**(1): p. 103-8.
2. Glover, J.R., et al., *Self-seeded fibers formed by Sup35, the protein determinant of [PSI+], a heritable prion-like factor of S. cerevisiae.* Cell, 1997. **89**(5): p. 811-9.
3. Maddelein, M.L. and R.B. Wickner, *Two prion-inducing regions of Ure2p are nonoverlapping.* Mol Cell Biol, 1999. **19**(6): p. 4516-24.
4. Schmuck, B., M. Sandgren, and T. Hard, *A Fine-tuned Composition of Protein Nanofibrils Yields an Upgraded Functionality of Displayed Antibody Binding Domains.* Biotechnol J, 2017.

Shedding light on miRNA targeting through structure

The miR34-a/CD44 complex as a model

Luis Silva[1] , Katja Petzold[1]

[1]Department of Medical Biochemistry and Biophysics, Karolinska Institutet, SE-171 77

Abstract: MicroRNAs, ~22nt RNAs, are endogenous RNAs that can act either as an on/off-switch or a fine tuner of gene expression[1], either through promoting degradation or translation repression of a target mRNA[2]. It is known that seed pairing is an important parameter for targeting[2], but there are still missing links on why it targets a certain mRNA and how the decision between degradation or translation repression is made. Previous studies have tried to shed light on this questions[3,4] probing into structural features of miRNA:mRNA complex or Argonaute:miRNA complex, but only extrapolated the importance of seed pairing. In our work, we use liquid state NMR spectroscopy, EMSA and UV melting experiments to biophysically characterise the complex of miRNA-34a with the target mRNA of CD44[5]. Here we present the NMR sample preperation as well as the first steps for the biophysical description of the mRNA:miRNA complex in question both with and without the Argonaut protein. CD44 will also be compared with another target to highlight targeting differences.

1.Mukherji, S. *et al.* MicroRNAs can generate thresholds in target gene expression. *Nat Genet* **43,** 854–859 (2011).
2.Bartel, D. P. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* **116,** 281–297 (2004).
3.Plavec, J. & Cevec, M. Solution Structure of miRNA:mRNA Complex. *MicroRNAs and the Immune System* (2010).
4.Nakanishi, K., Weinberg, D. E., Bartel, D. P. & Patel, D. J. Structure of yeast Argonaute with guide RNA. *Nature* **486,** 368 (2012).
5.Rokavec, M., Li, H., Jiang, L. & Hermeking, H. The p53/miR-34 axis in development and disease. *Journal of Molecular Cell Biology* (2014). doi:10.1093/jmcb/mju003

Structural and functional characterization of PsaBCA, the manganese transporter in *Streptococcus pneumoniae*

Jennie Sjöhamn[1], David Aragão[2], Norimichi Nomura[3, 4], So Iwata[3, 4, 5], Christopher McDevitt[6] and Megan J Maher[1]

[1]Department of Biochemistry and Genetics, La Trobe Institute for Molecular Science, La Trobe University, Melbourne, Australia. [2] Australian Synchrotron, 800 Blackburn Road, Clayton, Victoria, 3168, Australia. [3] Department of Cell Biology, Graduate School of Medicine, Kyoto University, Kyoto, Japan. [4] Research Acceleration Program, Membrane Protein Crystallography Project, Japan Science and Technology Agency, Kyoto, Japan. [5] RIKEN SPring-8 Center, SPring-8, Hyogo, Japan. [6] Research Center for Infectious Diseases, School of Biological Sciences, The University of Adelaide, Adelaide, South Australia, Australia

Streptococcus pneumoniae is the world's foremost bacterial pathogen, being responsible for more than one million deaths worldwide annually. In order to survive in the host environment, *S. pneumoniae* has an absolute requirement for the trace element manganese (Mn) Manganese is specifically acquired by the ABC transporter PsaBCA, which includes three subunits: a nucleotide binding domain (PsaB), a transmembrane domain (PsaC) and a substrate binding domain (PsaA).

I have successfully overexpressed the PsaBC complex in *Escherichia coli* with yields reaching 1 mg per litre culture. Purification of the protein complex in a number of different detergents have resulted in several crystallization conditions that are currently being further optimized. PsaBC crystals exhibit a variety of crystal morphologies growing in different conditions. However, in order to solve the structure to atomic resolution the crystals need optimizing.

To aid in the crystallization of PsaBC, a number of Fab antibody fragments have been generated and screened for crystallization. These different PsaBC:Fab complexes have revealed crystallization conditions that are distinctively different from the PsaBC on its own, and new crystal morphologies have been found, suggesting that it is indeed the PsaBC-Fab complex that is crystallizing. With the Fab fragment present in these crystals, it is possible that the larger soluble domains improve crystal contacts and aid in a better packing in the crystal which would give higher order in the crystal, resulting in higher resolution and quality of the diffraction observed. The Fab fragment can also be used as a model in molecular replacement, which would be practical in the phasing of the obtained data.

I will present our recent progress toward the structural and functional characterisation of PsaBCA, which will allow us to understand the mechanism of manganese transport and its specificity in *S. pneumonia*. The work presented here has implications for the potential treatment of *Streptococcus pneumoniae* infections.

Structural insights into the architecture of human Importin4_histone H3/H4_Asf1a complex and its histone H3 tail binding.

Jungmin Yoon[1], Sojin An[2], Alexander Leitner[3], Seung Joong Kim[4], Taeyang Jung[1,6], Ruedi Aebersold[3,5], Hans Hebert[6], Uhn-soo Cho[2], Ji-Joon Song[1,7]

[1]Department of Biological Sciences, entury, Korea Advanced Institute of Science and Technology (KAIST), Daejeon 34141, Korea. [2]Department of Biological Chemistry, University of Michigan Medical School, Michigan 48109, USA. [3]Department of Biology, Institute of Molecular Systems Biology, ETH Zurich, 8093 Zurich, Switzerland. [4]Department of Bioengineering and Therapeutic Sciences, Department of Pharmaceutical Chemistry, California Institute for Quantitative Biosciences, University of California, San Francisco, San Francisco, CA 94158, USA. [5]Faculty of Science, University of Zurich, Zurich, Switzerland. [6]Department of Biosciences and Nutrition, Karolinska Institutet, and School of Technology and Health, KTH Royal Institute of Technology, Novum, SE-141 57, Sweden

[7]Correspondence should be addressed to
Ji-Joon Song, songj@kaist.ac.kr

Importin4 transports histone H3/H4 in complex with Asf1a to the nucleus for chromatin assembly. Importin4 recognizes the nuclear localization sequence located at the N-terminal tail of histones. Here, we analyzed the structures and interactions of human Importin4, histones and Asf1a by cross-linking mass spectrometry, X-ray crystallography, negative-stain electron microscopy, small-angle X-ray scattering and integrative modeling. The XL-MS data showed that the C-terminal region of Importin4 interacts exclusively with the histone H3 tail. We determined the crystal structure of the C-terminal region of Importin4 bound to the histone H3 peptide, thus revealing that the acidic path in Importin4 accommodates the histone H3 tail and that histone H3 K14 is the primary residue interacting with Importin4. Furthermore, the structure of the Importin4_histone H3/H4_Asf1a complex computed through an integrative modeling approach reveals the overall architecture of the complex. Overall, this work provides structural insights into how Importin4 recognizes histones and their chaperone complex.

Structural studies of epoxide hydrolase mechanism of LTA_4H and its dynamic domain motion

Alena Stsiapanava and Jesper Z. Haeggström

Division of Physiological Chemistry II, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden

Leukotriene (LT) A_4 hydrolase/aminopeptidase (EC 3.3.2.6) is a bifunctional cytosolic zinc metallo-enzyme that was discovered through its ability to convert the highly unstable allylic epoxide LTA_4 into pro-inflammatory LTB_4 via an epoxide hydrolase (EH) reaction[1]. LTB_4 is a lipid mediator in innate immune response that is implicated in a number of human pathologies, particularly those involving chronic inflammation[2]. As aminopeptidase, the enzyme has an anti-inflammatory activity by cleaving the neutrophil attractant tripeptide Pro-Gly-Pro[3]. Hence, LTA_4H plays key roles in both the initiation and resolution phases of inflammation.

In this work, we describe for the first time two distinct conformations of human LTA_4H, suggesting that dynamic domain motion underlies its catalytic cycle. In its open conformation, the enzyme allows entry of the long LTA_4 substrate into its narrow hydrophobic pocket. After substrate binding, a domain movement both traps LTA_4 and aligns active site residues, so that the enzymatic reaction can proceed via a S_N1 mechanism involving formation of a carbocation intermediate. Two catalytic waters are implicated in the opening of the epoxide moiety and stereospecific insertion of a 12(R)-hydroxyl group into the intermediate product. Together with a rearrangement of the geometry of conjugated triene to cis-trans-trans configuration, this leads to the formation of physiologically active LTB_4. Finally, we found that LTA_4H uses the same water molecule for both enzymatic activities, EH and aminopeptidase. We believe that our studies of the EH mechanism provide important information for academic and industrial units engaged in the development of LTA_4H inhibitors that selectively block the synthesis of pro-inflammatory LTB_4.

1. Haeggström, J. Z., Wetterholm, A., Vallee, B. L. & Samuelsson, B. Leukotriene A4 hydrolase: an epoxide hydrolase with peptidase activity. *Biochem. Biophys. Res. Commun.* **173,** 431–437 (1990).
2. Peters-Golden, M. & Henderson, W. R., Jr. Leukotrienes. *N. Engl. J. Med.* **357,** 1841–1854 (2007).
3. Snelgrove, R. J. *et al.* A critical role for LTA4H in limiting chronic pulmonary neutrophilic inflammation. *Science* **330,** 90–94 (2010).

Structural studies on the Wag31 antigen from *Mycobacterium tuberculosis*

Adrian Suarez Covarrubias*, Terese Bergfors*, Yeasmeen Ali**, T. Alwyn Jones* and Sherry L. Mowbray*

From the *Department of Cell & Molecular Biology, Uppsala University, S-751 24 Uppsala, Sweden.
**University of Chittagong, Bangladesh

Abstract : Our group is committed to drug discovery, targeting infectious diseases such as tuberculosis and malaria. To enable structure-based design of inhibitory molecules that can provide new weapons in the fight against *Mycobacterium tuberculosis*, we engage in the structure determination of proteins from this organism, concentrating on ones that are essential for its survival.

The product of the *rv2145c* gene, Wag31 (also referred to as DivIVA or Antigen 84), is a 260-residue protein with roles in peptidoglycan biosynthesis and cell division. Its interactions with other proteins are critical in these roles.

Wag31 is predicted to be a highly helical protein; there is furthermore strong evidence for coiled-coil features that may be linked with oligomerization of Wag31, or associations with other proteins (1-4). This could be related to observations that a significant portion of the protein is found in cell wall and membrane fractions in *M. tuberculosis*. The similarity to proteins of the DivIVA class is found at the N-terminal end of the sequence referred to as the lipid-binding domain (LBD)

We obtained diffraction-quality crystals of the LBD (first 60 residues) of Wag31, and collected data to a resolution of 1.6 Å. The structure was solved by molecular replacement using the N-terminal region (60 aa) of *Bacillus subtilis* DivIVA (pdb code 2WUJ) as search model with 43% sequence identity. The structure will help us understand the anchoring to the membrane, and possibly interactions with proteins coded for by genes in an apparent operon with the Wag31 gene, which could be forming a complex.

1.-Lenarcic et al. EMBO J (2009). Localization of DivIVA by targeting to negatively curved membranes.
2.-Oliva et al. EMBO J (2010). Features critical for membrane binding revealed by DivIVA crystal structure.
3.- Meniche et al. PNAS (2014). Subpolar addition of new cell wall is directed by DivIVA in mycobacteria.
4.-Singh et al. Molecular Microbiology (2017). Identification of aminopyrimidine-sulfonamides as potent modulators of Wag31-mediated cell elongation in mycobacteria.

Production and characterization of microcrystals for serial femtosecond crystallography

Martin Söderström[1], Elin Claesson[1], Petra Edlund[1] and Sebastian Westenhoff[1]

[1]Gothenburg University, department for Biochemistry and Molecular Biology

Phytochromes are a family of red and far-red light sensing photoreceptors found in most plants, bacteria and fungi. They are highly important for the regulation of plant growth by light signals (photomorphogenesis) and control diverse processes such as seed germination and shade avoidance.

Light absorption occurs at a covalently attached tetrapyrrole chromophore that undergoes photoisomerization. This event propagates global structural changes in the protein and allows the protein to undergo a conformational switch between a red (Pr) and far red (Pfr) absorbing state upon light exposure. The photocycle has been characterized in detailed spectroscopic studies [summarized in our paper: Björling, Science Advances, 2016] but as of yet the initial conformational changes following photoisomerization are poorly understood.

The conformational changes occur at picosecond to millisecond time scales and can in principle be studied by time-resolved serial femtosecond crystallography (SFX) [Chapman, Nature, 2011]. In our previous work using SFX we determined the room-temperature structure of a truncated form of a phytochrome to 2.1 Å [Edlund, Scientific Rep, 2016]. Now we wish to extend this approach to studying the entire PCM module at pico- to millisecond time scales. For this it is mandatory to grow microcrystals (5 – 10 micrometer) which diffract to 2.5 Å resolution or better.

My master thesis work is focused on screening both various phytochrome homologs and surface engineered mutants [SER: Nagano, J. Biol. Chem. 2016] to find conditions for such microcrystals. We have so far identified a great number of conditions and mutants that yield microcrystals. Currently we are screening our obtained microcrystal for diffraction quality using synchrotron microfocus X-ray beams. This allows us to identify the microcrystals that would give high resolution snapshots with SFX and thus allows us to unravel the initial steps of phytochrome light absorption at atomic resolution.

Björling, A. et al., 2016. Structural photoactivation of a full-length bacterial phytochrome. *Science Advances*, 2(8), pp.e1600920–e1600920.

Chapman, H.N. et al., 2011. Femtosecond X-ray protein nanocrystallography. *Nature*, 470(7332), pp.73–77.

Edlund, P. et al., 2016. The room temperature crystal structure of a bacterial phytochrome determined by serial femtosecond crystallography. *Scientific Reports*, 6, p.35279.

Nagano, S. et al., 2016. The crystal structures of the N-terminal photosensory core module of Agrobacterium phytochrome Agp1 as parallel and anti-parallel dimers. *Journal of Biological Chemistry*, 291(39), pp.20674–20691.

The role of metal co-factors in DNA polymerase ε

Josy ter Beek, Vimal Parkash , Pia Osterman, Göran Bylund, and Erik Johansson

Umeå University, Medical Biochemistry and Biophysics, SE-901 87 Umeå, Sweden

DNA replication depends on numerous proteins that must work together to replicate each chromosome. Central in this process are the DNA polymerases that read the single stranded template DNA and incorporate, with high accuracy, the correct nucleotide into the growing DNA strand to avoid misincorporations, which can lead to a permanent change in the DNA sequence. In eukaryotes, DNA replication is carried out by three separate DNA polymerases, that all belong to the B-family of DNA polymerases.

The eukaryotic helicase unwinds duplex DNA to provide single-stranded template for DNA polymerases. DNA polymerase α (Pol α) initiates replication by forming a short RNA/DNA primer. Pol α is replaced by DNA polymerase ε (Pol ε) on the leading [1] and DNA polymerase δ (Pol δ) on the lagging strand [2]. Lagging strand synthesis occurs in short stretches known as Okazaki fragments. In contrast, the leading strand is built by Pol ε during a largely processive and continuous DNA synthesis.

Numerous DNA repair enzymes have been shown to contain Fe-S clusters. These redox centers have been proposed to scan large parts of DNA at once via charge transfer, since lesions in the DNA helix would prevent this [3]. However this idea is very controversial as such charge transfer could lead to radical formation near the DNA and thus DNA damage.

Recently it was shown by Netz et al. [4] that Fe-S clusters are also present in eukaryotic DNA polymerases. Evidence was presented for a [4Fe-4S] cluster in the C-terminal domain (CTD) of the catalytic subunits of polymerases α (Pol1), δ (Pol3), ε (Pol2) and ζ (Rev3) of *Saccharomyces cerevisiae*.

Another article described the presence of a Fe-S cluster in Pol2 from yeast without the CTD expressed in yeast or *E. coli*. They predicted that the Fe-S was bound via a cysteine rich motif conserved in Pol2, but not present in Pol1, Pol3 or Rev3. Mutations in the predicted cysteine binding residues decreased the polymerase, but not the exonuclease function of Pol2 [5].

We investigate where the Fe-S cluster is located in yeast Pol ε, what kind of Fe-S cluster is present and what its functional role is. We have investigated the importance of the cysteine ligands and presence of the metal center for complex formation and polymerase activity.

[1] Pursell et al. Science 2007
[2] McElhinny et al. Mol. Cell. 2008
[3] Sontz et al. Acc. Chem. Res. 2012
[4] Netz et al. Nat. Chem. Biol. 2012
[5] Jain et al. JMB 2014

Glucose transporters: production, crystallization and inhibition

Raminta Venskutonytė, Jesper S. Hansen, Peter Aasted Paulsen*, Serena Fortunato**, Carlotta Granchi**, Filippo Minutolo**, Bjørn Panyella Pedersen*, Karin Lindkvist-Petersson

Dept. of Experimental Medical Science, Faculty fo Medicine, Lund University, Sweden *Dept. of Molecular Biology and Genetics, Aarhus University, Denmark **Dept. of Pharmacy, University of Pisa, Italy

Glucose transporters (GLUTs) comprise a family of 14 membrane proteins that regulate glucose uptake into the cell. Different types of GLUTs are expressed in various tissues and play a crucial role in glucose metabolism[1]. Cancer cells are highly dependant on glucose and therefore GLUTs are possible drug targets for cancer therapy. In order to block the glucose uptake facilitated by GLUTs, various inhibitors are studied and both natural and synthetic compounds having an inhibitory effect on glucose uptake have been discovered[2, 3]. High resolution X-ray structure of the GLUT-inhibitor complex would provide a detailed understanding of protein-inhibitor interactions and contribute to facilitating the development of new derivatives.

The focus of this study is on a glucose transporter 1 (GLUT1). The GLUT1 has been produced and crystallization trials set up, which resulted in microcrystals. A series of salicylketoxime based compounds[4] have been shown to inhibit GLUT1 and two lead compounds displaying the highest inhibition have been identified in a giant vesicle assay[5]. The main goal of the study is to determine the structure of the GLUT1 with selected inhibitors. Moreover, studies on one more glucose transporter GLUT3 are carried out to investigate the selectivity of the salcylketoxime compounds.

[1]Deng, D. and Yan, N. (2016), Protein Science, 25: 546–558.

[2]Barron, C. C. et al. (2016), Metabolism, 65: 124-139.

[3]Granchi, C. et al. (2014), Bioorganic & Medicinal Chemistry Letters, 24: 4915-4925.

[4]Granchi, C. et al. (2015), ChemMedChem, 10: 1892-1900.

[5]Hansen, J. S. et al., (2015), Chem. Commun., 51: 2316-2319.

Towards Structural and Functional Characterization of Zinc transporters

Anders Wiuf[1], Kamil Gotfryd[1] and Pontus Gourdon[1,2]

[1] Department of Biomedical Sciences, University of Copenhagen, Blegdamsvej 3, 2200 Copenhagen N, Denmark
[2] Department of Experimental Medical Science, Lund University, Sölvegatan 19, 221 84 Lund, Sweden

Zinc is an essential heavy metal involved in a large range of physiological and cellular processes, including cellular signalling and cell proliferation. Consequently, zinc homoeostasis has to be carefully controlled, as dysregulation is toxic to cells and can cause pathological conditions like growth retardation. The family of ZIP transporters partake in regulation of intracellular Zn^{2+} concentrations. Dysregulation of these proteins has been associated with several cancer types and is directly linked to severe genetic disorders such as acrodermatitis entreropahtica and Ehlers-Danlos syndrome.

Despite their significance for human health, very little functional and structural data is available for the ZIP family, as these membrane proteins are difficult to overproduce and purify and therefore also to characterize. The overarching aim of this project is to determine the first structure of a ZIP family member, and to provide mechanistic models for zinc transport. Currently several targets are being optimised for overproduction and purification using *E. coli* as a host.

Understanding Viral Entry Mechanisms Through Multi-Level Structural Analysis

Eleonore von Castelmur[1]

1 Department of Physics Chemistry and Biology, Linköping University

Picornaviruses are a major cause of infections in humans and animals, including common cold, poliomyelitis, hepatitis A, and foot-and-mouth disease. To identify and characterise novel viral host factors will help us gain a better understanding of the lifecycle of these biomedically important viruses, and hopefully yield candidates for the development of novel antiviral therapeutics. By employing insertional mutagenesis in haploid human cells, we identified a poorly-studied phospholipid-modifying enzyme, PLA2G16, as a common host factor for multiple picornaviruses[1]. We could show that this enzyme is required in the early steps of viral entry, enabling virion-mediated genome delivery into the cytoplasm (ahead of a viral pore-formation triggered clearance pathway involving the autophagy machinery). Remarkably, there is a group of picornaviruses whose 2A proteins share conserved sequence motifs with the human protein host factor[2], which led us to ask whether these viruses had acquired and evolved the host protein so as to become independent from the host factor.

As a structural biologist, I am currently characterising representative examples of this protein from all branches of the phylogenetic tree of viral $2A^{H\text{-}box}$ proteins, to try and trace the possible evolutionary steps of this protein. The crystal structures of H-box containing proteins we have elucidated to date[3,4] reveal a remarkable structural plasticity. Addressing how this structural plasticity correlates with the functional repurposing of the protein in the different picornaviruses will help answer still open questions on the molecular details of the picornavirus life cycle, contribute to our basic understanding of protein folding and function, and help guide rational enzyme design efforts. In this presentation, I will describe my recent and ongoing work and some of the questions I plan to address in establishing my research group at Linköping University.

---

[1] Staring, J., von Castelmur, E., Blomen, V.A., van den Hengel, L.G., Brockmann, M., Baggen, J., Thibaut, H.J., Nieuwenhuis, J., Janssen, H., van Kuppeveld, F.J.M., Perrakis, A., Carette, J.E. and Brummelkamp, T.R. "PLA2G16 represents a switch between entry and clearance of *Picornaviridae*", *Nature*. (2017) **541**(7637):412-416

[2] Hughes, P. J. and Stanway, G. "The 2A Proteins of Three Diverse Picornaviruses Are Related to Each Other and to the H-Rev107 Family of Proteins Involved in the Control of Cell Proliferation.," *The Journal of General Virology* (2000) **81**(1): 201–7.

[3] von Castelmur, E., Staring, J., van den Hengel, L., Heidebrecht, T., Leonard, G., Fry, E.E., Stuart, D.I. and Perrakis, A. "Exploring evolutionary relationships of a viral host factor and its picornavirus 2A protein homologs", manuscript in preparation

[4] von Castelmur, E., Staring, J., Zhu, L., Ren, J., Fry, E.E., Stuart, D.I. and Perrakis, A. "Structural plasticity of Parechovirus family H-box 2A proteins", manuscript in preparation

Identifying the TAILS complex component(s), a microtubule intraluminal interrupted helix found in human spermatozoa

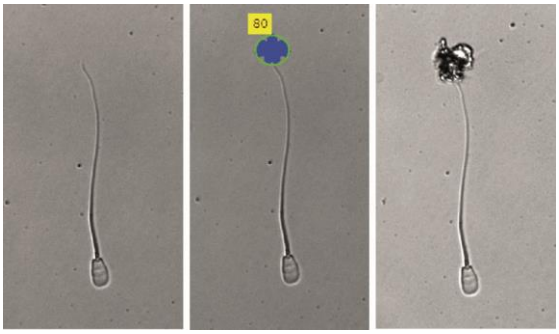
Davide Zabeo*, Mandy Rettel**, Linda Sandblad***, Per Widlund****, Mikhail Savitski**, Johanna Höög*

*Department of Chemistry and Molecular Biology, University of Gothenburg, Gothenburg, Sweden, **EMBL, Heidelberg, Germany ***Department of Molecular Biology, Umeå University, Umeå, Sweden, ****Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden

Microtubules are an important part of the cellular cytoskeleton, as they play a role in multiple fundamental cellular processes, for example in cellular motility. A large number of microtubule-associated proteins (MAPs) are used by the cell to regulate their dynamic instability, for example by promoting growth or triggering depolymerisation. While the vast majority of MAPs associate with the outer surface of microtubules, relatively little is known about the microtubule lumen and the proteins inside of it. Microtubule inner proteins (MIPs) are found inside the microtubule lumen, and bind at specific locations on the doublet microtubule lattice[1].

We identified a several micrometers long repetitive structure inside the microtubule lumen in sperm tails, by performing cryo-electron tomography (cryo-ET) on healthy human spermatozoa, frozen intact in their ejaculate. Sub-tomogram averaging revealed the structure to be an interrupted helix. This is, to our knowledge, the first time a complex structure is seen inside the microtubule lumen and we named it TAILS[2]. We speculate that TAILS might have a role in microtubule stabilization either by preventing dynamic instability or stiffening the fibers in the spermatozoon end piece.

To further investigate the role of the TAILS complex, we need to identify which protein(s) it is composed of. However, eukaryotic flagella consist of over 1000 proteins, and the human sperm tail consists of approximately 700 tail-specific proteins[3]. Our cryo-ET showed that TAILS is one of the major components of the sperm end piece with ~40.000 copies/end piece (if consisting of one protein). Therefore, we laser dissected end pieces from bull spermatozoa (image) and performed mass spectrometry of this outmost part of the sperm tails, which is so small that it was not even visible in the laser dissection set-up. A list of candidate proteins is now being assessed.



1. Nicastro, D. The Molecular Architecture of Axonemes Revealed by Cryoelectron Tomography. *Science* 313, 944–948 (2006).
2. Heumann, J. M. *et al.* Microtubules with a twist: a lumenal interrupted helix in human sperm tail microtubules. doi:10.1101/088476
3. Baker, M. A. *et al.* Head and flagella subcompartmental proteomic analysis of human spermatozoa. *Proteomics* 13, 61–74 (2013).

Cryo-EM Imaging of the Packaged Genome in the Native and Swollen Tomato Bushy Stunt Virus Particles

Daniel S. D. Larsson, Kerstin Mühlig, Anders Kvarnheden* and Janos Hajdu

Dept. of Cell and Molecular Biology, Uppsala University, Box 596, 751 24 Uppsala, Sweden, * Dept. of Plant Biology, Swedish University of Agricultural Sciences, Box 7080, 750 07 Uppsala, Sweden

Tomato bushy stunt virus was the first virus particle imaged to atomic resolution[1]. Since crystal packing is determined by particle contacts, X-ray crystallography (XRC) by necessity average over all 60 icosahedral orientations. Likewise, most electron cryo-microscopy (cryo-EM) structures of virus particles have averaged over the icosahedral symmetry. This enhances the sampling tremendously, but perhaps even more importantly, orientation determination of highly symmetric objects is very difficult. Recent improvements in cryo-EM technology with direct electron detectors now allows for the asymmetric reconstruction of virus particles to near-atomic resolution. This allows for the reconstruction of the packaged genome as well as minor proteins[2].

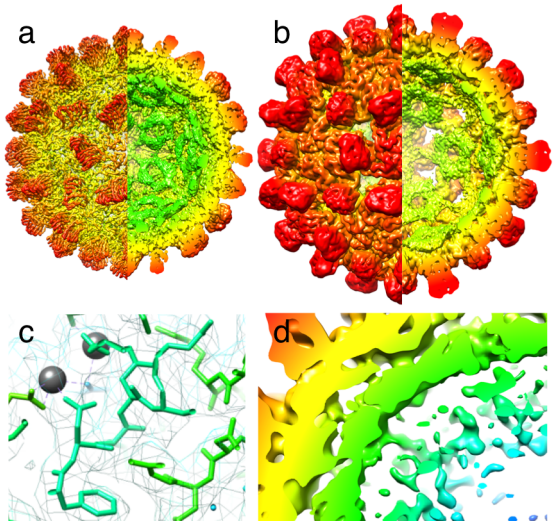


Figure 1. Reconstructions of TBSV (**a**) Native particle at 3.2 Å with radial color scale (capsid in red-yellow and RNA in green). (**b**) Swollen particle at 4.6 Å (same coloring scheme). (**c**) Detail of map in (a) showing a region of the capsid with two calcium binding sites. (**d**) Detail from an asymmetric reconstruction of the native particle showing RNA density at the center of the particle (same coloring scheme as in (a)).

In this study, we purified virus particles of tomato bushy stunt virus (TBSV) from plants and imaged them by cryo-EM using a Titan Krios microscope with a Gatan K2 Summit direct electron detector and energy filter. A dataset with approximately 32,000 particles was reconstructed with symmetry averaging to 3.2 Å resolution (Fig. 1a,c). A mesh of previously unreported density was found beneath the capsid. This density likely corresponds to structured duplex RNA in associated with the N-terminal RNA-binding domain of the coat protein. An asymmetric reconstruction of the same dataset reached 4.5 Å resolution and revealed additional RNA density at lower radii (Fig. 1d).

By mimicking the conditions of the cytoplasm of the host cell (by removing bound Ca^{2+} ions and raising the pH to slightly alkaline conditions), one can induce a swollen state of the virus particle. An 8 Å resolution XRC structure of this disassembly intermediate shows that the particle radius increase by ~10% and large pores form through the capsid[3]. We collected a dataset with approximately 6,000 particles of swollen particles and the symmetry averaged reconstruction reached 4.6 Å resolution (Fig. 1b). The structure revealed interesting structural rearrangements of the coat proteins and that the RNA cage remained associated with the capsid.

1. Harrison *et al.* (1978) Tomato bushy stunt virus at 2.9 Å resolution. *Nature* **276**: 368–373
2. Dai *et al.* (2017) In situ structures of the genome and genome-delivery apparatus in a single-stranded RNA virus. *Nature* **541**: 112–116
3. Robinson *et al.* (1982) Structure of the expanded state of tomato bushy stunt virus. *Nature* **297**: 563–568

Svenska Kemisamfundet
Wallingatan 24, 3tr • 111 24 Stockholm
08-411 52 60 • service@kemisamfundet.se
www.kemisamfundet.se