24th Swedish Conference
on

Macromolecular Structure and Function

Virtual Reality, 20-23 June 2021

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ABSTRACTS

Around The Corner

creoptix sensors

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Sprint Bioscience

SARomics

Keynote speakers

Tuesday 22, 09:00-09:40
"Structural bases of the pathological threshold in Huntington's disease. An integrative structural and chemical biology approach"
Prof. Pau Bernado**,** Centre de Biochimie Structurale (CBS), University of Montpellier, France
http://www.cbs.cnrs.fr/index.php/fr/personnel?PERS=Pau%20Bernado

Tuesday 22, 10:30-11:10
"Structural basis of protein misfolding diseases"
Prof. Dr. Marcus Fändrich, Institute of Protein Biochemistry, Ulm University, Germany
https://www.uni-ulm.de/en/nawi/institute-of-protein-biochemistry/

Monday 21, 14:00-14:40
"*Charting the organization of a human cell with proximity-dependent biotinylation"*
Dr Anne-Claude Gingras, Lunenfeld-Tanenbaum Research Institute, Toronto, Canada
http://www.lunenfeld.ca/?page=gingras-anne-claude

Tuesday 22, 10:30-11:10
"*The awesome power of Fluorine NMR"*
Prof. Angela Gronenborn**,** University of Pittsburg, USA
https://www.structbio.pitt.edu/index.php/12-faculty/2-angela-gronenborn

Sunday 20, 18:30-19:10
"*Animating molecular machines*"
Dr. Janet Iwasa**,** University of Utah, USA
https://bioscience.utah.edu/faculty/iwasa/iwasa.php

Monday 21, 09:00-09:40
"*The HIV capsid pretends to be a karyopherin to enter the nucleus"*
Dr David Jaques**,** Structural Virology, University of New South Wales, Sydney, Australia
https://sms.unsw.edu.au/david-jacques

Suggested pre-reads

Below please find suggested articles for you to dwell on before the Keynote lectures in order to prepare your minds!

Pau Bernado
Flanking Regions Determine the Structure of the Poly-Glutamine in Huntingtin through Mechanisms Common among Glutamine-Rich Human Proteins
https://pubmed.ncbi.nlm.nih.gov/32402249/

Evidence of the Reduced Abundance of Proline cis Conformation in Protein Poly Proline Tracts
https://pubmed.ncbi.nlm.nih.gov/32266815/

Marcus Fändrich
AA amyloid fibrils from diseased tissue are structurally different from in vitro formed SAA fibrils
https://pubmed.ncbi.nlm.nih.gov/33579941/

Cryo-EM structure of a transthyretin-derived amyloid fibril from a patient with hereditary ATTR amyloidosis
https://pubmed.ncbi.nlm.nih.gov/31676763/

Anne-Claude Gingras
A proximity-dependent biotinylation map of a human cell
https://pubmed.ncbi.nlm.nih.gov/34079125/

The GATOR-Rag GTPase pathway inhibits mTORC1 activation by lysosome-derived amino acids
https://pubmed.ncbi.nlm.nih.gov/33060361/

Angela Gronenborn
^{19}F-modified Proteins and ^{19}F-containing Ligands as Tools in Solution NMR Studies of Protein Interactions. https://pubmed.ncbi.nlm.nih.gov/26577728/

^{19}F Paramagnetic relaxation enhancement: A valuable tool for distance measurements in proteins.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4715678/

Janet Iwasa
Using 3D Animation to Visualize Hypotheses
https://pubmed.ncbi.nlm.nih.gov/32423744/

Preparing scientists for a visual future: Visualization is a powerful tool for research and communication but requires training and support
https://pubmed.ncbi.nlm.nih.gov/31608553/

David Jacques
Kinetics of HIV-1 capsid uncoating revealed by single-molecule analysis
https://pubmed.ncbi.nlm.nih.gov/29877795/

Rapid HIV-1 Capsid Interaction Screening Using Fluorescence Fluctuation Spectroscopy
https://pubmed.ncbi.nlm.nih.gov/33593049/

SELECTETED PRESENTATIONS

The carboxysomal carbonic anhydrase encapsulation mechanism

Cecilia Blikstad[1,3], Eli Dugan[1], Thomas Laughlin[1], Sophie Shoemaker[1], Mira Liu[1], Jonathan Remis[3] and David Savage[1]

[1] Department of Molecular and Cell Biology, University of California, Berkeley, United States,
[2] California Institute for Quantitative Biosciences (QB3), University of California, Berkeley, United States
[3] Present affiliation: Department of Chemistry – Ångström, Uppsala University, Sweden

Rubisco, the primary CO_2-fixing enzyme of the Calvin–Benson–Bassham cycle, is notorious, for being a relatively slow and nonspecific enzyme. Aside from carboxylation, it also catalyzes a reaction with O_2 that results in a costly process called photorespiration. To overcome this problem, cyanobacteria, eukaryotic algae, and some plants have developed different types of CO_2-concentrating mechanisms (CCMs), systems that increase CO_2 levels near Rubisco to inhibit photorespiration and accelerate CO_2 fixation (Figure 1B). The key component of the cyanobacterial CCM is the carboxysome, a proteinaceous bacterial organelle found in all cyanobacteria and some proteobacteria (1). Carboxysomes range from 100 to 400 nm in diameter, have a protein shell, and encapsulate carbonic anhydrase (CA) together with Rubisco (Figure 1A). Since CCMs accelerate Rubisco, there is a great interest in transplanting cyanobacterial CCMs into crop plants to increase photosynthetic efficiency (2). However, to be successful understanding fundamental principles underlying carboxysome assembly and function are essential (3).

Here I will discuss our work on elucidating the carboxysomal CA's encapsulation mechanism. First, we used biolayer interferometry (BLI) to screen all carboxysome proteins against binding to the CA and successfully identified its interaction partner. We show that the interaction and encapsulation into carboxysomes is dependent on CA's unique intrinsically disordered peptide. We further solved a sub 2 Å cryoEM structure of the interaction partner in complex with the CA peptide, revealing the binding site. Our work identifies a previously unknown supercomplex found inside the carboxysome and highlights a surprising flexibility in the scope of protein-protein interactions which lead to its self-assembly.

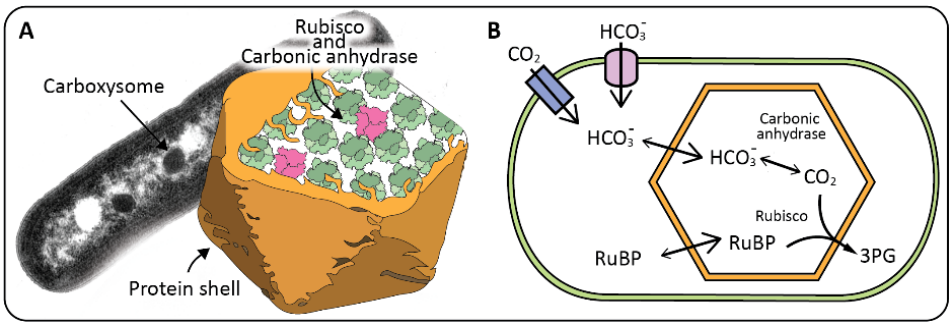

Figure 1: (A) TEM of *S. elongatus* with carboxysomes and model of the carboxysome showing the protein shell and the encapsulated Rubisco and CA. (B) Schematic of the CO_2 concentration mechanism in cyanobacteria.

References:

1. Kerfeld, C. A., and Melnicki, M. R. (2016) *Curr. Opin. Plant Biol. 31*, 66–75.
2. Long, B. *et al*., (2018) *Nat. Commun. 9*, 3570.
3. Flamholz A.I., Dugan E, Blikstad C, et al., (2020) *eLife* 2020;9:e59882.

Interaction of a human monoclonal antibody recognizing two different epitopes in a conserved region of streptococcal M proteins

Lotta Happonen[1,2], Wael Bahnan[1], Hamed Khakzad[3,4], Maria Gourdon[5], Oonagh Shannon[1], Lars Björck[1], Lars Malmström[1], Johan Malmström[1,2] and Pontus Nordenfelt[1]

[1] Lund University, Faculty of Medicine, Department of Clinical Sciences Lund, Infection Medicine, Lund, Sweden; [2] Swedish National Infrastructure for Biological Mass Spectrometry BioMS, Lund, Sweden; [3] Equipe Signalisation Calcique et Infections Microbiennes, Ecole Normale Superieure Paris-Saclay, Gif-sur-Yvette, France; [4]Institut National de la Sante et de la Recherche Medicale (INSERM) U1282, Gif-sur-Yvette, France; [5] Lund University, Lund Protein Production Platform LP3, Lund, Sweden

Group A streptococcus (GAS) is an important human-specific pathogen causing both mild infections in the upper respiratory tract as well as acute invasive diseases, and ranks globally among the top ten causes of mortality from infectious diseases. GAS has evolved an extensive array of measures to counteract the human immune response, including resistance to phagocytosis and several antibody-targeting mechanisms. Currently, there are few candidates for anti-bacterial monoclonal antibody therapy in general, and none available for GAS. Much effort has been allocated to developing vaccines against GAS, with the prime immunizing antigen being the M protein. Yet, no effective vaccine against GAS has been approved to date.

Here, we have generated antibodies derived from the memory B cells of an individual who had successfully cleared a group A streptococcal infection. We demonstrate using targeted crosslinking mass spectrometry in combination with Rosetta modeling that the antibodies bind with high affinity to the central region on the M protein. One of these antibodies, Ab25, effectively promotes vital immune functions, including phagocytosis and *in vivo* protection in mice. Due to its highly protective nature, the structure of the Ab25 Fab-domain was determined by X-ray crystallography for future therapeutic applications. Remarkably, Ab25 only interacts through dual-Fab cis mode, where the Fabs bind to two distinct epitopes on the M protein, and with conserved binding across different streptococcal strains. In contrast, another antibody isolated here binding to a single epitope in the same region does not bypass the M protein's virulent effects.

A broadly-binding, protective monoclonal antibody is a strong candidate for anti-streptococcal therapy. It also highlights the concept of dual-Fab binding and the accessibility of conserved regions for immune antibody targeting.

REFERENCE

Bahnan W, Happonen L, Khakzad H, Ahnlide VK, de Neergaard T, Wrighton S, Bratanis E, Tang D, Hellmark T, Björck L, Shannon O, Malmström L, Malmström J, Nordenfelt P. 2021. Protection induced by a human monoclonal antibody recognizing two different epitopes in a conserved region of streptococcal M proteins. bioRxiv 2021.03.01.433494.

The first structure of an enteric human adenovirus, HAdV-F41

Karim Rafie, Lars A. Carlson

Umeå University, Sweden

Abstract

Human adenovirus (HAdV) types F40 and F41 are a prominent cause of diarrhea and diarrhea-associated mortality in young children worldwide, with a roughly 500,000 deaths per year. These enteric HAdVs differ notably in tissue tropism and pathogenicity from respiratory and ocular adenoviruses, but the structural basis for this divergence has been unknown. Here, we present the first structure of an enteric HAdV—HAdV-F41—determined by cryo–electron microscopy to a resolution of 3.8 Å. The structure reveals extensive alterations to the virion exterior as compared to nonenteric HAdVs, including a unique arrangement of capsid protein IX. The structure also provides new insights into conserved aspects of HAdV architecture such as a proposed location of core protein V, which links the viral DNA to the capsid. Localised asymmetric reconstructions allowed the identification of assembly-induced conformational changes in the penton base protein. Our findings provide the structural basis for adaptation of enteric HAdVs to a fundamentally different tissue tropism.

Structural and functional characterization of UvrD mediated transcription-coupled DNA repair

Ashish A. Kawale, Björn M. Burmann

Wallenberg Centre for Molecular and Translational Medicine, Department of Chemistry and Molecular Biology, University of Gothenburg, Sweden

Maintaining a steady genome composition is one of the most critical task for the survival of any living organism. Environmental factors, such as UV irradiation, can lead to deleterious changes of the genetic material, posing a serious threat to cell survival. In order to cope with this threat cells have developed evolutionary conserved DNA–repair pathways, which mend DNA lesions and thereby, ensure genomic stability and integrity. Nucleotide Excision Repair (NER) is one of the major pathways of the DNA–repair in both prokaryotic and eukaryotic cells, which restores diverse types of DNA lesions up to 10–12 nucleotides long [1]. In bacterial cells, NER is mediated by the multistep assembly of the multiprotein UvrABCD complex, which localizes and cleaves damaged base-pairs in a synergized manner [2]. Transcription coupled repair (TCR) is an important sub-pathway of the NER, where concurrent scanning for DNA lesions is performed by the transcribing RNA Polymerase (RNAP), resulting in stalled transcription complexes at damaged sites [3].

UvrD is a multifunctional DNA Helicase (II) protein which belongs to the superfamily 1 helicases performing 3'–5' helicase function. Recent studies show that UvrD, a member of the UvrABCD NER machinery, also plays a more active role in TCR pathway based on genetic studies corroborating that UvrD mutant bacterial strains show high sensitivity towards UV-induced DNA-damage [4]. UvrD has been proposed to function by back-tracking stalled RNAP in order to recruit NER machinery facilitating DNA-repair and the subsequent restart of transcription.

Our aim is to understand the structure-function relationship of the UvrD helicase mediating TCR by employing an integrated structural biology approach with more focus on solution NMR spectroscopy. We are interested in deciphering domain dynamics of the UvrD protein and transient interactions within different TCR complexes in order to get better understanding of the atomic level details of TCR pathway. We have recently discerned the solution NMR structure of the UvrD's carboxy-terminal region harbouring a Tudor-domain fold, crucial for RNAP interaction, acting as a binding hub [5]. A compare-&-contrast backbone dynamics analysis benchmarked against other Tudor domains revealed the presence of extensive large amplitude slow timescale motions embedded within UvrD Tudor domain corroborating it's inherent binding promiscuity.

[1] J.J. Truglio, et al., *Chem. Rev.*, 106 (2006)

[2] A. Sancar and W.D. Rupp, *Cell*, 33 (1983)

[3] S. Tornaletti, P.C. Hanawalt, *Biochimie*, 81 (1999)

[4] V. Epshtein, et al., *Nature*, 505 (2014)

[5] Kawale and Burmann, *Commun Biol*, 607 (2020)

Structural changes in intrinsically disordered peptides upon adsorption to solid surfaces

Mona Koder Hamid[*], Linda Månsson[*] , Viktoriia Meklesh[**], Per Persson[**] and Marie Skepö[*,†]

*Division of Theoretical Chemistry, Department of Chemsitry, Lund University, Naturvetarvägen 14, 223 62 Lund, Sweden, **Centre for Environmental and Climate Research, Lund University, Sölvegatan 37, 223 62 Lund, Sweden, †Lund Institute of Advanced Neutron and X-ray Science (LINXS), Scheelevägen 19, 223 70 Lund, Sweden

Intrinsically disordered peptides (IDPs) are a class of proteins that have widespread occurrence but are not yet well understood. It is hypothesized that IDPs become functional from changes in their surroundings that promote secondary structure formation. To test this hypothesis, we need to improve our understanding of the conditions that induce structural changes in IDPs. Here, we use a combination of computer simulations and experiments to gain detailed insight on the mechanism of adsorption of cationic IDPs to anionic solid surfaces and the structural changes that follow. The IDPs we investigate include the cathelicidin antimicrobial peptide LL-37 (37 amino acids, 4.493 kDa) and variants obtained from exchanging amino acids. In addition, we study the peptide referred to as KEIF (33 amino acids, 3.871 kDa), which corresponds to the natively disordered, N-terminal region of Magnesium transporter A found in bacteria.[1] Results from circular dichroism and Fourier transformed infrared spectroscopy indicate an increase in helical content in IDPs upon adsorption. Atomistic molecular dynamics are in agreement with experiments and suggest that arginine binding is central in the adsorption process. These results together with ongoing studies of the adsorption of IDPs to lipid bilayers will help to elucidate the role of IDPs.

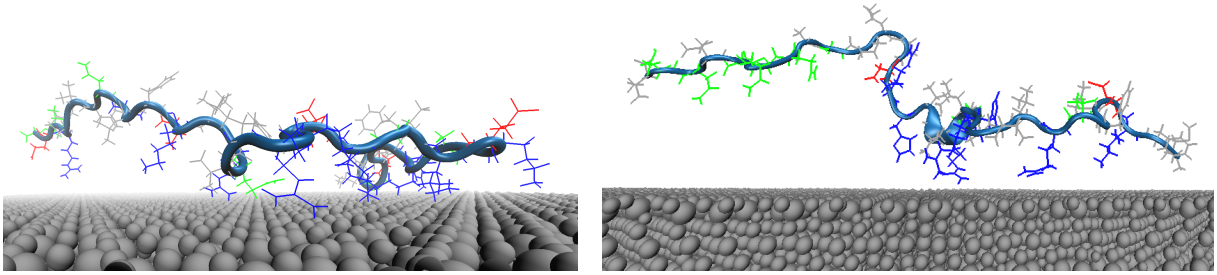

Figure 1. LL-37 (left) and KEIF (right) adsorbed to a negatively charged Laponite® clay surface (gray). Peptide residues color code: positive (blue), negative (red), polar (green) and non-polar (light gray).

References

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Cryo-EM reconstructions of LiCl core particles mimic early assembly intermediates

Daniel S. D. Larsson, Sandesh Kanchugal P. and Maria Selmer

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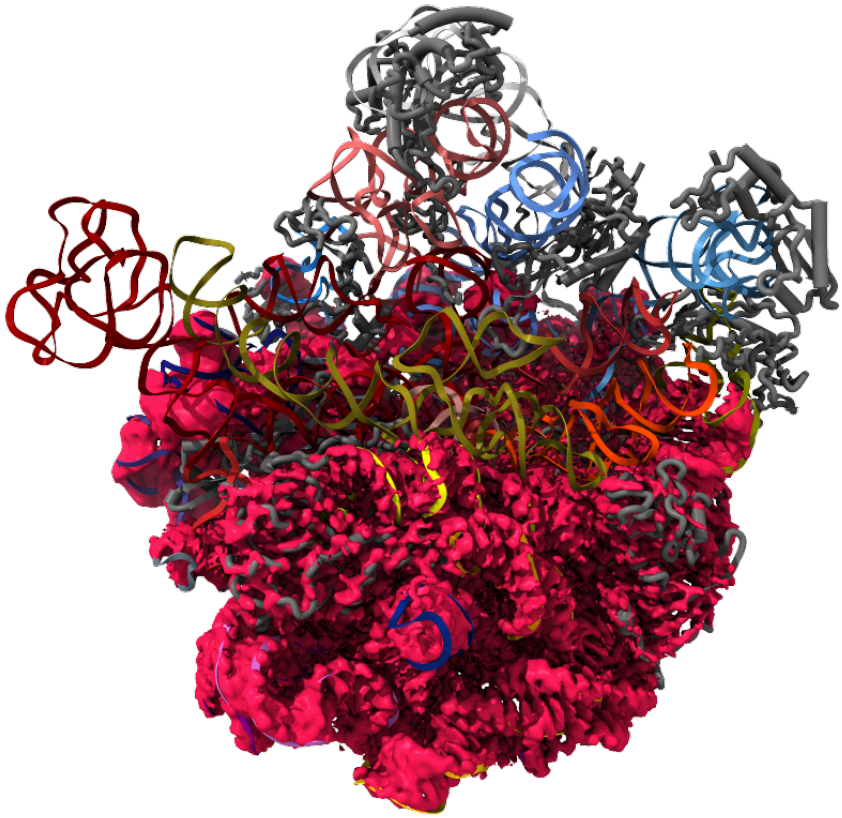
Bacterial ribosome assembly is a complex process, and experimentally challenging to study. Purified 50S ribosomes subjected to high-salt wash that removes some of the ribosomal proteins (r-proteins), were early shown to be competent for *in vitro* assembly into functional 50S subunits, but their exact structures have remained hidden. We here used cryo-electron microscopy to determine the structure of such LiCl core particles derived from *E. coli* 50S subunits. A wide range of complexes with large variation in the extent of ordered 23S rRNA and occupancy of ribosomal proteins could be identified, and resolved to between 2.8 Å and 9 Å resolution. Many of these particles showed high similarity to previously identified *in vivo* and *in vitro* assembly intermediates, supporting the inherent stability of these states. In addition, smaller core particles that may mimic earlier stages of 50S biogenesis were discovered. Particle comparison enabled us to identify structural dependencies between rRNA secondary structure elements and r-proteins and to reconstruct possible assembly pathways. Our data support a multi-pathway assembly process based on independent folding blocks. The study supports the physiological relevance of using LiCl core particles as a mimics of early ribosome assembly intermediates.



Solution structure of the pentameric ligand-gated ion channel GLIC probed by small-angle neutron scattering

Marie Lycksell, Urška Rovšnik, Cathrine Bergh*, Nicolai T Johansen**, Anne Marel***, Lionel Procar***, Lise Arleth**, Rebecca J Howard, and Erik Lindahl

Stockholm University, *KTH Royal Institute of Technology, **Niels Bohr Institute, ***Institut Laue-Langevin

Pentameric ligand-gated ion channels undergo subtle conformational cycling to control electrochemical signal transduction in many kingdoms of life. Several crystal structures have now been reported in this family, but the functional relevance of such models remains unclear. We used small-angle neutron scattering (SANS) to probe ambient solution-phase properties of the pH-gated bacterial ion channel GLIC under resting and activating conditions. Data collection was optimized by inline paused-flow size-exclusion chromatography, and exchanging into deuterated detergent to hide the micelle contribution. Resting-state GLIC was the best-fit crystal structure to SANS curves, with no evidence for divergent mechanisms. Moreover, enhanced-sampling molecular dynamics simulations enabled differential modeling in resting versus activating conditions, with the latter corresponding to an intermediate ensemble of both the extracellular and transmembrane domains. This work demonstrates state-dependent changes in a pentameric ion channel by SANS, an increasingly applicable method for macromolecular characterization as neutron source brilliance increases and inline SEC-SANS set-ups become increasingly accessible.

Site-Specific Incorporation of Two ncAAs for Two-Color Bioorthogonal Labeling and Crosslinking of Proteins on Live Mammalian Cells

The pyrrolysyl-tRNA/pyrrolysyl-tRNA synthetase (PylT/RS) pair from archaeon *Methanosarcina mazei* (*Mma*) is widely used in protein engineering to site-specifically introduce noncanonical amino acids (ncAAs) through nonsense codon suppression. Here, we engineer the PylT/RS pair encoded by *Methanogenic archaeon ISO4-G1* (*G1*) to be orthogonal to *Mma* PylT/RS and alter the *G1* PylRS active site to accept a complementary ncAA spectrum. We combine the resulting mutual orthogonal pairs for site-specific dual ncAA incorporation of two lysine-analogs with high selectivity and efficiency. Demonstrating the robustness of the system, we incorporate two ncAAs with compatible bioorthogonal reactivity into a Notch receptor, as well as a G-protein coupled receptor. We show that selective and site-specific incorporation of two ncAAs allows for two-color bioorthogonal labeling as well as chemical-controlled crosslinking of surface proteins on live mammalian cells.

X-ray spectroscopy on biological samples at the Balder beamline at MAX IV

Susan Nehzati and Kajsa G. V. Sigfridsson Clauss

MAX IV Laboratory, Lund University

Metal ions are a vital part of life's machinery, as structurally and functionally important parts of proteins. With hard X-ray absorption and emission spectroscopy (XAS and XES), we take the perspective of the metal ion and look out on the surrounding protein to follow changes in its local atomic and electronic structure. The Balder beamline (1) for XAS and XES at MAX IV will provide the tools you need to learn more about the rich chemistry of metal cofactors in biology. In order to do so, we need to adopt strategies to not damage the delicate biological samples with the high intensity X-ray beam (radiation damage). Typically, X-rays ionize the water matrix in the sample, which in turn can create everything from bubbles (via radiolysis) to aqueous electrons capable of, for example, reducing high valent metal ions. To minimize these problems: i) investigate the sample frozen, or ii) remove water by drying (lyophilisation), or iii) constantly refresh your sample by flowing it in the beam. Balder beamline provides specialized sample environments to alleviate these common difficulties; a 10 K closed cycle cryostat and a microfluidic flow cell (AdaptoCell). In addition, Balder is equipped to reduce the X-ray dose by minimizing the beam exposure to only data acquisition: fast scanning capabilities in fluorescence, a fast shutter, and methods to modulate the beam intensity. Examples of different biological samples measured at Balder will be presented.

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New insights into the interaction of Class II dihydroorotate dehydrogenases with ubiquinone in lipid bilayers as a function of lipid composition

Juan Manuel Orozco Rodriguez[1], Hanna Wacklin-Knecht[2], Luke Clifton[3], Giovanna Fragneto[4] and Wolfgang Knecht[1]

[1]Department of Biology & Lund Protein Production Platform, Lund University, Sweden, [2]Department of Chemistry, Lund University & European Spallation Source ERIC, Sweden, [3]ISIS Neutron and Muon Source, Didcot, UK, [4]Institut Laue-Langevin, Grenoble, France

In humans, the fourth enzymatic reaction in the *de novo* pyrimidine biosynthesis, the oxidation of dihydroorotate to orotate, is catalyzed by dihydroorotate dehydrogenase (DHODH). Enzymes belonging to the DHODH Class II are membrane-bound proteins that use ubiquinone as their electron acceptor[1, 2]. We designed this study to understand the interaction of an N-terminally truncated version of human DHODH (*Hs*Δ29DHODH), a target for anti-inflammatory drugs[3], and wild-type bacterial DHODH from *Escherichia coli* (*Ec*DHODH), with ubiquinone (Q_{10}) in supported lipid membranes using neutron reflectometry (NR) (**Fig. 1**). NR allowed us to determine *in situ,* under solution conditions, how the enzymes bind to lipid membranes and to resolve the location of Q_{10} within the membrane. We can show that *Ec*DHODH binds more efficiently to simple bilayers consisting of palmitoyl oleoyl phosphatidylcholine (POPC) and tetraoleoyl cardiolipin (TOCL) than *Hs*Δ29DHODH. Q_{10} is exclusively located at the center of all the lipid bilayers investigated, including more complex lipid mixtures mimicking either bacterial or mitochondrial membranes. Incorporation of Q_{10} into lipid bilayers also increases the efficiency of DHODH binding to the lipid bilayers, as shown by increased enzyme retention upon rinsing. We therefore show that the interaction between the enzymes located at the bilayer-water interface and the membrane is mediated by Q_{10}. Our results highlight the importance of Q_{10} as well as lipid composition on enzyme binding and enzyme retention.

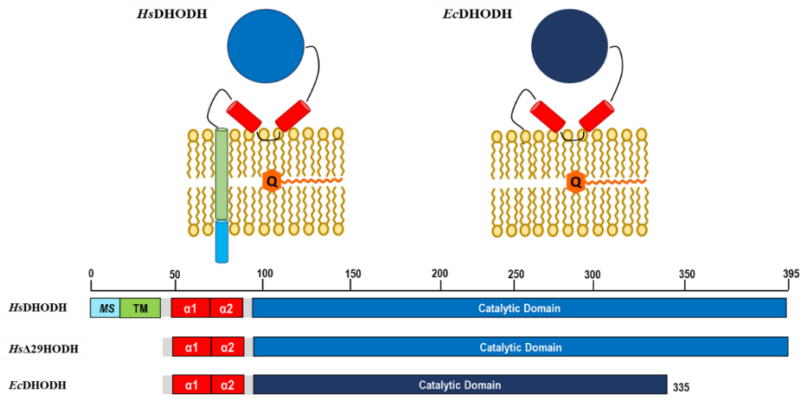

Figure 1. Schematic representation of the structure and location of *Hs*DHODH and *Ec*DHODH in a lipid bilayer, and a comparative presentation of the proteins used in this study (*Hs*Δ29DHODH, *Ec*DHODH) with full-length *Hs*DHODH.

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3. Sykes, D.B., et al., *Inhibition of Dihydroorotate Dehydrogenase Overcomes Differentiation Blockade in Acute Myeloid Leukemia.* Cell, 2016. **167**(1): p. 171-186.e15.

Towards a structural understanding of the Type 4 Secretion System ATPases in Gram-positive bacteria

Saba Shahzad[1] and Ronnie Bernsttsson[1,2]

[1]Department of Medical Biochemistry and Biophysics, Umeå University, Umeå, Sweden, [2]Wallenberg Centre for Molecular Medicine, Umeå University, Umeå, Sweden

Antibacterial resistance is becoming a major challenge worldwide. The World health organisation in its first-ever report on antibacterial surveillance, 2014, classified antibiotic resistance as a threat that could devastate modern health care. This emphasizes the need to find alternative strategies to control the spread of multi-resistant bacterial infections.

Bacteria mainly acquire resistance by a process called conjugation. There, megadalton sized protein machineries called Type 4 secretion system (T4SS) facilitate the horizontal gene transfer from bacterial donor cells to recipient cells. T4SSs are thus the main contributors to antibiotic resistance (3). However, we know very little of the T4SS due to the lack of structural information from a variety of bacteria. A few model systems from Gram-negative bacteria are well-studied (4,5), but we only have extremely limited data about the T4SSs from Gram-positive bacteria. This is problematic, since most of the proteins in Gram-positive T4SSs do not display any homology to their Gram-negative counterparts. Only the proteins involved in the DNA processing and the ATPases that fuel the transport are proposed to have functional homology, despite their very limited sequence identity. The ATPases of the T4SSs provide energy both for the build-up of the channel and for the transfer of the substrate from the donor bacteria into the recipient cell.

Here, we present the Cryo-EM structures of two core ATPases from a T4SS from *Enterococcus faecalis*. They are essential proteins in the T4SS, and have been proposed to be potential targets for inhibitors that target the function of T4SSs. In the presentation, along with exhibiting the structure-function relationship of the two ATPases in T4SS, the challenging process of obtaining the structure will also be emphasized. Further I will discuss our current hypothesis of how these proteins function in concert to fuel the DNA transfer through the T4SS channel.

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1. Antimicrobial Resistance, Global report on Surveillance, World health organisation, 2014(https://www.who.int/antimicrobial-resistance/publications/AMR_report_Web_slide_set.pdf?ua=1)
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POSTER PRESENTATIONS

Structural basis for the interaction of MYC with PNUTS, the regulatory subunit of the PP1:PNUTS phosphatase complex

Ahlner A*, Wei Y**, Redel C**, Lemak A**, Åkhe-Johansson I*, Houliston S** , Morad V*, Resetca D**, Wallner B*, Arrowsmith CH**, Penn LZ**, Sunnerhagen M**

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**University Health Network, Toronto, Canada

cMyc is one of the most dysregulated oncogenes in all cancer forms and therefore it has for long time been one of cancer researchers "holy grails" to inhibit cMyc. To our knowledge, no-one has been able to develop a drug with this purpose, probably due to cMyc's intrinsically disordered regions. To address this, we have previously investigated the proxiome of cMyc to find interactions with the potential to inhibit cMyc's interactions crucial for cancer. One of the identified interactors were the protein phosphatase-1 nuclear-targeting subunit (PNUTS). (Kalkat, 2018) If this interaction is inhibited cMyc gets hyperphosphorylated and degraded. (Dingar, 2018). To have a good starting point in designing drugs for this interaction we have used Nuclear Magnetic Resonance Spectroscopy (NMR), biolayer interferometry (BLI) and modelling using energy-based docking with Rosetta to structurally investigate this interaction.

We have determined the first NMR structure of PNUTS 1-148 revealing an alpha helical structure with slightly disordered N-terminal. An N-terminal part of cMyc called myc box zero binds in a highly dynamical way to the C-terminal part of PNUTS, shown both by titration experiments with NMR and BLI and mutation studies. The PNUTS cMyc complex have thereafter been modelled by Rosetta to further understand the interaction. The part of PNUTS involved in the interaction reveals a small but hopefully druggable pocket.

Our structure of PNUTS together with our titration studies of PNUTS interaction to the highly potent oncoprotein cMyc reveals a specific interaction which if targeted has potential to inhibit cMyc cancer activity.

References

Dingar D. et al, MYC dephosphorylation by the PP1/PNUTS phosphatase complex regulates chromatin binding and protein stability, Nat Commun. 9(1):3502 (2018)

Kalkat M. et al MYC Protein Interactome Profiling Reveals Functionally Distinct Regions that Cooperate to Drive Tumorigenesis Mol Cell. 72(5):836-848 (2018)

Protein Science Facility, Karolinska Institutet

Emilia Strandback, Henry Ampah-Korsah, Martin Moche and Tomás Nyman

Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Solna 171 77, Sweden

The Protein Science Facility provides protein production services and user training and access to equipment for macromolecular crystallography and biophysical characterisation of proteins.

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PSF offers expert service and advice at all levels of protein production.

- Advice, discussion and evaluation of new projects.
- Molecular biology services and assistance in expression construct design and ordering.
- Small-scale expression and solubility tests in bacterial, HEK, and CHO cells.
- Liter scale (1- 20 liter) production cultures in bacterial, HEK, and CHO cells.
- Protein purification using a range of established protocols.

Mammalian cell work is done in collaboration with Juni Andréll and the Eukaryotic Protein Production (EPP) lab at SciLifeLab Solna.

The produced proteins are used in a wide range of research areas, such as structural biology, biochemistry studies of protein function and interactions, enzyme activity, cell biology, assay development, drug lead development, imaging studies, and antigen and antibody production.

PSF also provides its user base with many utility enzymes of different categories, for instance DNA/RNA polymerases, ligases and reverse transcriptases, proteases, Cas enzymes, Tn5 transposase, and sortase A variants.

During the COVID-19 pandemic of 2020 and 2021, PSF has been involved in multiple projects for virus testing, antibody screening, drug lead and method development toward the understanding of and fight against the SARS-CoV-2 virus.

PSF is part of the protein production network Sweden (PPNS) that aims to share resources and expertise to the benefit of the user community. In 2021, PSF and groups from UmU, KTH, GU, and LU submitted a joint application to VR for the funding of a national infrastructure for protein production in Sweden (PPS). A decision is expected in September.

High resolution single particle cryoEM studies of NrdA-NrdB complex of RNR from *L.blandensis*.

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We are studying the role of the 100 amino acid domain called the ATP cone domain in the allosteric regulation of ribonucleotide reductases(RNRs) using single particle cryoEM as the main technique. RNRs are almost ubiquitous in all life forms and are essential for ribonucleotide reduction to deoxy-ribonucleotides. The overall activity of RNR is regulated allosterically, being up-regulated by ATP and down-regulated by dATP at the ATP cone domain which is found at the N-terminus of many RNRs. Here, we are studying the class I RNR from *L. blandensis* (LB), which has a catalytic subunit (NrdA)(α) and a radical generating subunit (NrdB)(β). The catalytic domain, in this sub-class of RNR has the ATP cone domain which acts as the allosteric switch. (1) One important question that still remains unresolved is how the tetramerization of LBNrdB and NrdBs with similar ATP cone fusions inhibits enzymatic activity as in the dATP induced tetramer, the interaction face of NrdA with NrdB in the active complex remains exposed. Here, we show the first high resolution structure of NrdA and also show the dATP inhibited NrdA-NrdB complex from *L.blandensis*.

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Characterization of ribonucleotide reductases and their transcriptional regulator NrdR

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Organisms store their genetic information in DNA, thus making deoxyribonucleotides (dNTPs) essential components of the cellular replication and proliferation machinery. The enzyme ribonucleotide reductase (RNR) catalyses the reaction that provides new DNA building blocks. There are three different classes of RNRs (I, II, III) different in reactivity towards oxygen, cofactor requirement, and quaternary structure, even within subgroups of each class. A fine-tuned allosteric regulation occurs within the enzyme to control the amount of dNTPs available in the cell. Allosteric effectors ATP or dATP bind to an N-terminal regulatory domain called ATP-cone that acts as an on/off switch of the enzyme. Although RNRs are widely studied, their mechanisms of regulation are largely unknown.

The details of dATP-inhibition have so far only been described for class I RNRs, in which case large oligomeric complexes are formed, excluding formation of the enzymatically active dimer-of-dimers between the two RNR subunits. In contrast, class II and III RNRs consist of a single subunit, in most cases forming a homodimeric enzymatically active complex, suggesting that the mechanism of dATP-inhibition is different from that of class I.

We are also interested in the mechanism of genetic regulation of RNRs by the transcription factor NrdR, which binds to the RNR promoter regions to repress transcription. NrdR is found in the majority of bacteria and some archaea and acts as a universal transcriptional regulator of RNRs.

We investigate the molecular mechanisms and allosteric regulation of RNRs and their transcriptional repressor NrdR, which share the same regulatory domain - the ATP-cone.

Oxygen-free fixed target SSX with Hemoglobin

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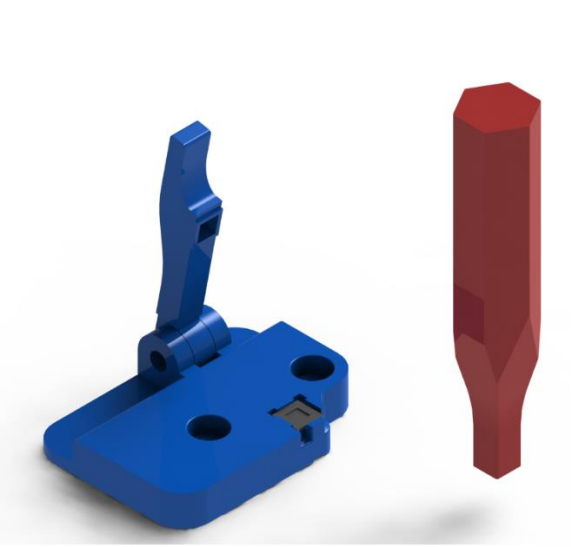
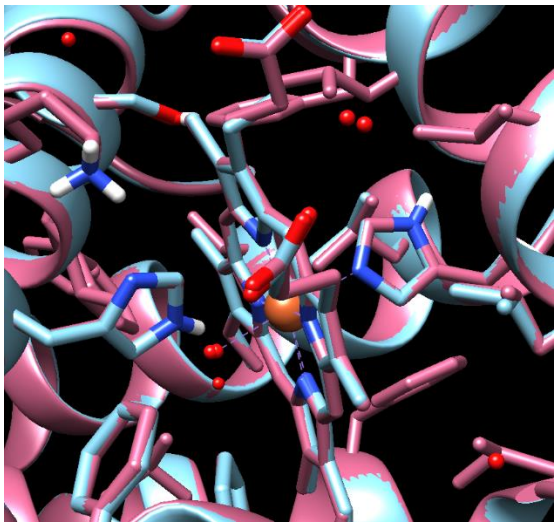
Serial Synchrotron Crystallography (SSX) is an emerging data-collection approach in which diffraction data are collected from multiple protein microcrystals. Some of the most common sample delivery setups for SSX include LCP extruder, GDVN , and fixed target. Fixed target is attractive as it potentially offers high hit rates coupled with modest sample consumption. Commercially available chips for crystals in solution have porous texture and are therefore not suited for oxygen-sensitive protein crystals . Silicon-nitride chips assembled in a chip-sandwich principle ensures proper sealing needed for handling oxygen-sensitive protein crystals. We have developed a novel protocol for the chip assembly in an oxygen-free environment (glove box) based on using a custom 3D-printed assembling device and accessories. The protocol was validated by X-ray absorption spectroscopy at the Balder beamline revealing a two hour time-stamp of no oxygen leak. A SSX fixed target experiment using deoxyhemoglobin crystals was conducted at the BioMAX beamline. Data collected on chips assembled using this protocol agrees with previously published deoxyhemoglobin structures.





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Structural and functional characterization of the relaxase TraI – Implications for Gram-negative Type 4 Secretion Systems

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Rising levels of antibiotic resistance and nosocomial infections represent a great threat to human health. Type 4 Secretion Systems (T4SS) allow bacteria to transfer DNA between each other and spread resistance genes via conjugative plasmids. T4SSs are a highly variable superfamily of secretion systems, which can be found in many bacterial species including Gram-negative and Gram-positive ones. In order for plasmid transfer to occur, the DNA needs to be linearized and unwound through the formation of the relaxosome. The relaxosome consists of the accessory factor TraK, the relaxase TraI and the plasmid DNA. TraI consists of a trans-esterase domain, which is responsible for nicking the DNA and a helicase domain for unwinding the strands. Although structures of a few relaxases are known, there are substantial differences in their composition, something we aim to shed more light on with structural and functional studies of these proteins.

In this project we aim to elucidate the structure of the relaxosome using both single particle cryo-EM and crystallography, while complementing both *in vitro* and *in vivo* functional assays. While the work on the full-length TraI, alone and in combination with the other components of the relaxosome is ongoing, we here present the structure of the TraI trans-esterase domain both in its apo form and in complex with bound substrate DNA. The structure shows that overall mechanism of DNA processing is likely conserved between relaxases from different families.

Understanding human CLC proteins: channels and transporters in chloride ion flux

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The CLCs are a family of chloride flux membrane proteins that contribute to cell ion balance in many organisms. The 9 members of this family present in humans help regulate cell volume, muscle relaxation and resorption in the kidney. Some are ubiquitously expressed, while others are tissue specific, and several of them are directly linked to disorders such as osteopetrosis (ClC-7) (1) and Dent's disease (ClC-5) (2). The family is also interesting as it consists of both channels and transporters, two functions previously thought to require very different protein architectures. Of the human CLCs, 4 are channels, and 5 are transporters. In addition, several human CLCs are regulated by interaction partners, including barttin (ClC-Ka and ClC-Kb) (3), GlialCAM (ClC-2) (4) and Ostm1 (ClC-7) (5). While the structures of two human CLCs have been determined (ClC-1 and ClC-7) (6, 7), one including an interaction partner (ClC-7/Ostm1) (7), several questions remain. Here I present an overview of the human CLCs, and the early stages of a project to use cryo-EM to gain further structural insights into these important proteins. Questions this project will aim to address are both of a general nature, such as delineating the minimal structural differences between a channel and a transporter, but also specifics, like the structural effects of GlialCAM on ClC-2, as it is the only known interaction partner that is not required for function.

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Proteins as keys to understanding SARS-Cov-2 – Contributions of the Protein Production Network Sweden (PPNS) to an ongoing pandemic

Malin Bäckström, on behalf of the Protein Production Network Sweden (PPNS)

Mammalian Protein Expression core facility, University of Gothenburg

The understanding of SARS-CoV-2 viral proteins has been the focus of research during the on-going Covid-19 pandemic. The virus infects human hosts through protein contacts. The viral polyprotein needs to be processed by the main protease, which gives rise to other important enzymes, as well as several structural capsid components such as the spike protein. Much of recent research has focused on the understanding of these proteins.

The different protein production platforms at Swedish universities, which are part of the Protein Production Network Sweden (PPNS, https://ppns.ki.se/), have during the last year contributed with the production and purification of several SARS-CoV-2 proteins, including the spike protein, the non-structural protein Nsp10 and the main protease Mpro. The proteins have been used to determine their structures, to identify inhibitors that can serve as drug candidates, as antigens for the development of therapeutic antibodies and have also served as serological antigens to detect antibodies in Covid-19 patients. Research using these proteins has already led to several publications from different research groups (see references below).

PPNS is a nation-wide collaboration of Swedish protein production and purification platforms. The overall aim is to provide highly specialized competences and advanced protein production methodologies for the Swedish academic life science community in order to make best use of available resources. Within the network the nodes can transfer projects and help scientists to find the best suited approach for solving a specific project.

Please feel free to contact any of the facilities with your needs of recombinant proteins. A listing of the platforms in PPNS and contact details can be found on the PPNS homepage: https://ppns.ki.se/ppns-facilities-2/ .

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SAS studies on regulation of MYC_{303}-MAX DNA binding in cancer

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Linköping University, *Linköping University, ** Institute Laue Langivin

Small Angle Scattering (SAS) is a powerful tool for analysing the shape and conformational changes of macromolecules, and it is beneficial in case the variability of conformation or intrinsical disorder or dimensions preclude structural determination via NMR or crystallography[1]. With SAS, we can explore non-crystallisable protein-DNA complexes in solution, without restrictions of artificially symmetrised DNA and restriction of protein sequence, it allows us thus exploring hitherto unchartered territories of regulatory protein-DNA interactions.

The MYC family oncogene is deregulated in more than 50 % of human cancers[2]. MYC is a central hub in almost every oncogenic aspect, regulating apoptosis proliferation, differentiation and metabolism. Although directly targetting MYC would be a powerful and effective method for treating several cancer types, unfortunately, it has been a challenge due to the missing information regarding the protein structure. One alternative to block the MYC signalling is the destruction of the MYC-MAX complex because MYC strictly depends on its partner MAX to regulate gene transcription. Consequently, interrupting the MYC-MAX complex is an efficient strategy to interrupt MYC signalling[3].

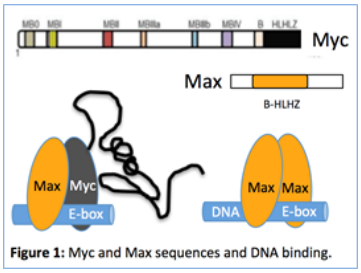

Figure 1: Myc and Max sequences and DNA binding.

At the current status, the precise role of the MAX homodimer is still unknown; there are suggestions that the induction of MYC upon mitogenic stimulation and the subsequent formation of MYC-MAX heterodimers may activate promoters under transcriptional repression by constitutively expressed MAX homodimers[4]. To bind DNA, the c-terminal region of MYC must form a heterodimer with the protein MAX. Crystal structures describing the MYC-MAX and MAX-MAX dimers have so far only included the core DNA-binding motif, including the bHLHzip region[5].

We have resolved with Small-Angle Scattering the structure of the two complexes: MAX-MAX and MYC_{303}-MAX, both bound with the E-box DNA. The technique of SAS was chosen due to the extreme flexibility of the protein and, in particular, SANS to be able to use the contrast matching technique to recognise each element of the complex: MYC, MAX and DNA. Exploring the difference between homodimer and heterodimer will give us an excellent step to understanding this whole complex for human cancerogenesis.

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Biological Support Laboratory (Biolab) at MAX IV

Yang Chen

MAX IV laboratory, Lund University

The Biolab at MAX IV is the central laboratory facility for the support of life-science synchrotron experiments at BioMAX, Balder, CoSAXS, NanoMAX, FemtoMAX, MicroMAX, SoftiMAX, MedMAX. The lab complements the sample preparation facilities at these beamlines by offering extended instrumentation access and support.

Biolab offers visiting scientists with specialist equipment and expertise for preparation, characterization and handling of biological and biochemical samples, including proteins, nucleic acids, lipids and macromolecular complex samples. Currently Biolab offers dynamic and static light scattering, gel electrophoresis, UV/Vis spectrometry, chromatography, anaerobic sample preparation, optical microscopy etc.

We focus on the development of experimental methods and assisting users with optimization of their samples for structural studies. In collaboration with beamline staff and user communities, we are working to develop new sample environments, serial-crystallography, and sample preparation techniques. An off(beam)line test station of the microfluidic setup for the AdaptoCell project is placed at Biolab. The setup is tuned in and tested with protein samples prior to beamtime. The microfluidic platform is applied at Balder and CoSAXS for delivering protein solution samples, and will be at MicroMAX for delivering micro crystals in novel *in situ* and time-resolved experiments. A station of the HVE injector for serial crystallography at BioMAX and MicroMAX is also being tested and developed at the Biolab.

ProLInC - The Protein folding and Ligand Interaction Core facility

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Understanding protein function and their mechanisms within expanded pathways in general often requires, for practical purposes, the ability to fully characterise the individual proteins and their interactions in isolation. Such 'targeted' experimentation is plagued with assay artefacts and subsequent complications.

Each technique only examines a biased view of the question addressed. What if the technique is inappropriate or the test subjects flawed? Or if the original question was wrong? All assays have shortcomings - artefacts and bias from one technique can only be circumvented with orthogonal methodology.

The **PRO**tein folding and **L**igand **IN**teraction **C**ore facility (ProLInC) at Linköping University has been established as a resource integrating a diverse (and complementary) array of biophysical techniques for the complete characterization of proteins and their complexes: shape, structure, stability, affinity etc. Together with on-site expertise, the facility aims not only to provide access to instrumentation but also to facilitate experimental design and assist in the interpretation of results.

Visiting researchers can utilize the ProLinC facility in a variety of ways: simple access to the diverse array of equipment (with technical support and advice on hand if required); the ability to verify (and complement) previously obtained results; complete investigation/characterisation of a protein or protein complex; a user focused open discussion addressing generalised questions – advising on appropriate/best technique(s) to answer the question at hand and assistance in design and optimization of experiments.

ProLinC is key player in the newly formed EU-program for molecular biophysics: **MO**lecular-**S**cale **B**iophysics **R**esearch **I**nfrastructure **(**MOSBRI). This is a consortium of 2 companies and 13 academic centres of excellence offering international access to instrumentation and expertise for molecular-scale biophysical approaches. https://www.mosbri.eu

ProLinC also forms part of SMILE: an initiative to improve Small and Medium Enterprises (SME) access to experimental and computational laboratory infrastructures and deepen collaboration between academia and business. https://liu.se/en/research/smile

https://liu.se/en/research/prolinc

Site-Specific Incorporation of Two ncAAs for Two-Color Bioorthogonal Labeling and Crosslinking of Proteins on Live Mammalian Cells

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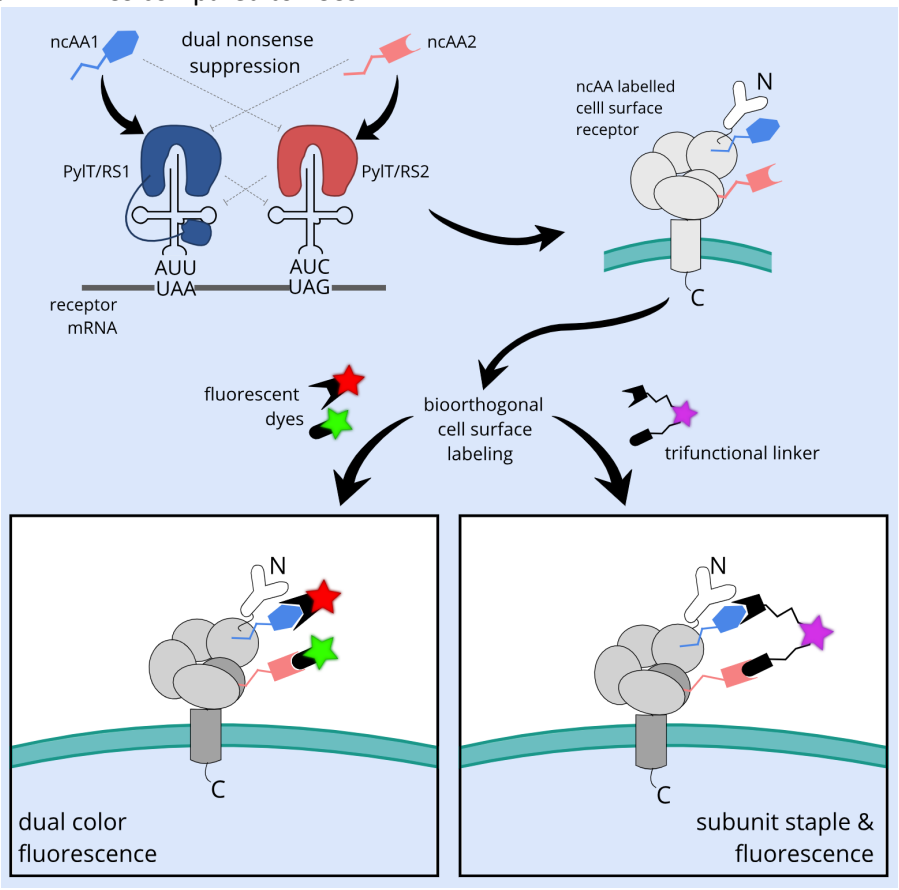
* equal contribution

The pyrrolysyl-tRNA/pyrrolysyl-tRNA synthetase (PylT/RS) pair from *Methanosarcina mazei* (*Mma*) is widely used in protein engineering to site-specifically introduce noncanonical amino acids (ncAAs) through nonsense codon suppression. Here, we engineer the PylT/RS pair encoded by *Methanogenic archaeon ISO4-G1* (*G1*) to be orthogonal to *Mma* PylT/RS and alter the G1 PylRS active site to accept a complementary ncAA spectrum. We combine the resulting mutual orthogonal pairs for site-specific dual ncAA incorporation of two lysine-analog ncAAs with high selectivity and efficiency. Demonstrating the robustness of the system, we incorporate two ncAAs with compatible bioorthogonal reactivity into a Notch receptor, as well as a G-protein coupled receptor. We show that selective and site-specific incorporation of two ncAAs allows for two-color bioorthogonal labeling as well as chemical-controlled crosslinking of surface proteins on live mammalian cells. We further characterized the dual labeled receptors with FRET-FCS compared to FCCS.



Self-crowding of IDPs probed with SAXS and coarse-grained simulations

Eric Fagerberg, Linda K. Månsson, Samuel Lenton and Marie Skepö

Lund University

Intrinsically disordered proteins (IDPs) lack a distinct equilibrium structure, being best described as ensembles of structures, while still being biologically important. Here, the crowding of IDPs is investigated. In biological settings, the macromolecular concentration can be up to 400 mg/ml[1], yielding a crowding effect not always present in experimental settings. Considering protein structure, the effect is non-monotonic[2], with different outcomes possible, why measurements in dilute conditions may not reflect the actual structure *in vivo*.

The short, antimicrobial saliva-protein Histatin 5 (Hst5) and its dimer $(Hst5)_2$ were used as model-IDPs to capture the crowding effect with small-angle X-ray scattering (SAXS). The introduction of the dimer allows for the deduction of length effects on crowding.

To assist the interpretation of results, coarse-grained modelling is employed. Despite some disagreement between experiment and simulation, no change in average structure of Hst5 is found, behaving like a stiff rod up to a protein concentration of 50 mg/ml, whereafter non-specific aggregation is observed[3]. The stiff rod behaviour and the lack of response to crowding was hypothesized to be a consequence of its short length, however, also the dimer does not seem to exhibit any change in structure upon crowding, up to a point where non-specific aggregation is observed. At dilute conditions, the dimer was also found to have smaller dimensions than expected from scaling laws obeyed by Hst5 and even atomistic modelling predicted larger structures.

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A Microfluidic Platform for Synchrotron X-ray Studies of Proteins

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New tools are needed to allow for complex protein dynamics studies, especially to study proteins in their native states. In the AdaptoCell project[1] a microfluidic platform for academic and industrial users at MAX IV Laboratory is being developed. MAX IV is a Swedish national laboratory providing brilliant synchrotron X-rays for research. Due to the high photon flux, sensitive samples such as proteins are prone to rapid radiation damage; thus, it is advantageous to have the liquid sample under flow to refresh the sample continuously. This, in combination with small volumes, makes microfluidics a highly suitable sample environment for protein studies at MAX IV. The AdaptoCell platform is being integrated at three beamlines: Balder (X-ray absorption/emission spectroscopy), CoSAXS (small angle x-ray scattering) and MicroMAX (serial synchrotron crystallography). Currently, the platform is fully available at Balder, under commissioning at CoSAXS and being developed for MicroMAX.

In Figure 1a an illustration of the platform is shown. As well as at the beamlines the microfluidic equipment is also available at the off-line test station in the BioLab. This facility allows for the offline testing and optimisation for the devices with user specific samples and to "health check" the protein samples before the beam time. Suitable AdaptoCell devices are now commercially available via a mainstream manufacturer. The majority of the devices are made of COC (cyclic olefin copolymer), which have high X-ray transmission and high resistance to X-ray induced damage. One key challenge is to avoid contact of the sample with the channel walls in order to prevent fouling, current investigations are focussing on different methods of avoiding this contact (Fig. 1b and 1c). In addition to the sample presentation from the devices at the beamlines, different add-on techniques are also being developed, such as on-chip mixing (Fig. 1d) to e.g., induce rapid pH changes. Later in the project, UV-vis spectroscopy read-out will be integrated to allow additional research data and superior sample control.

The AdaptoCell platform is a convenient sample environment available for MAX IV users that the project team believes will open up for new types of structural and functional studies of proteins.

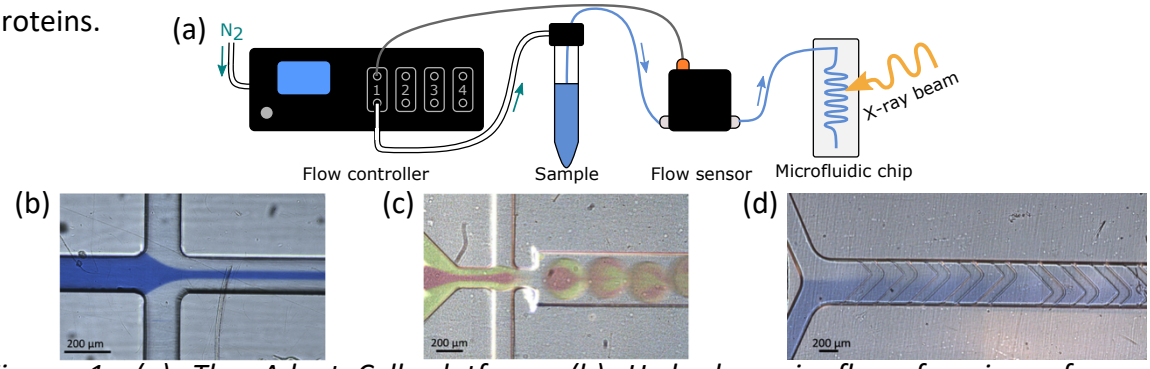

Figure 1. (a) The AdaptoCell platform. (b) Hydrodynamic flow focusing of sample. (c) Generation of water-in-oil droplets. (d) On-chip mixing in a herringbone micromixer.

[1]The AdaptoCell project is funded by SSF ITM-17 (grant ITM-0375).

MacroGreen, a simple tool for detection of ADP-ribosylated proteins

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Enzymes in the PARP family partake in the regulation of vital cellular signaling pathways by ADP-ribosylating their targets. The roles of these signaling pathways in disease development and the de-regulation of several PARP enzymes in cancer cells have motivated the pursuit of PARP inhibitors for therapeutic applications. In this rapidly expanding research area, availability of simple research tools will help assess the functions of ADP-ribosylation in a wider range of contexts. Here, we used the ADP-ribose binding protein Af1521, a so-called macrodomain from the archaea *Archaeoglobus fulgidus*, as template. We prepared a multi-mutant macrodomain fused to green fluorescent protein (GFP) to generate a high affinity ADP-ribosyl binding reagent. The resulting tool – which we call MacroGreen – is easily produced by expression in *Escherichia coli* strains and can detect both mono- and poly-ADP-ribosylation in diverse proteins. It can also report ADP-ribosylation in cells in response to DNA damage. MacroGreen can be used to quantify modification of target protein in ELISA-type assays, and to screen for PARP inhibitors in high-throughput format with excellent assay statistics. We expect that this tool will facilitate ADP-ribosylation related discoveries by laboratories who do not specialize in ADP-ribosylation.

High-throughput screening combining bimolecular fluorescence with flow cytometry reveals constructive membrane protein complex formation

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Interactions between membrane proteins in their cellular environment are determinants of the fine-tuned regulation crucial for all living cells. To shed light on the molecular mechanism of protein interactions, robust methods to screen and analyse protein complexes are of utmost importance where most available methods are designed for soluble proteins. The bimolecular fluorescence complementation (BiFC) assay is an approach which visualizes complex formation involving protein:protein interaction (PPI) partners *in vivo*, also for membrane protein complexes. In this study, we report the development of a high-throughput screening approach, using fluorescence-activated cell sorting (FACS) to characterize membrane protein interaction partners in the host *Saccharomyces cerevisiae*, allowing the discrimination between constructive complexes and random interaction. The approach developed in this study will make the use of manual screening obsolete and give the opportunity to efficiently screen in living cells for novel protein interaction partners *in vivo* in a high-through put setup.

BioMAX, a macromolecular crystallography facility at MAX IV

Ana Gonzalez, Oskar Aurelius, Roberto Appio, Monika Bjelčić, M. Eguiraun, Thomas Eriksson, Ross Friel, Ishkhan Gorgisyan, Andrea Gross, Vahid Haghighat, Elmir Jagudin, Tobias Krojer, Julio Lidon-Simon, Gustavo de Lima, Mirko Milas, Uwe Mueller, Jie Nan, Anastasiia Shilova, Vladimir Talibov, Marjolein Thunnissen, Johan Unge, Thomas Ursby

BioMAX is the first operational Macromolecular Crystallography (MX) beamline at the MAX IV facility.[1] The design goal was to create a stable and reliable multipurpose beamline. The beamline optics consist of a double crystal Si(111) monochromator that provides an energy range between 5 and 25 keV and a pair of KB mirrors; the beam can be focused down to 20 x 5 µm2 FWHM at the sample position with a photon flux of about 5x1012 photons/s at a ring current of 250 mA and an energy of 13 keV. Changes of energy and beam focus (up to 100 x 100 µm2) are automated. The experimental hutch is equipped with a MD3 microdiffractometer, an IRELEC Isara sample changers and a 16M Eiger detector. MXCuBE3 is used for user beamline control and data collection.[2]

A wide range of MX experimental techniques are available at the beamline, from standard oscillation data collection at cryo-temperatures, optimized SAD and MAD experiments, humidity-controlled room temperature data collection and Serial Crystallography experiments, both fixed target and injector based.[3] A fragment-based drug discovery facility (FragMAX) is also based a BioMAX.[4] Conventional experiments at cryotemperatures can be done fully remotely.

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Mechanism of substrate binding and metal catalysis in *Mycobacterium tuberculosis* phosphatidylinositol phosphate synthase

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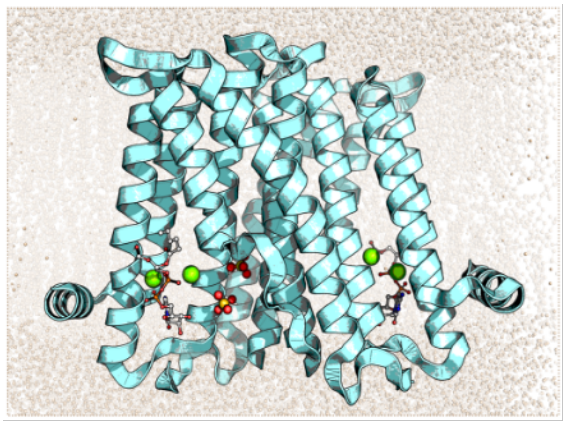
Mycobacterium tuberculosis is the most medically relevant pathogen of its genus, causing tuberculosis in humans and killing over one million people worldwide annually. Using X-ray crystallography, we have characterised one of the essential *M. tuberculosis* membrane-embedded CDP-alcohol phosphotransferase family enzymes – phosphatidylinositol phosphate synthase, also called PgsA1.



Cartoon representation of the PgsA1 in complex with CDP-DAG, Mg^{2+} and sulfate in a lipid bilayer.

PgsA1 catalyses a crucial step of inositol-derived phospholipid biosynthesis, producing structural precursors for biogenesis of structurally complex components of the *M. tuberculosis* cell envelope. In addition, because of significant differences in inositol phosphate biosynthesis pathways in mycobacteria and eukaryotes, PgsA1 was identified as a promising drug target.

We have determined three high-resolution crystal structures of PgsA1: without any ligands bound (2.9 Å), in complex with CDP-DAG - one of the PgsA1 natural substrates (1.8 Å), and in complex with Mn^{2+} together with serendipitously bound citrate in the active site of the enzyme (1.9 Å). The structures reveal atomic details of substrate binding and flexibility of catalytic metal site. In conjunction with obtained structural information, molecular docking and mutagenesis data suggested a binding mode for the second PgsA1 substrate, D-*myo*-inositol-3-phosphate. Together, data suggest a refined general catalytic mechanism, including substrate-induced carboxylate shift, for class I CDP-alcohol phosphotransferases.

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

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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.

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Recombinant human transthyretin from *Vibrio natriegens* – a promising source for protein production for protein crystallography

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Transthyretin (TTR) is a highly abundant protein in blood plasma and cerebrospinal fluid. TTR carries the thyroid hormone thyroxine and retinol-binding protein. The TTR protein is the culprit protein in the amyloid disease transthyretin amyloidosis. *Vibrio natriegens* has recently gained attention because of its quick growth, which is between two to three times faster than *Escherichia coli*, and possibly higher protein synthesis rate, making it a candidate for an enabling molecular biology platform [1]. Here, human wild-type TTR was produced in *Vibrio natriegens* to investigate the possibility of using this new host for recombinant protein expression and further crystallography. Recombinant human TTR produced in *Vibrio natriegens* provided high yield of protein. After purification, the resulting protein was crystallized to verify the structure from this novel expression host. The crystal structure was the same as for recombinant TTR produced and crystallized from *Escherichia coli* which is the same as that for TTR purified from human serum. These results indicates that the protein synthesis of recombinant proteins in *Vibrio natriegens* can successfully be used in crystallography applications.

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Investigating the structural and dynamical implication of the P44L mutation in the N-Myc oncoprotein

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The Myc proteins are master regulators involved in numerous pathways important for cellular growth and function. However, when deregulated these vital transcription factors are also drivers of many human cancers. The N-Myc oncoprotein has been linked to both neuronal and nonneuronal cancers and is especially known for its involvement in pediatric cancers[1]. This protein is thus an important target for furthering our understanding of tumor development and progression, however due to its intrinsically disordered nature, only limited biophysical analysis on N-Myc have been performed[2].

We have successfully produced the intrinsically disordered N-terminal region of the N-Myc protein, spanning residues 1-69 and encompassing the conserved MB0 and MBI. For this construct, we were able to investigate the structural and dynamical properties of N-Myc using dynamic light scattering (DLS), multi angle light scattering (MALS) and nuclear magnetic resonance spectroscopy (NMR). These methods were then used to investigate the effects on structure and dynamics by the N-Myc mutation P44L, a known somatic mutation that has been seen to increase cellular N-Myc levels 2-4-fold compared to wild-type protein in different cancer cells [3].

Here, we present NMR data that yielded a near-complete backbone assignment of the N-Myc construct spanning residues 1-69. The HSQC spectra display the intrinsically disordered nature of N-Myc and multiple minor peaks indicate that the protein has alternate conformational states due to cis-trans isomerization. Introducing the mutation P44L induces changes in chemical shift to selected residues throughout the studied construct, indicating that changes are transmitted via side chain interactions rather than through the backbone. The mutation also results in an increased chemical exchange in the N- and C-terminal of the construct, indicating that they partake in intra- or intermolecular interactions in higher degree than in the wild-type.

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Structural Comparison of LolA and LolB Protein across Gram-Negative Bacterial Phylum

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The bacterial cell envelope plays an important role in maintaining viability, growth, and pathogenicity. Gram-negative bacteria contain double cell membranes in contrast to Gram-positive bacteria. The outer membrane of Gram-negative bacteria is often associated with pathogenicity and serves as a barrier against antibiotics. Lipoproteins are one of the most important structural and functional components of the cell envelope. They perform various physiological functions and have virulence-associated roles like antigenicity and colonization. **L**ocalization **o**f **l**ipoproteins is essential for outer membrane biogenesis which is carried out by the **Lol** pathway. Lol pathway consists of - an integral membrane complex LolCDE (ABC transporter), a periplasmic chaperone LolA and an outer membrane-anchored receptor Lol B. LolB is anchored on the outer membrane and transfer lipoproteins from LolA to the inner leaflet of the outer membrane. We have solved the crystal structure of LolA from *Vibrio cholerae* and *Porphyromonas gingivalis* and LolB from *Vibrio cholerae.* This has broadened the present repertoire of LolA/B structures across the broad phylum of gram-negative bacteria and enabled us to examine distinct structural features. We have compared the LolA/B from *Vibrio cholerae* and *Porphyromonas gingivalis* with solved structures from various gram-negative bacteria -*E.coli, Y.pestis, P.aeruginosa, N.europa*. We have done pairwise structural alignment using the DALI program to look for conserved/different residues and secondary structures, which could not be done by mere sequence analysis. We have also analyzed the surface charge especially the distribution of hydrophobic patches as it will show if acyl chains could bind on the outer surface of Lol proteins. This broadens the perspective of acyl chain binding and transfer modalities from LolA to LolB, across gram-negative bacteria. As the emergence of multidrug resistance demands the search for new antibacterial targets, structural insights into key interactions of Lol proteins would serve as prospective candidate targets for new antibiotics.

Crystal Structure of Iron Assimilating Protein 1 (FEA1) from *Chlamydomonas reinhardtii*

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The well-characterized green alga *Chlamydomonas reinhardtii* expresses various iron-dependent proteins essential in electron pathways, reactive oxygen detoxification, fatty acid metabolism, and amino acid biosynthesis.[1] The concentration of iron in the cell is tightly controlled. During iron deficiency, the abundance of iron assimilating protein 1 (FEA1) mRNA and protein increases.[2,3] The mechanism of FEA1 in the iron uptake pathway is unknown. The structural study of FEA1 will lead to a better understanding of its function and role in the iron assimilation pathway. Currently, protein structures are solved mainly by molecular replacement, but protein with no homology to Protein Data Bank (PDB) structures requires experimental phasing. One such phasing method is single-wavelength anomalous dispersion (SAD). Native SAD phasing can be challenging and dependent on accurate data collection. A long-wavelength X-ray can increase the anomalous scattering signal; however, both crystal thickness and the solvent surrounding the crystal may cause strong absorption effects during data collection.[4,5] In this study, FEA1 was successfully crystallized, and the phasing was solved using S-SAD at a long wavelength of 2.7 Å coupling with laser cutting technology. The initial model was determined by authoSHARP followed by autoBuster. It was used as a search model for the native data set collected at a higher resolution of 1.9 Å. The FEA1 crystal belongs to *C*2 space group and contains three molecules in the asymmetric unit. FEA1 forms dimers that were supported by a size exclusion chromatography result. FEA1 structure is mostly composed of α-helices. Based on DALI server result, there are no similar structures found in PDB; therefore FEA1 structure reveals a new fold.

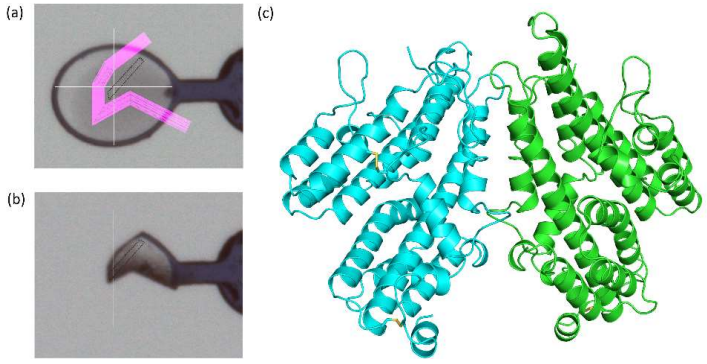

Figure 1 (a) Original loop before laser cutting and cutting line pattern (pink line); (b) the same loop after laser cutting. (c) The dimeric form of FEA1 is shown as a cartoon representation (image from *PyMOL*).

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LP3 and DEMAX

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Proteins are 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. Most drugs act on proteins. The structures and mechanisms of proteins are therefore prominent topics in life science research.

Access to both state-of-the-art X-ray (MAX IV) and neutron sources (ESS) will increase the capacity for innovation in the life sciences. To enable efficient use of these unique and powerful facilities by Lund researchers, Lund University hosts the protein production platform, LP3 (www.lu.se/lp3). LP3 assists users with: 1) recombinant protein production, 2) biophysical protein characterisation 3) high-throughput crystallization and structure determination, and 4) stable isotope labelling and bio-deuteration of biological macromolecules.

Since 2016, the DEuteration and MAcromolecular Xtallization (DEMAX) platform of the ESS is co-localized with LP3. DEMAX and LP3 are coordinating in their efforts [1-4] to develop cost-effective production of deuterated proteins for macromolecular crystallography, enable crystallization of interesting proteins for neutron work, and for the production of labelled proteins/lipids for neutron reflectometry.

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

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The FragMAX facility for crystal-based fragment screening at MAX IV Laboratory

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Fragment-based lead discovery (FBLD) is by now an established drug development strategy, which has so far delivered four novel drugs and more than 40 additional molecules in clinical trials. Starting points for FBLD are usually found by biophysical screening of fragment libraries with several hundred and up to a few thousand compounds. The reduced logistics make it an ideal method for academic groups with an interest in drug development or chemical biology. Crystal-based fragment screening has become increasingly popular over the last few years, facilitated by automation, improvements in beamline instrumentation and software development. The FragMAX facility provides a new user platform for crystallographic fragment screening at BioMAX, the first operational beamline for macromolecular crystallography at MAX IV Laboratory. The FragMAX platform started serving external users in 2019 and has since established an international user program that is open to academic and industrial research groups. The platform consists of three main components: (i) a crystal preparation facility, (ii) diffraction data collection at BioMAX and (iii) FragMAXapp, an intuitive web-based tool for large-scale data processing. The facility provides free access to several fragment libraries, notably, the in-house developed FragMAXlib. The screening processes have been adjusted throughout the COVID-19 pandemic and our aim is to further develop the platform so that users with different levels of experience can routinely achieve actionable screening hits for their targets. Our poster provides an overview of the screening process, describes different modes of access and outlines planned developments.

Suppression of amyloid fibrillation by the molecular co-chaperone HSP10

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Alzheimer's disease (AD) is a neurodegenerative disease that has devastating effects on patients, relatives and society as a whole. The aggregation of Amyloid beta (Aβ) has for a long time been associated with the development of the disease and several treatments targeting the Aβ have been and are proposed (1). But currently, there are no cures for the disease. Targeting proteostasis is a new approach as a disease modulating treatment (1), and it is also a research tool, providing insight into fundamental mechanisms of Aβ aggregation. Heat shock protein 10 (HSP10) is an evolutionary well conserved co-chaperone that is mostly known to interact with Heat shock protein (HSP60). Currently, HSP10 is believed to mainly assist HSP60. Yet, HSP10 has been shown to interact with α-synuclein fibrils in Parkinson's disease, and overexpression of HSP10 has also been seen in AD (2,3), and the *E. coli* homolog (GroES) has been reported to possess unfoldase activity during folding (4). Herein, we investigate how the co-chaperone HSP10 from humans, as well as its homologs from *D. melanogaster* and *E. coli*, affects the aggregation of Aβ42. *In vitro* aggregation of Aβ42 was shown to be inhibited by Human HSP10 and Drosophila HSP10, while GroES did not show any inhibitory effect on Aβ42 aggregation. Different structural polymorphs of Aβ42 fibrils were observed when Aβ42 was fibrillated in the presence of the different chaperones. Thicker, bundled fibrils could be observed when Human HSP10 was present during fibrillation and more amorphous aggregates were formed when GroES and Drosophila HSP10 were present. These results suggest that the co-chaperone interacts with Aβ42 during fibrillation. This, in turn, leads to changes in aggregation kinetics as well as the fibrillar morphology. We verified the anti-aggregation activities of the Hsp10s by monitoring fibril formation kinetics of human prion protein with a similar outcome as for Aβ42. These results suggest that HSP10 has an intrinsic chaperone function that independent of HSP60 and provides new mechanistic insights into chaperone-mediated mitigation of amyloid fibril formation in neurodegenerative diseases.

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Enterococcal PrgU provides additional regulation of pheromone-inducible conjugative plasmids

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Antibiotic resistance and hospital acquired infections present major problems in the world, and they are on the rise. One of the most clinically relevant issues is transferable resistance linked to conjugative plasmids, which transfers DNA horizontally through Type 4 Secretion Systems (T4SS). Here we focus on the regulation of the T4SS in the conjugative plasmid pCF10 from the Gram-positive bacteria *Enterococcus faecalis*. All the genes important for the T4SS in *E. faecalis* pCF10 are under the regulation of one promoter named PQ. Conjugation in Gram-positive bacteria in plasmid-containing donors are often induced by peptide sex pheromones produced by recipient cells (1). However, a couple of years ago a new protein involved in the regulation of pCF10 was found: PrgU. PrgU inhibits the transcription from the PQ promoter, and it has also been indicated to be important for cell survival (2). The structure of PrgU is known and it has been shown to contain a PUA-domain, which makes it likely to bind RNA-like structures (2). Upon inspection of the PQ operon, an intergenic region (IGR) is found between the first and second gene on the operon. This IGR has been proposed to contain rRNA structural fold (3).

Here we show by combining biochemical assays such as EMSAs of PrgU binding to DNA and RNA, in vivo experiments, and single-cell fluorescence that PrgU binds to the IGR after the PQ promoter. By doing so it terminates transcription of the operon, causing the T4SS protein levels to decrease as compared to a system where PrgU has been deleted. Deleting PrgU causes pCF10 containing cells to produce higher mRNA levels of the entire operon, as observed by increases in both the *prgB* and *prgG* transcripts (early and late part of the operon, respectively). We propose that PrgU functions as a regulatory protein in order to keep the production of the virulence factor PrgB within acceptable limits, as too many copies of PrgB are lethal to the cell.

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Novel approaches to determine solubility of amyloid beta peptides

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Background: Alzheimer's disease (AD) is a devastating illness currently affecting more than 25 million people worldwide, and incidence is projected to triple by 2050[1]. One of the major hallmarks of AD is the accumulation of aggregated amyloid beta peptide (Aβ)[2–4]. Several studies have shown that the aggregation of Aβ is closely linked to the disease. However, no clinical trials focused on removing Aβ have so far been successful enough to generate an approved drug towards the disease[5]. Thus, it is increasingly important to elucidate what modulates the solubility of Aβ to potentially find new therapeutic strategies. **Methods:** The methodology to study Aβ's solubility can be divided into three main steps: reaching the end state of fibrils formation (equilibrium), separating the soluble monomers from the fibrils and determining the concentration of monomers left in solution. This rests upon the assumption that the concentration of the monomeric peptide at equilibrium with fibrils is equal to that system's solubility.[6] A successful strategy established in our group was to reach the equilibrium under quiescent conditions by fibrillating $A\beta_{m1\text{-}40}$ at different initial monomers concentrations (0.25-25 μM range) for a given time and incubation temperature. $A\beta_{m1\text{-}40}$ monomers were then separated from the fibrils by centrifugation at 22640 x g for 15 min and their concentration was determined by MALDI-MS (with an internal isotope standard) and microfluidic diffusional sizing (MDS). **Results:** The solubility for recombinant $A\beta_{m1\text{-}40}$ was determined to be 0.36 ± 0.15 μM in an aqueous solution of 20 mM sodium phosphate buffer at pH 7.4 and 37°C. **Future prospects:** Currently we are in the process of extending our repertoire of methods to facilitate reliable measurements of solubility for numerous new variants and conditions. One example is to use ninhydrin combined with phenylacetaldehyde for the concentration determination, for increased dynamic range and sensitivity.

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DNA replication in staphylococcal genomic islands

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The initiation of DNA replication is a highly controlled mechanism where the ring-shaped replicative helicase needs to be loaded onto the DNA at the origin of replication. When no additional factors are needed for helicase loading, called self-loading helicases, the mechanism for the self-loading process remains unexplored. Here we study all three different replicative helicases called Rep found in SaPIs, a family of pathogenicity islands present in the medically relevant *Staphylococcus aureus*. We demonstrate that all three Reps are functional homologs of MCM-replicative helicases with 3' to 5' polarity and show that different Reps have different preference for nucleotide usage as energy source. Our cryoEM structures of two of these Reps, one from SaPI5 at 3.24 Å and a second one from SaPI1 at 3.9Å resolution, show that in both cases the double stranded DNA binding domain moves respect the ATPase domain with two distinct and continuous movements: rotation and tilting. This conserved flexibility is ATP independent and provides the starting point to understand how these pathogenicity islands replicate as good as the viruses they parasite.

PReSTO status report 2021

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The Swedish structural biology ecosystem consists of several large-scale research infrastructures such as BioMAX beamline at MAX IV, SciLifeLab cryoEM, Swedish NMR Centre and upcoming neutron crystallography beamline at ESS, supported by numerous small sample preparation facilities spread across Swedish universities. In the strategic plan for 2023-2026, Swedish National Infrastructure for Computing (SNIC) aim to engage in dialogues with Swedish research infrastructures creating best possible compute and data management environment for their research communities.

PReSTO coined in 2013 started as MX only however in 2018 the Swedish Research Council granted funds to involve other Swedish structural biology branches. Since 2016, we use Easybuild to keep build recipes readable and simple, logged build processes and resolved software dependencies that simplify sharing of installations across new hardware.

Now in 2021, we have a working MX software setup available at NSC Tetralith, LUNARC Aurora and the MAX IV compute cluster supporting MX and BioMAX users. The MAX IV staff is pushing PReSTO towards serial crystallography applications preparing for MicroMAX beamline in construction.

In collaboration with cryoEM staff, we installed Scipion, Relion, EMAN2, CisTEM, Gctf, Gautomatch, MotionCor2, and CTFFIND, however it remains to install CryoSPARC, CryoDRG, eTomo, Dynamo, EMClarity and PEET. PReSTO is not available at SciLifeLab-cryoEM compute clusters and despite 170 compute nodes at NSC Tetralith equipped with NVIDIA T4 GPUs, data transfer to Tetralith is considered a major hurdle for the cryoEM community. Swedish NMR centra has not installed PReSTO and only a few NMR software's are installed at NSC Tetralith such as NMRPipe and MddNMR. The neutron crystallography beamline at ESS will be able to use the MX part of PReSTO out of the box.

The PReSTO master installation is located at NSC Tetralith and new releases are pushed to LUNARC and MAX IV cluster. PReSTO training and documentation are available both from SNIC and MAX IV perspective for MX researchers, however training and documentation is absent for all other structural biology branches! Test data from EMPIAR and coordination with DDLS data repositories is required by CryoEM. CryoEM may develop a PReSTO branch ahead of NSC Tetralith and the easybuild framework would later be helpful in moving their software setup to next local compute cluster or DDLS Berzelius resource in Linköping run by PReSTO staff.

We look for researchers and staff scientists from our large research infrastructures that are willing to engage by using the current PReSTO installation, adding software to PReSTO, develop new or GPU adapt existing software creating a joint platform for structural biology.

Welcome to PReSTO!

Fluorescent fusion protein as a structural probe to characterize Aβ amyloid fibril polymorphism associated with Alzheimer's disease (AD)

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Alzheimer's disease (AD) is the most common neurodegenerative disease worldwide. AD is associated with misfolding and aggregation of Aβ and tau proteins into amyloid fibrils in the brain. Various *in vivo* and *in vitro* studies have established the link between Aβ and tau amyloid fibril structural polymorphism and AD progression. In this context is it import to study and understand real-time Aβ and tau aggregation in animal models and in *in vitro*.

We made fusion constructs in which the N-terminal of Aβ1-42 is labelled with mNeon Green (mNG). mNG-Aβ1-42 was expressed in bacteria and purified as per standard protocol. Aggregation kinetics was monitored using ThT dye binding assay and aggregates were further characterized using transmission electron microscopy (TEM). To study Aβ1-42 amyloid fibril polymorphism *in vivo*, transgenic Drosophila with gene insertion of UAS-mNG-Aβ1-42, UAS-mNG, were generated. Further to study the effect of mNG-Aβ1-42 on Aβ1-42 aggregation *in vivo*, transgenic fly lines were made co-expressing mNG-Aβ1-42 and Aβ1-42.

Aggregation kinetics data *in vitro* of Aβ1-42 suggest that mNG-Aβ1-42 slightly delays while mNG alone does not affect the Aβ1-42 fibrilization. TEM analysis reveals that Aβ1-42 fibrils formed in presence mNG-Aβ1-42 were straight, homogeneous, and more bundled than fibrils formed by Aβ1-42 alone. Hyperspectral imaging data show that mNG-Aβ1-42 gets incorporated in Aβ1-42 fibrils *in vitro*. Survival data from mNG-Aβ1-42 or mNG expressing flies shows more decline in lifespan of mNG-Aβ1-42 compared to mNG. Further ifly assay analysis showed decrease is climbing speed of mNG-Aβ1-42 compared to age matched mNG flies. It was observed that mNG-Aβ1-42 expressing flies a shows presence of non-amyloidogenic aggregates. Data on flies co-expressing mNG-Aβ1-42 together with Aβ1-42 showed partial attenuation of Aβ1-42 induced toxicity. The amyloid dye X-34 was used to stain whole brain and cryosections from these flies. Spectral analysis revealed mNG positive amyloid aggregates of Aβ1-42 corroborating the *in vitro* results of incorporation of mNG-Aβ1-42 into Aβ1-42 fibrils also *in vivo.* Spectral data suggest that X-34 and mNG is an excellent donor-acceptor pair for FRET analysis. We hence used confocal microscopy with FLIM to study aggregates formed in different cell types. FRET analysis of co-aggregated mNG-Aβ1-42 with Aβ1-42 fibrils formed from two different subtypes of cells (glia versus neurons) revealed easily quantifiable FRET efficiency between X34 and mNG. FRET efficiency in glial aggregates compared to neuronal aggregates was dependent on genotype and age. Our FLIM data suggest that different Aβ1-42 fibril polymorphs are produced in different cell types corroborating previous results from our group (Jonsson 2018). This new reporter protein allows us to do in depth (low resolution structure analysis) of temporal and genetic analysis of fibril structural polymorphism in real time *in vivo*.

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Regulation of Myc-Max DNA binding in cancer

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Myc proteins are multifunctional, as they play a role in cell cycle progression, apoptosis, and cellular transformation processes. Myc proteins act as a universal upregulator of gene expression, except early genes in cells. Deregulated Myc proteins lead to an unregulated expression of many genes that results for transforming normal cell to cancer cell (Meyer and Penn 2008). Thus, Myc proteins are strongly considered as a promising target for anti-cancer drugs. These proteins belong to the Myc family of transcription factors and comprises a large intrinsically disordered region, comprising conserved so called Myc Box (MB0 – IV) regions, and a bHLHzip DNA-binding motif (basic helix-loop-helix and leucine zipper motif). These regions play an important role in Myc interactions with DNA and other transcription factors. Our research mainly focuses on the understanding of the interactions between Myc and its interaction partner proteins like Max. Myc-Max heterodimer assembly is known to play a central hub in cellular growth control. Uncontrolled Myc expression disturbs the finely tuned balance of cell growth regulation, which turns the Myc-MaxMax heterodimer into an oncoprotein multimodular platform and acts a key contributor to the development of numerous cancers.

Solved crystal structures of Myc-Max and Max-Max dimers are very similar and only included the bHLHzip DNA-binding motif without flanking regulatory regions. Our group has previously shown by circular dichroism that regions flanking the Max bHLHZip core significantly add helical propensity to the dimer fold (Pursglove 2004). We are focusing on investigating the structure envelope of DNA-bound complexes of Max-Max and Max-Myc dimers using small angle scattering methods (SAXS and SANS). As a first step, we aim to target the SAS of Max-Max-DNA complex, which would provide the full structural envelope of Myc-regulating dimer. As a second step, we aim to target the structure of Myc-Max heterodimers, which provides critical information regarding the modeling the Myc-Max heterodimer with its regulatory flanking regions. To achieve our aims, we have been working on the two versions of Myc, longer c-terminal constructs like $Myc_{303-434}$ which contains DNA binding domain and MBVI, and $Myc_{126-434}$ which contains DNA binding domain and MBVI, MBIII and MBII. We have established the expression and the purification platforms for the Myc versions. Further we have developed the biochemical and biophysical characterization of the tertiary complexes, Myc-Max-DNA complexes, and this kind of characterization is a prerequisite step of achieving our aims.

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Investigating the dynamic interaction of the N-Myc oncoprotein and the protein kinase Aurora A

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The N-Myc protein is a master transcription factor involved in the regulation of numerous pathways important for normal cellular growth and function. Deregulation of N-Myc is a well-known risk factor and driver in both neuronal and nonneuronal tumour types, making the N-Myc oncoprotein a key player in several human cancers[1]. Tight regulation of N-Myc at multiple levels including transcriptional, translational and/or post-transitional levels, is thereby essential to avoid tumour development and progression. The protein levels of N-Myc are tightly controlled by the ubiquitin proteasome pathway, however this path is thought to be disrupted by the protein kinase Aurora A, which stabilizes N-Myc upon binding[2]. The direct interaction between Aurora A and parts of N-Myc is well documented[3], but the exact nature of this stabilizing effect is still unclear and further studies are required.

Here, we investigate the interaction between the intrinsically disordered N-terminus (TAD) of N-Myc (two constructs of different length, both including both MB0 and MBI) and the kinase domain of Aurora A (wt and C290A:C393A, a more stable protein), using several biophysical methods such as nano differential scanning fluorimetry (nano-DSF), Isothermal titration calorimetry (ITC) and Nuclear magnetic resonance spectroscopy (NMR). Our preliminary date revealed a slight difference in binding affinities between N-Myc and the different Aurora A constructs. The binding affinity to the longest N-Myc construct was much stronger.
The NMR data reveals a highly dynamic interaction involving residues spanning MB0 to MBI and the nano-DSF data showed that N-Myc stabilizes Aurora A in a concentration dependent manner. This stabilization continues past a concentration ratio of 1:1 which can be explained be the highly dynamic nature of this interaction seen in the NMR data.

These results expand on previous knowledge about the interaction between N-Myc and Aurora A and takes us one step closer to understanding the nature of this oncogenic partnership.

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Biophysics and MX at Protein Science Facility

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Protein Science Facility (PSF) was founded in 2011 and provides protein production services, user training and access to equipment for biophysical characterisation including macromolecular X-ray crystallography (MX) in yearly use by around 60 different academic and non-academic research groups. PSF has been acknowledged in more than 160 research publications, and more than 200 unique research groups from all large Swedish universities, Finland, Denmark, Norway, Germany, Ghana, UK, and the United States have used PSF over the last ten years.

This poster aims to present PSF biophysical characterization and macromolecular X-ray crystallography platforms mainly intended for self-service after user training however, PSF offers full service when staff and time allows.

PSF biophysical characterisation platform consists of:

- ITC200. Isothermal titration calorimeter is a non-label method for the characterisation of affinity, stoichiometry, and entropy of interaction between biomolecules.
- Biacore 2000. Surface Plasmon Resonance is suitable for protein-protein, protein-ligand, protein-DNA/RNA, and antibody evaluation and requires very low amount of material.
- DSF (thermofluor) and Stargazer. For thermal stability characterization of proteins. A cheap and quick assay for screening of ligand interactions or stabilising buffer conditions.
- CD. Circular dichroism to evaluate the integrity or change in protein secondary structure.

PSF is always available for discussion and evaluation of result data, and guidance for continued experiments. PSF MX offers training and access to state of the art equipment for crystal screening, optimisation, and access to infrastructures for X-ray diffraction data collection and 3D structure determination

- Phoenix and Mosquito nano dispenser for crystallisation experiments in 96-well format.
- Tecan Freedom Evo. A programmable 8-needle dispense robot for 24-well optimizations, cryo-solutions, and crystal screen reformatting from 10 ml tubes to 96 deep well blocks.
- 4C and 20 C Formulatrix imaging plate hotels, allows UV and visible light imaging and remote monitoring of crystallisation experiments.
- Synchrotron BAGs services for X-ray data collection at MAX IV, DIAMOND and BESSY
- 3D structure determination via PReSTO (see separate PReSTO poster)

PSF offers a wide range of pre-dispensed crystal screens in 96 well format, and all necessary solutions for optimisations of initial crystal hits. Tools for crystal handling, freezing and shipment to synchrotrons are maintained and available. User training and supervision in all above steps are offered for self-service after user training researchers however, in recent years PSF noticed a growing interest in full-service 3D structure determination from gene of interest to deposited 3D model and we are adapting accordingly.

Neutron structures of Leishmania mexicana triosephosphate isomerase complexes with reaction intermediate mimics shed light on the proton shuttling steps

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Triosephosphate isomerase (TIM) is a key enzyme in glycolysis that catalyses the interconversion of glyceraldehyde-3-phosphate (GAP) and dihydroxyacetone phosphate (DHAP). This simple reaction involves the shuttling of protons mediated by protolysable side chains. The catalytic power of TIM is thought to stem from the ability to facilitate the deprotonation of a carbon next to a carbonyl group to generate an enediolate intermediate. The enediolate intermediate is believed to be mimicked by the inhibitor 2-phosphoglycolate (PGA) and the following enediol intermediate by phosphoglycolohydroxamate (PGH). We have determined the neutron structure of *Leishmania mexicana* TIM with both inhibitors and performed joint neutron-X-ray refinement followed by quantum refinement. The structures show that in the PGA complex, the postulated general base Glu-167 is protonated, while in the PGH complex it remains deprotonated. The deuteron is clearly localized on Glu-167 in the PGA–TIM structure, suggesting an asymmetric hydrogen bond instead of a low-barrier hydrogen bond. The full picture of active site protonation states allows us to investigate the reaction mechanism with density functional theory calculations.

Classification of Aβ amyloid fibril structures in *Drosophila* and mouse models

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Formation of fibrillar aggregates deposits composed of misfolded proteins known as amyloids, is a pathological hallmark of several neurodegenerative diseases. Alzheimer's disease (AD) pathology is characterized by extracellular senile amyloid plaques, composed of accumulated fibrillar Aβ peptides and intracellular neurofibrillary tau tangles, constituting misfolded microtubule associated tau proteins. Even if the disease-causing protein is composed of the same primary sequence, the tertiary and quaternary fibrillar structures can be polymorphic. Several studies showed that this structural polymorphism may correlate with different clinical phenotypes of AD patients (Rasmussen et al., 2017). To elucidate the correlation between the polymorphism in the structure of Aβ fibrils and the disease phenotype, we utilize different model systems – *Drosophila melanogaster*, mouse models as well as recombinantly expressed proteins in *in-vitro* experiments. Previous studies from the lab showed that expressing the Aβ peptide in either neurons or glia in *Drosophila melanogaster* gives rise to different fibril structures and toxicity (Jonson et al., 2019).

We now aim to further explore how different AD mouse models vary in respect to amyloid morphology in Aβ plaques. With the aid of amyloid conformation sensitive dyes LCOs (Luminescence Conjugated Oligothiophenes) and hyper spectral imaging fluorescence microscopy, we observed that different mouse lines exhibit different structures depending on transgenic genotype. This observation was confirmed by ROI (region of interest) analysis along with a more unbiased approach to analyze every pixel in the images by imaging analysis software developed in RStudio. Also, we ask whether these structures have different kinetic profiles while forming the aggregates. Preliminary data imply that distinct kinetic differences depend on seed origin and Aβ peptide substrates. These structural studies may shed light on better classifying amyloid polymorphs and their impact on clinical phenotypes of AD. Finally, these may help to identify disease relevant structures for high resolution studies by CryoEM to determine druggable target and design biomolecules that can be used for therapeutic purposes (Fändrich et al. 2018).

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Expression, refolding, and purification of recombinant *Bombyx mori.* Cocoonase using *E. coli*

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The silk fiber is harvested from the cocoon of the silk moth, *Bombyx mori*. The cocoon is predominantly built up from the fiber protein, fibroin, and an adhesive protein called sericin that connects the fibroin fibers. Cocoonase is a serine protease enzyme secreted by silk moth to soften the cocoons to enable their escape with the following development into the adult silk moth. Cocoonase is secreted by specific glands on the head of the moth and specifically digests sericin. Removal of sericin, i.e. degumming, is an initial step in silk processing to release the fibroin component. This process has been traditionally performed with energy-demanding procedures, and there is a need to find green alternatives for this process. Using cocoonase as a degumming agent in industrial processes has great potential, as it is more efficient and less damaging to the silk fiber. We here report the production and purification of *Bombyx mori.* cocoonase using an *E. coli* expression system. Cocoonase can be highly expressed in *E. coli*; however, it predominantly remains as insoluble inclusion bodies. To recover protein from these inclusion bodies, we have developed a protocol that can refold protein in a buffer with a balanced redox potential, composed of 1:5 of oxidized and reduced glutathione. The soluble and refolded proteins are subjected to further three-step purification. Based on our reproducible protocols, we can produce the functional cocoonase up to 15 mg/L scale of bacterial cell culture. Therefore, this *E. coli* based production is suitable for scaling up to provide a sufficient amount of protein for structure determination and modification of cocoonase in terms of catalytic function and stability. Cocoonase was found to be highly effective in hydrolysis of the cocoons by means of a NMR based functional assay where solid cocoons were subjected to hydrolysis by cocoonase.

Key words: Silk moth (*Bombyx mori.*), cocoonase, serine protease, refolding, and *Escherichia coli*

Structural and functional characterization of viral 2A$^{H\text{-}box/NC}$ proteins

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The Picornaviruses are a large family of non-enveloped, small RNA viruses, responsible for numerous human and animal diseases including the common cold, hepatitis A as well as foot-and-mouth disease[1]. During infection, the virus attaches to susceptible cells via specific receptors on the cell membrane, which triggers its internalization. After pore-mediated transfer to the cytoplasm, the monopartite ss(+)-RNA viral genome serves as mRNA. The Internal Ribosome Entry Site (IRES) in the 5'-UTR guides cap-independent translation of the single open reading frame. The encoded polyprotein is approximately 200-250kDa in size and exhibits a conserved organization, where the N-terminal region codes for the structural (capsid) proteins, followed by the non-structural proteins required for viral replication. Already during translation, the polyprotein gets processed in a multi-step cascade into a range of precursor and mature proteins through the action of virally encoded proteases. While several of the non-structural proteins are quite well conserved, especially 2C, 3C^{pro} and 3D^{pol} involved in polyprotein processing and RNA replication, other proteins are less well conserved. Of these, the 2A proteins display the most notable divergence in sequence and function across the different genera. In *Enterovirus*, the 2A protein is a chymotrypsin-like protease that cleaves its own N-terminus from the P1 precursor. This 2A^{pro} is also known to cleave eiF-4G and thus, shut down the cap-dependent translation of the cell. In *Aphtovirus* like the Foot-and-Mouth Disease Virus (FMDV), 2A^{npgp} triggers polyprotein processing through ribosome skipping. A third type of 2A protein has been described in a range of other picornaviruses, including *Parechovirus and Aichivirus*. This 2A is fascinating to study due to its previously identified similarities[2] and with the recently identified picornavirus host factor PLA2G16[3]. Indeed, these 2A proteins share sequence homology with H-rev107 family through a region called H-box/NC motif[2], suggesting that these viruses might have acquired the protein through horizontal gene transfer to become independent of the human host factor. However, its role in the viral life cycle remains unclear. In this study, we aim to use structural and functional studies of this class of 2A proteins to investigate their role in the viral life cycle and how they might confer independence of the human host factor.

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Structural investigation of full-length deubiquitinating enzyme USP14 in solution

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The ubiquitin proteasome system (UPS) is a promising area for drug development targets such as E1/E2 ubiquitin enzymes and deubiquitinases (DUBs), all containing functional cysteines. Ubiquitin-specific protease 14 (USP14), one of three DUBs associated to the 26S proteasome, is a dual-domain protein consisting of a ubiquitin-like (Ubl) domain and a catalytic core domain. The Ubl-domain is responsible for the association to the proteasome while the core domain binds ubiquitin conjugated to proteins sent for proteasomal degradation and cleaves the isopeptide chain. Expression levels of USP14 is significantly higher in cancers such as lung, breast and prostate cancer when comparing to healthy cells and is therefore an interesting therapeutic target[1]. In collaboration with our pharmacology collaborators, we characterized 10 different active drugs and found evidence of selective inhibition of USP14 where 7 of these showed significant antineoplastic activity in zebrafish embryos2. As USP14 gets activated upon binding to the proteasome, we wanted to investigate the behaviour of free USP14 in solution. Here we present a structural analysis of USP14 in solution by combining small angle x-ray scattering (SAXS), nuclear magnetic resonance (NMR) and modelling using Rosetta. We collected experimental data on full-length protein as well as the isolated domains and used these as constrains for selecting the generated models. From our analysis we draw the conclusion that the two domains move independently. However, differences in chemical shifts and rotational correlation time (τc) of the Ubl-domain, as part of the full-length protein or individual, suggest slight interdomain interactions of USP14 when free in solution.

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In situ investigation into Langat virus (LGTV) replication using cryo-electron tomography of infected mice and cells

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Flaviviruses are enveloped positive-sense RNA viruses that infect humans and cause serious disease. Flaviviruses like those part of the tick-borne encephalitis virus (TBEV) complex including the antigenically related Langat virus (LGTV) can cause permanent neurological damage or even death. These viruses manipulate host cell membranes, hijack several other cellular processes and induce the formation of an organelle for genome replication called a replication complex (RC) within the endoplasmic reticulum (ER). An RC assembles via "non-structural viral proteins" (NS1-5) which are expressed as a polyprotein and then cleaved into individual proteins in which each serve a particular function in viral RNA replication. Despite their crucial role in flavivirus replication, virtually nothing is known about the structural architecture of RCs. I use *in situ* cryo-electron tomography (cryo-ET) to tackle this crucial gap in the understanding of flavivirus biology. Human cells and mice were infected with LGTV and these cells and the brain tissue from the infected mice were subjected to cryo-focused ion-beam milling and cryo-ET. The resulting tomograms of LGTV RCs revealed insights into the organization of this unique viral organelle. This work provides a structural basis for molecular virology of flaviviruses, paving the way for new approaches to antiviral development as well as serving as a model for similar studies into other human viruses.

Structure and mechanism of a phage- encoded SAM lyase revises catalytic function of enzyme family

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The first bacteriophage-encoded S-adenosyl methionine (SAM) degrading enzyme (SAMase) was discovered in bacteriophage T3 in the 1960s. It was found to protect the phage against the bacterial restriction-modification system, and annotated as a SAM hydrolase forming 5'-methyl-thioadenosine (MTA) and L-homoserine. From environmental phages, we recently discovered three SAMases with barely detectable sequence similarity to T3 SAMase or to each other, and without homology to proteins of known structure [1]. Here, we present the very first phage SAMase structures, in complex with a substrate analogue and the product MTA. The structure forms a trimer of alpha–beta sandwiches similar to the GlnB-like superfamily, with active sites formed at the trimer interfaces. Molecular dynamics failed to identify a water atom that could participate in hydrolysis, and quantum-mechanical calculations suggested that SAM within the active site could instead react with itself in a lyase reaction forming MTA and L-homoserine lactone. These two reaction products were confirmed using thin-layer chromatography and nuclear magnetic resonance spectroscopy. Sequence analysis and *in vitro* and *in vivo* experiments with mutants of the different SAMases support that they all belong to the same structural family and utilize the same reaction mechanism. The conclusion is that phage-encoded SAMases are not hydrolases but lyases forming MTA and L-homoserine lactone in a unimolecular reaction mechanism [2].

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Identification of non-ubiquitin-binding USP14 mutants

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The ubiquitin proteasome system is responsible for the controlled degradation of proteins, a process crucial for the maintenance of cellular homeostasis and viability. Proteins are tagged for proteasomal degradation by the small protein ubiquitin. At the proteasome, ubiquitin chains are removed by deubiquitinating enzymes, such as ubiquitin specific protease 14 (USP14), to regulate protein degradation and allow recycling of ubiquitin. USP14 not only regulates proteasomal degradation by its catalytic activity, it also exerts allosteric regulatory control over the proteasome.[1] Since USP14 is overexpressed in several cancer types it represents a novel target for anti-cancer drug development.[2] A better understanding of the catalytic and regulatory roles of USP14 is imperative for future drug design.

It is common to use the catalytically inactive mutant C114A to study the allosteric effects of USP14 at the proteasome. However, C114A binds ubiquitin, potentially with a higher affinity than wildtype.[3] Since ubiquitin binding alters the effects of USP14 on the proteasome[1], studying wildtype and C114A is not sufficient to give a complete picture. The aim of this project is to identify and characterize non-ubiquitin-binding USP14 mutants in order to study how USP14 regulates proteasome activity.

Eight binding pocket mutants were produced, and their activity was evaluated in two assays. Mutant Y333V was proficient for di-ubiquitin cleavage but not ubiquitin-propargylamide binding, indicating reduced ubiquitin affinity, whereas D199A and E202K did not bind nor cleave ubiquitin. Circular dichroism spectroscopy was used to investigate the tertiary structure of the mutants. Native fold could be confirmed for Y333V and E202K, but not with certainty for D199A. However, fluorescence thermal shift assay confirmed that all three mutants were thermally stable, indicating that the reduced ubiquitin affinity observed was not due to protein unfolding. Lastly, using proteasome substrates it was shown that the ubiquitin binding mutants behave similarly to wildtype USP14 in decreasing proteasome gate opening, indicating that the mutations do not prevent proteasome binding.

Thus, three interesting mutants were identified: E202K and D199A which are non-ubiquitin binding and Y333V which binds ubiquitin with reduced affinity. These mutants will be valuable tools for studying the role of USP14 at the proteasome.

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Protein Science Facility, Karolinska Institutet

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The Protein Science Facility provides protein production services and user training and access to equipment for macromolecular crystallography and biophysical characterisation of proteins.

PSF started in 2011 and has served its user community for 10 years. PSF is open to all academic and non-academic user groups and works with groups from all major Swedish universities, a range of companies, and also international user groups from Finland, Denmark, Norway, Germany, Ghana, UK, and USA.

PSF works with 60-70 user groups yearly, and has worked with over 200 unique groups since the start. The work of PSF has so far contributed to over 160 published papers.

This poster presents the protein production services of PSF.

PSF offers expert service and advice at all levels of protein production.

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- Molecular biology services and assistance in expression construct design and ordering.
- Small-scale expression and solubility tests in bacterial, HEK, and CHO cells.
- Liter scale (1- 20 liter) production cultures in bacterial, HEK, and CHO cells.
- Protein purification using a range of established protocols.

Mammalian cell work is done in collaboration with Juni Andréll and the Eukaryotic Protein Production (EPP) lab at SciLifeLab Solna.

The produced proteins are used in a wide range of research areas, such as structural biology, biochemistry studies of protein function and interactions, enzyme activity, cell biology, assay development, drug lead development, imaging studies, and antigen and antibody production.

PSF also provides its user base with many utility enzymes of different categories, for instance DNA/RNA polymerases, ligases and reverse transcriptases, proteases, Cas enzymes, Tn5 transposase, and sortase A variants.

During the COVID-19 pandemic of 2020 and 2021, PSF has been involved in multiple projects for virus testing, antibody screening, drug lead and method development toward the understanding of and fight against the SARS-CoV-2 virus.

PSF is part of the protein production network Sweden (PPNS) that aims to share resources and expertise to the benefit of the user community. In 2021, PSF and groups from UmU, KTH, GU, and LU submitted a joint application to VR for the funding of a national infrastructure for protein production in Sweden (PPS). A decision is expected in September.

Selective targeting of a core protein interaction for insulin release

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Elevated insulin release induced hyperinsulinemia plays a causal role in obesity and insulin resistance which increase the risk for type 2 diabetes. Insulin is released from pancreatic β-cells via vesicle-mediated exocytosis that is regulated by intracellular calcium (Ca^{2+}) concentration-dependent signaling. The signal of increased intracellular Ca^{2+} concentration is transmitted by Ca^{2+}-binding proteins, so called Ca^{2+}-sensors. Such Ca^{2+}-sensor, which is abundantly expressed in β-cells, is secretagogin. Secretagogin undergoes conformational changes upon Ca^{2+}-binding that promotes the formation of protein-protein interactions modulating insulin secretion. Secretagogin loss-of-function leads to a diabetic phenotype but the exact molecular mechanisms of its action remain elusive.

To elucidate how secretagogin influences insulin secretion, we targeted its core interaction in vesicle-mediated exocytosis. We have identified about a hundred of putative interacting partners of secretagogin including SNAP-25 (synaptosomal-associated protein of 25 kD), which is a member of the SNARE (soluble NSF-attachment protein receptors; NSF, N-ethylmaleimide-sensitive fusion protein)-complex mediating vesicle docking and release (1). Using direct probing of recombinant proteins of secretagogin and SNAP-25 we confirmed their Ca^{2+}-dependent binding with high affinity. Moreover, we identified the interaction motif in SNAP-25 that forms the secretagogin binding site. Mapping the binding interface allows us to screen for small molecules that mimic the binding partner and can interfere with secretagogin interactions. This will enable the investigation of secretagogin's impact on vesicle docking and insulin release. Revealing the exact molecular mechanism and providing small molecules to target this interaction will make drug development possible to address impairments in insulin resistance and type 2 diabetes. In addition, as vesicular release of other hormones shares the same molecular machinery, our findings may have implications in novel therapies for disorders where secretagogin-dependent Ca^{2+}-signaling takes center stage.

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MicroMAX – for microcrystals, serial crystallography and time-resolved studies

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MicroMAX at the first 4th generation storage ring [1] at MAX IV Laboratory is a new beamline providing the macromolecular crystallography field with a new powerful tool. The main applications are serial crystallography, time-resolved science, and micro-crystallography.

The beamline will offer different sample delivery systems for serial crystallography, in particular fixed-target and injector-based systems but be flexible to accommodate other setups. In addition, the experiment setup will also provide a highly automated mode for oscillation data collection including a robotic sample changer. The setup will include a chopper providing short X-ray pulses (down to microseconds) and instrumentation for different time-resolved experiments. The detector stage will host two area detectors, a photon-counting and an integrating detector.

The X-ray beam at the sample will have 10^{13} photons/second in monochromatic mode (5-25 keV energy range) and up to 10^{15} photons/second using a wider energy bandpass mode (10-13 keV energy range). The X-ray beam size will be flexible and easily tailored to the experimental needs, initially focused down to 10 µm and in a second stage down to 1 µm.

The possibility to combine all these different modes and instrumentation in a flexible way will allow to cater a wide range of experiments in structural biology including methods not yet developed.

X-ray commissioning of MicroMAX is planned to start in 2022. MicroMAX is funded by the Novo Nordisk Foundation.

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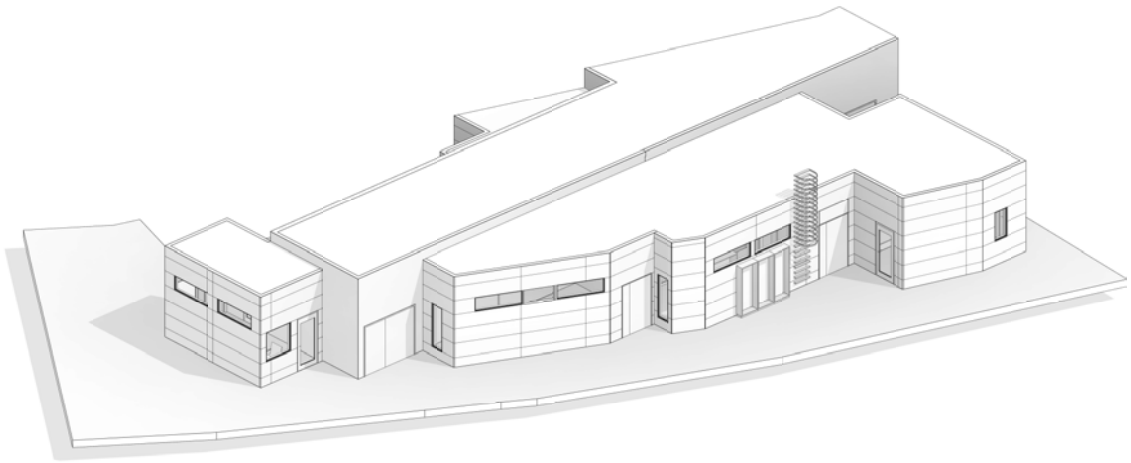

Structural and functional characterization of PLAAT3

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PLAAT3 is a small, intracellular phospholipase initially characterised as a class II tumour suppressor[1] and recently described to have an essential role in organelle clearance during lens development.[2] The enzyme is composed of an N-terminal catalytic domain, followed by a C-terminal hydrophobic region of 30 amino acids. Prior structural studies of the catalytic domain have revealed a conserved catalytical triad in an arrangement compatible with catalysis.[3,4,5] However, the C-terminal is required for proper enzymatic function. This hydrophobic region, which is not constitutively membrane associated, is rather involved in the recruitment of PLAAT3 to sites of membrane damage.[2,6]

PLAAT3 was recently discovered as an essential host factor for certain enteroviruses where PLAAT3 is involved early in the viral life cycle, facilitating genome delivery into the cytoplasm.[6] The role as a host factor is reliant on the catalytic activity and recruitment of PLAAT3 through its C-terminus.[6] Therefore, cells lacking the catalytic function of PLAAT3 (either through mutation or inhibition) should be resistant against infections by these biomedically important viruses, making PLAAT3 an attractive target for the development of novel antiviral therapies.

In this study, we aim to clarify the role of the C-terminal region. To gain a better understanding of PLAAT3's mechanism of action at the molecular level, we aim to elucidate the structure and dynamics of the full-length protein by NMR and X-ray crystallography. This will be complemented with enzymatic assays to probe the catalytic function of PLAAT3.

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Expressing and Purifying TRPA1 from *Hylobius abietis* to study its Structure and Activation

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Pine weevil (*Hylobius abietis*) is a pest that causes huge problems for the forest industry as it destroys lots of newly planted trees. This problem can hopefully be stopped by the use of repellents that could deter the bugs from attacking the trees. We are investigating the possibility to use the nociceptor TRPA1 (Transient Receptor Potential Ankyrin 1) as a target for agonist activation which could result in an antifeedant response in *H. abietis*.

TRPA1 is expressed in *Pichia pastoris* to study the activation and structure. The protein is truncated in a similar fashion to what has been done on human and mosquito previously in our group (Moparthi et al., 2014), (Survery et al., 2016), with the hope of increased expression levels and stability to make handling easier. The removed part mainly consists of the ankyrin repeat domain situated at the N terminus, and brings the protein size from around 134 kDa to 55 kDa. We have identified the detergent foscholine-12 as a good choice for the solubilisation of the protein. The use of a zwitterionic detergent gives good extraction from the membrane, but is not very specific which will force us to rely more on later purification steps.

The structure of human TRPA1 was first solved in 2015 by Paulsen et al. using cryo-EM (Paulsen et al., 2015). Since then more structures have been published but not from any other species than human. Suo et al. recently published three structures where they used nanodiscs to stabilise the protein in a more native environment (Suo et al., 2020). We hope to use nanodiscs in a similar fashion to help the structure determination.

We also want to study the activation of TRPA1 by developing a potassium assay, which could allow for simple activity measurements and agonist screens.

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