23rd Swedish Conference on Macromolecular Structure and Function

Tällberg, 14-17 June 2019

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Oral presentations

Illuminating Genome Organization through Integrated Microscopy and Sequencing

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In human cells, two meters of DNA sequence are compressed into a nucleus whose linear size is five orders of magnitude smaller. Deciphering how this amazing structural organization is achieved and how DNA functions can ensue in the environment of a cell's nucleus represent central questions for contemporary biology.

Here, I embrace this challenge by establishing a comprehensive framework of microscopy and sequencing technologies coupled with advanced analytical approaches, aimed at addressing three fundamental highly-interconnected questions: *1) What are the design principles that govern DNA compaction? 2) How does genome structure vary between different cell types as well as among cells of the same type?* 3) *What is the link between genome structure and function?* To this aim, we have devised a powerful method for Genomic loci Positioning by Sequencing (GPSeq), which allows genome-wide measurements of the distance of genomic loci to the nuclear periphery. Using GPSeq, we are generating reproducible maps of radial genome organization in human cells, in order to reveal radial gradients of genetic and epigenetic features as well as gene expression in various cell and tissue types. In parallel, we are developing high-end microscopy tools for simultaneous localization of dozens of genomic locations at high resolution in thousands of single cells.

We are generating the first-ever genome-wide maps of radial positioning of DNA loci in the nucleus, and combining them with available DNA contact probability maps in order to build 3D models of the human genome structure in different cell and tissue types. Using microscopy, we are visualizing chromosomal shapes at unprecedented resolution, and use these rich datasets to discover general DNA folding principles. Our study shall illuminate the design principles that dictate how genetic information is packed and read in the human nucleus, while providing a comprehensive repertoire of tools for studying genome organization.

Dynamic conformational ensembles of proteins in vitro and in cells

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Confined by the Boltzmann distribution of the energies of states, motion is inherent to biomolecules. This omnipresent dynamics is believed to be involved in the function of proteins including enzyme activity and protein allostery. For a detailed understanding of a protein's function, not only the 3D structure but also the description of its dynamics is thus required.

Using experimentally-derived exact nuclear Overhauser effect (eNOE) liquid state NMR we studied the enzyme cyclophilin. The 3D structure of the ensembles of states reveals the presence of an open and a closed state of cyclophilin, which is indicative of large-scale correlated motion. In the open state, the catalytic site is preorganized for catalysis, thus suggesting the mechanism of action to be conformational sampling, while the ligand-binding loop appears to act through an induced fit mechanism. This finding is supported by affinity measurements of a cyclophilin designed to be more open. Overall, more than 60–70% of the side-chain conformations of cyclophilin appear to be correlated.

Protein allostery describes the long range coupling between two active sites of a protein far apart from each other as exempliefied on the WW domain of the proline cis/trans isomerase Pin1, with a coupling between the ligand binding site and the inter-domain interaction site. Here, the multi-state-structures of the WW domain of the free form and in complex with two antogonizing ligands, one which is strengthening the allostery and one which suppresses it, has been determined. It is found that in the absence of ligands, the protein undergoes a micro-second exchange between two states, of which one is believed to be fit to interact with the catalytic domain, while the other one is not. In presence of the positive allosteric ligand, the equilibrium between the two states is shifted highlighting the mode of ligand action to be conformational selection as proposed by Monod. In contrast, the allostery-surpressing ligand is decoupling the side chain arrangement at the inter phase into an anti-correlative orientation and dynamics thereby reducing the inter-domain interaction. Its action can thereby regarded to be of dynamic allostery nature. The presented distinct modes of action highlight the power of the dynamics-structural interplay in the biological activity of proteins.

Finally, first steps into the elucidation of the dynamics and structures of proteins in cells are documented.

Towards better understanding of MYC regulation, focusing on myc boxes 0-II

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The intrinsically disordered oncoprotein c-Myc (MYC) is a master regulator of cell viability and is overexpressed in more than half of human cancers. Located within the intrinsically disordered N-terminus part of MYC are the highly conserved so-called MYC boxes (MB0-IV), which are crucial for MYC function and interaction with other transcription factors. In collaboration, we have used BioID to identify several hundreds of MYC dynamic interaction partners and used deletion mutants to identify MB0 and MBII as critical for tumor growth. (Kalkat et al. 2018). The Sunnerhagen group has previously thoroughly studied MYC(aa1-88) containing MB0-MBI, both alone and together with interactors (Andresen et al. 2012, Helander et al. 2015). We will now present structural and dynamical data for a longer construct containing MB0-MBI-MBII. For better understanding of MYC transcription factor activity we will also present results that describe the interaction between this construct and TBP. Most target genes regulated by MYC are transcribed by RNA polymerase II (Pol II). Pol II forms a pre-initiation complex (PIC), together with TFIID, a multimeric protein assembly composed of TBP and 13 TBP-associated factors (TAFs). We show that the interaction between MYC and TBP is of significant importance to understand MYC's role as an oncoprotein.

Andresen et al., Nucleic acids research 2012. *Transient structure and dynamics in the disordered c-Myc transactivation domain affect Bin1 binding.*

Helander et al., Structure 2015. *Pre-Anchoring of Pin1 to Unphosphorylated c-Myc in a Fuzzy Complex Regulates c-Myc Activity.*

Kalkat et al., Molecular Cell 2018. *MYC Protein Interactome Profiling Reveals Functionally Distinct Regions that Cooperate to Drive Tumorigenesis.*

Yong et al., Nature SMB in review 2019. *Multiple direct interactions of TBP with the MYC oncoprotein*.

The substrate selectivity of human adenylate kinase 3

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Adenylate kinase 3 (AK3) is a human adenylate kinase that interconverts GTP + AMP into GDP + ADP. Previously, the lab has investigated the mechanisms for the selectivity of *Escherichia coli* adenylate kinase (eADK), which is ATP-selective. By coupling kinetics assays with structural studies, we have now elucidated a model for the mechanism of substrate selectivity for AK3, which can be contrasted with the observations with eADK. In doing so, we have identified a key selectivity loop that differs between the ATP-selective eADK, and GTP-selective AK3. This discovery uncovered a key structural indicator that potentially determines the substrate selectivity across the large NMP kinase family of enzymes. Furthermore, kinetic inhibition studies using AK3 and ATP identifies the molecular mechanism through which ATP inhibits AK3 and further reinforces the significance of the selectivity loop motif.

NMR, a molecular microscope to characterize the peroxiredoxin-Hsp70 interplay slowing down aging

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Peroxiredoxins (PRDXs) are thiol active peroxidases responsible for the regulation of peroxides in the cell thanks to the presence of a peroxidatic cysteine within their active site. Upon elevated peroxides levels, they are subject to catalytic inactivation by hyperoxidation, which involves structural modifications and leads to the exhibition of chaperone function.[1] Recently, the major cytosolic PRDX in *S. cerevisiae*, Tsa1, was shown to recruit the molecular chaperone Ssa1 to aggregated proteins through an H_2O_2-specific redox switch. Interestingly, the sulfinylation of Tsa1 supports Ssa1 binding to damaged proteins accumulated in the aging process and its subsequent reduction triggers the disaggregation of misfolded and aggregated proteins.[2,3] This molecular switch between peroxidase and chaperone functions seems to be a key aspect of this functional mechanism. However, besides the fact that Tsa1 and Ssa1 can physically interact, the structural and dynamical details of this interaction at the molecular level, as well as the functional consequences for both enzymes, remain elusive.

Using advanced high-resolution NMR-spectroscopy, we are studying the mechanism of the Tsa1-Ssa1 interaction, in order to characterize this complex in detail and to decipher the functional consequences for both enzymes. As classical solution state NMR-approaches are limited regarding the size of the system (300 kDa), two approaches are considered. First, the characterization of stable smaller Tsa1-variants using sire directed mutagenesis. Then, the application of methyl-TROSY NMR experiments in combination with methyl-specific isotopic labeling for the study of the larger wild-type protein complex.[4] Finally, for the characterization of the chaperone Ssa1, a divide-and-conquer approach is implemented to study both sub-domains of Ssa1 separately.[5] Here, we present the initial characterization of Tsa1-WT and Tsa1-S78D variant correlated with first NMR experiments on specific-methyl-labeled Tsa1, as well as preliminary results on Ssa1 subdomains.

1. Wood, Z. A., Poole, L. B. & Karplus, P. A. Science (2003).
2. Molin, M., Yang, J., Hanzen, S., Toledano, M. B., Labarre, J., & Nystrom, T. Mol Cell (2011).
3. Hanzen, S. Cell (2016).
4. Burmann, B. M., and Hiller, S. Chimia (Aarau) (2012).
5. Zhuravleva, A., Clerico, E. M. & Gierasch, L. M. Cell, (2012).

Structure, assembly and reaction chemistry of the DNA replisome

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DNA replication is essential for cell proliferation. In this presentation, I will first present a prototypical structure of a simple DNA replication fork, where leading and lagging strand DNA syntheses are coupled to downstream DNA unwinding and primer synthesis. Replication fork arrangement indicates how lesions may be detected by DNA polymerase and helicase. Secondly, by combining *in crystallo* catalysis with time-resolved X-ray diffraction analysis, we have observed reaction intermediates of DNA synthesis at unprecedented atomic detail. Contrary to the conventional view that DNA synthesis occurs by a two-Mg^{2+}-ion mechanism, we have discovered that two Mg^{2+} ions bound to the polymerase active site are insufficient for product formation. A third Mg^{2+} ion must be captured by the enzyme-substrate complex en route to product formation. This third metal ion is free of enzyme coordination and appears to drive phosphoryl-transfer by breaking the existing phosphodiester bond in dNTP. Lastly, we find that cation trafficking in the DNA synthesis reaction is not an exception to the rule of the transition state theory, but it also drives RNA hydrolysis by nucleases.

Structural basis for sequence-specific DNA binding by the iron-dependent transcriptional regulator IdeR from *Saccharopolyspora erythraea*

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Metal ions play a crucial role in the survival of all organisms. They serve as cofactors for proteins, enabling them to perform reactions or bind molecules that proteins alone cannot accomplish. The great functional variety of metalloproteins is a result of the different physical and chemical properties of metal ions. Incorporation of the wrong metal ion can have a toxic effect for the cell by altering the protein's activity or function. It is therefore essential for cell survival to avoid mismetallation, which is ensured by regulating the concentrations of the available metal ions in the cell. In prokaryotes, metal homeostasis is controlled by metal-sensing transcription factors, which, upon metal-binding, repress or activate the expression of certain sets of genes. This enables the cell to react to the lack or excess of a certain metal by, for example, inducing the gene expression of metal transporters or storage proteins.

In this project, we are investigating the structure-function relationship of the iron-dependent regulator (IdeR) in the Gram-positive actinobacterium *Saccharopolyspora erythraea*. IdeR belongs to the Diphteria toxin repressor family. Homologs of IdeR can be found in many other actinobacteria, including human pathogens, such as *Mycobacterium tuberculosis*, which requires IdeR for virulence.

We recently determined the three-dimensional structure of metal-bound IdeR with and without its DNA recognition sequence bound by X-ray crystallography to 2.3 and 2.4 Å resolution, respectively. Together with the results of DNA-binding assays, the structure of the ternary complex revealed that interactions with particular bases and recognition of the overall DNA shape is needed for sequence-specific DNA-binding upon protein-metallation. We were able to pinpoint two nucleotides in the recognition sequence that are required for specific base interactions with the iron sensor.

We are now investigating the effects of different metal ions on the structure of IdeR in order to understand how it differentiates between iron and other d-metals, such as Mn^{2+} and Zn^{2+}, and determine their affinities for IdeR. Furthermore, we are attempting to solve the apo-protein structure, which will reveal how metal-binding activates the protein for DNA-binding.

Turning *E. coli* into a "photosynthetic" H_2 producing biocatalyst

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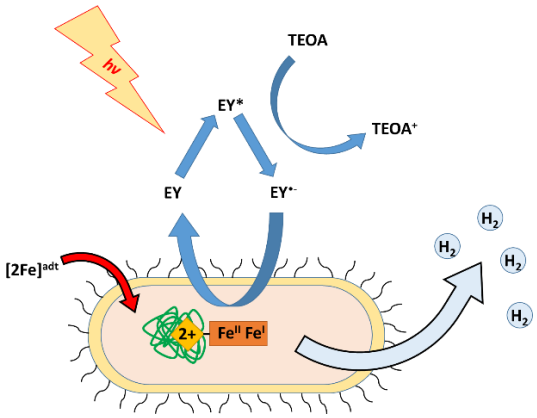
Hydrogen is arguably the most promising **energy carrier** to be employed in the energy transition that will inevitably take place in the next one or two decades. In order for it to be a viable option, an **efficient and sustainable hydrogen production** method must be developed.

Among molecular catalysts, **[FeFe] Hydrogenases** have attracted the attention of the community by virtue of their **high turnover frequency** for hydrogen evolution (up to 10,000 s^{-1}) in combination with low overpotential requirements.[1] Their major drawback is their **oxygen-sensitivity**, which currently prevents us from using them as industrial-scale catalysts.

A way to **protect** these enzymes from O_2 inactivation would be to exploit the microoxic conditions that can be found in the cytoplasm of many aerobic bacteria, such as the widely employed *E.coli*. Our group developed a method for ***in vivo* artificial maturation** of [FeFe] Hydrogenases, where the enzyme is expressed in its *apo*-form and then matured by providing an artificially synthetized cofactor mimic ($[2Fe]^{adt}$) to the cell culture. This method allows us to generate semi-synthetic enzymes under *in vivo* conditions, enabling both spectroscopic investigations and *in vivo* H_2 production.[2, 3]

Here, I will present my work on transforming *E. coli* into a **light-driven hydrogen producing whole-cell biocatalyst**. This is achieved through the *in vivo* artificial maturation of the **[FeFe] hydrogenase from *C. reinhardtii* (*Cr*HydA1)**, in combination with the addition of a synthetic photosensitizer. When compared to glucose as a fermentable electron source, the use of the organic dye Eosin Y with TEOA as sacrificial electron donor results in a **relatively high and sustained H_2 production**, even under dim light conditions.



(1) W. Lubitz, H. Ogata, O. Rudiger, E. Reijerse – Hydrogenases ***– Chem. Rev., 2014****,* 114(8), 4081-4148

(2) N. Khanna, C. Esmieu, L. S. Meszaros, P. Lindblad and G. Berggren, **Energy Environ. Science**, **2017**, 10, 1563-1567.

(3) *L. S. Meszaros, B. Nemeth, C. Esmieu, P. Ceccaldi and G. Berggren*, **Angew. Chem., Int. Ed.**, **2018**, *57*, 2596-2599

Targeted protein aggregation

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The most common mechanism for protein aggregation results from the propensity of proteins to form amyloid-like interactions between identical protein chains. Indeed, most protein polypeptide sequences possess short aggregation prone regions (APRs) have the intrinsic propensity to self-associate by beta-strand interactions. Our laboratory has pioneered the development of several bioinformatics algorithms, including TANGO [1] and WALTZ [2], allowing the prediction of APRs in proteins. The extremely ordered, in-register packing of side chains in amyloid fibrils explains why the assembly of such structures is highly sequence specific and thus selective [3]. We investigated whether the sequence-specificity of APRs can be used to specifically knockdown protein function by induced protein aggregation. In a first example, Pept-Ins were designed based on the APRs found in a negative regulator of the brassinosteroid (BR) signaling pathway (BIN2) of *Arabidopsis thaliana* [4, 5]. These Pept-Ins induced the aggregation and subsequent inactivation of the target protein, resulting in a constitutive BR response as seen from the growth phenotype. Second, we developed Pept-Ins whose sequences were based on APRs of bacterial proteomes (*Staphylococcus epidermidis* and *E. coli*) that showed a strong anti-bacterial effect in vitro and in vivo by inducing cell-wide aggregation and the subsequent formation of lethal inclusion bodies [6, 7]. Finally, an anti-tumoral Pept-In was designed whose sequence is based on an APR found in the human vascular endothelial growth factor receptor 2 (VEGFR2) [8]. It was shown that this peptin can induce the aggregation of VEGFR2, thereby knocking down its function *in vitro*. When applied intravenously, vascin reduces VEGFR2-dependent tumor growth in mice xenografts. Taken together these data indicate that target protein aggregation is possible and may be interesting to exploit for therapeutic purposes.

References

[1] Fernandez-Escamilla AM, Rousseau F, Schymkowitz J, Serrano L. Prediction of sequence-dependent and mutational effects on the aggregation of peptides and proteins. Nature Biotechnology. 2004;22:1302-6.

[2] Maurer-Stroh S, Debulpaep M, Kuemmerer N, de la Paz ML, Martins IC, Reumers J, et al. Exploring the sequence determinants of amyloid structure using position-specific scoring matrices. Nature Methods. 2010;7:237-U109.

[3] Ganesan A, Debulpaep M, Wilkinson H, Van Durme J, De Baets G, Jonckheere W, et al. Selectivity of aggregation-determining interactions. J Mol Biol. 2015;427:236-47.

[4] Betti C, Schymkowitz J, Rousseau F, Russinova E. Selective Knockdowns in Maize by Sequence-Specific Protein Aggregation. Methods Mol Biol. 2018;1676:109-27.

[5] Betti C, Vanhoutte I, Coutuer S, De Rycke R, Mishev K, Vuylsteke M, et al. Sequence-Specific Protein Aggregation Generates Defined Protein Knockdowns in Plants. Plant physiology. 2016;171:773-87.

[6] Khodaparast L, Khodaparast L, Gallardo R, Louros NN, Michiels E, Ramakrishnan R, et al. Aggregating sequences that occur in many proteins constitute weak spots of bacterial proteostasis. Nature communications. 2018;9:866.

[7] Bednarska NG, van Eldere J, Gallardo R, Ganesan A, Ramakers M, Vogel I, et al. Protein aggregation as an antibiotic design strategy. Mol Microbiol. 2015.

[8] Gallardo R, Ramakers M, De Smet F, Claes F, Khodaparast L, Khodaparast L, et al. De novo design of a biologically active amyloid. Science. 2016;354.

Human Dihydroorotate Dehydrogenase (*h*DHODH) as a new target on Acute Myelogenous Leukemia (AML): Targeting Myeloid Differentiation using Potent and Innovative *h*DHODH Inhibitors

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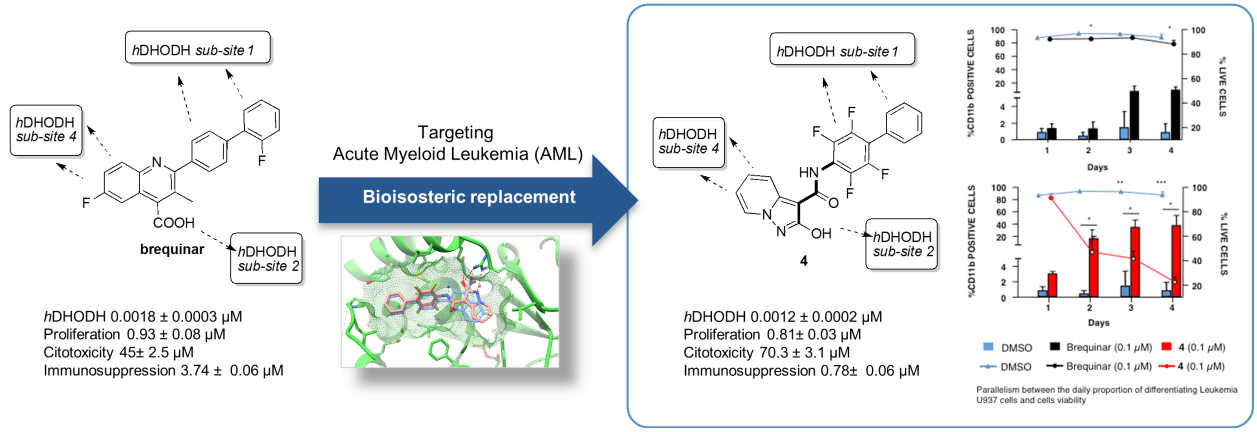
Acute myelogenous leukemia (AML) is a clinically devastating disease with dismal prognosis and survival rate. Efforts to identify new therapeutic targets to overcome *myeloid differentiation* blockade were largely unsuccessful until the fall 2016 breakthrough study by Sykes *et al.*[1] who demonstrated that inhibition of human *dihydroorotate dehydrogenase* (*h*DHODH) enables myeloid differentiation in both human and mouse AML models. These findings have significantly increased the interest in *h*DHODH-targeted therapy for cancer, in particularly as potential new strategy for the treatment of AML.[2] In these days, around two years after the Sykes discovery, three *h*DHODH inhibitors are in AML related clinical trials: *Brequinar* (from *Clear Creek Bio,* Phase I), *ASLAN003* (from *Aslan Pharmaceuticals*, Phase II) and *BAY2402234* (*Bayer*, Phase I). Starting from *innovative bioisosteric hypothesis* and using state-of-the-art designing paradigms, we have discovered[3] a novel small molecule (cpd **4**), representative of a novel class of *h*DHODH inhibitors based on a *hydroxylpyrazole-pyridine* scaffold, that is able to restore the myeloid differentiation in AML leukemia cell lines (U937 e THP1) at a 1-log lower concentration compared to the *lead* brequinar, causing a massive death of leukemic cells. To our knowledge, cpd **4** is one of the most potent and safe *h*DHODH inhibitor so far discovered. In this occasion, the recent studies we dedicated to cpd **4** optimization and *in vivo* behaviour in AML models will be presented and fully discussed.



Bibliography

1) D.B. Sykes, *et al Cell* **2016**, 167, 171. 2) J.T. Madak *et al Pharmacology & Therapeutics* **2019**, 195, 111-131. 3) S. Sainas *et al* J. *Medicinal Chemistry* **2018**, 61 (14), 6034-6055.

Structure of an algae picorna-like virus and the evolution of *Picornaviridae* viruses

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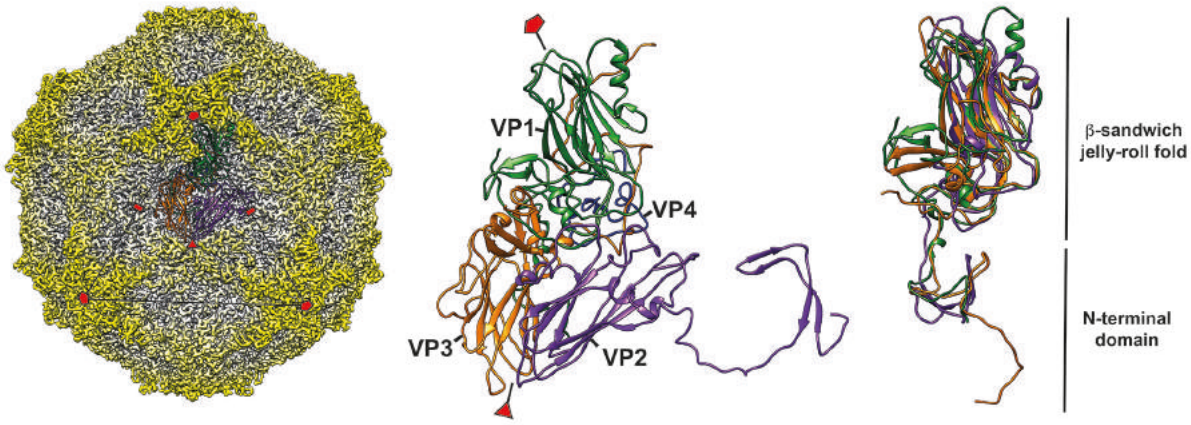
There is an uneven distribution of the major classes of RNA, DNA and retroid viruses among branches of host organisms. The RNA virome of marine metagenomic samples consists almost exclusively of the positive-sense single-stranded RNA ((+)ssRNA) viruses: the picornavirus-like superfamily. This implies that these viruses are an ancestral group from which the (+)ssRNA viruses of multicellular eukaryotes have evolved [Koonin, 2015].

The aim was to determine the structure of the diatom CtenRNAV-II virus and investigate the evolutionary relationship with members of the vertebrate *Picornaviridae* family as well as with other picorna-like viruses of insects and mammals.

The structure of CtenRNAV-II was determined with cryo-electron microscopy to a resolution (overall) of 3.1Å (FSC=0.143). This is the first high-resolution structure of an algae picorna-like virus. All four capsid proteins VP1, VP2, VP3 and VP4 were identified in the structure. The VP2 protein of CtenRNAV-II has a N-terminus domain swap, which is believed to be a primitive feature of the *Picoranviridae* viruses [Wang, 2015]. The domain swap as well as a structure-based phylogeny suggests that CtenRNAV-II belongs to an ancestral group of the *Picornaviridae* viruses. Additionally, VP1 exhibits a surface loop and does not follow the so-called canyon hypothesis that is proposed for many of the human pathogenic picornaviruses, suggesting that CtenRNAV-II has a unique way of recognising its host receptor.



References:
Koonin, E.V, *et al.* The Big Bang of picorna-like virus evolution antedates the radiation of eukaryotic supergroups (2008) *Nat Rev Microbiol* **6**:925-39
Wang, X. *et al.* Hepatitis A virus and the origins of picornaviruses (2015) *Nature* **517**:85-88

The Chase for Virus X - a Pipeline to find Novel Viral Proteins

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The virus X project is funded by the H2020 EU program and is aimed at discovering novel virus enzymes for biotechnological applications. The work is spanning over four years and is done as a collaboration between academia and three companies, involving a total of thirteen partners. The project started with DNA isolation and will be finished with commercialization of novel enzymes. SARomics Biostructures´ part in this project involve protein characterization, crystallization and structure determination. So far around forty proteins have entered the crystallization pipeline built by us for the purpose. Dynamic light scattering and differential scanning fluorimetry have been used to guide the choice of buffers and potential ligands facilitating crystallization. Eleven structures have been determined so far in the project using a combination of different phasing techniques such as ab initio phasing, SAD and molecular replacement. The structures belong to known classes of proteins - endolysines, helicases and DNA polymerases, with the exceptions of two novel proteins, XepA and YomS. Both proteins were cloned from *Bacillus subtilis* prophages and share an overall sequence identity of 38 %. XepA, shown in Figure 1A, may possesses a putative function in DNA insertion to the bacterial host. YomS, Figure 1B, is very similar to the C-terminal domain of XepA, although only the C-terminal domain was cloned. We show that XepA but not YomS, have lytic activity and can break the cell wall. The structure of XepA reveal a pentameric fold of two discs each consisting of five β-sandwich monomers. The discs are connected via a small domain with two cross-β-sheets involving all five chains in each sheet and separated by a helical turn. All through the XepA structure (100 Å) spans a 10 Å wide channel that we think may be used for DNA insertion.

A B

Figure 1. Ribbon presentation of Virus-X proteins XepA (A) and YomS (B) with a 5-fold symmetry

Pulling type three secretion systems apart to put them back together - high resolution views of complexes from flagellar and non-flagellar type three systems

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Type three secretion systems are crucial for virulence of many human pathogenic bacteria either in their role as bacterial flagellar or as toxin injection systems. Despite many decades of study our understanding of the core complexes required for function in these nano-machines had largely stalled. Recent advances in single particle cryo-em methodologies now allow us to build de novo atomic models for many of the complexes involved in type three secretion systems that have been recalcitrant to crystallisation over the years. Structures of these complexes are revealing major holes in our understanding from mis-assigned symmetries to supposed membrane proteins that adopt out of membrane-locations within the intact assemblies requiring re-analysis of earlier functional work in the context of the architecture revealed. New structures will be presented and their implications for function discussed.

A novel helical structure in the lumen of microtubules in human sperm flagella tips revealed by cryo-electron tomography

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Eukaryotic flagella are an important organelle involved in signalling, sensing, and motility. Motile flagella are made up of a ninefold symmetric arrangement of doublet microtubule filaments, surrounding two singlet microtubules called the central pair. This structure is referred to as the axoneme, and is generally conserved throughout evolution. However, near the tip region the doublet microtubules terminate to become singlets, and this region is where the flagellum grows and shrinks. Flagellum tip morphology differs considerably between organisms, and by applying cryo-electron tomography to human sperm flagella our lab discovered a novel structure we named TAILS decorating the lumen of microtubules at the distal tip of the flagellum.

The protein(s) that forms TAILS has yet to be identified, and we plan to scan the tips of sperm from organisms throughout evolution in order to identify which organisms contain TAILS, and which do not, and use comparative genomics to narrow down our list of candidate genes. Subtomogram averaging was used to reveal the structure of TAILS, however, the resolution of our model was limited to around 4 nm. Consequently, a protocol was developed to remove the membrane of sperm flagella at the tips and splay the microtubules apart so that they can be imaged directly by cryo-EM. By plunge freezing cells prepared in this way, and imaging the exposed microtubules containing TAILS at the tips by cryo-EM, we plan to use single particle analysis to generate a high resolution of model of TAILS. In this way we can gain information on how many subunits TAILS comprises, secondary structure, and if possible regions in which the amino acid sequence can be read directly. This will help elucidate the function and identity of TAILS, and provide insight to how proteins interact with the lumen of microtubules and affect their structure.

In situ structural studies of enterovirus replication using FIB milling and cryo-electron tomography

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Enteroviruses (EVs) are a major genus of positive-sense single-stranded RNA ((+)ssRNA) viruses and include human pathogens such as Poliovirus (PV), Coxsackieviruses (CVs) and Rhinoviruses. EVs are thus responsible for a wide variety of diseases such as poliomyelitis, myocarditis, gastroenteritis and respiratory infections. As all (+)ssRNA viruses, Enteroviruses drastically reorganize the internal membranes of a cell within hours of infection, generating *replication complexes* (RCs), which are the sites of viral genome replication. Some molecular determinants of RC morphogenesis have been identified, and resin-embedding EM has indicated the drastic membrane remodeling involved in RC formation. However, such approaches fail to reveal the macromolecular structural organization of RCs in cells. Hence, the aim of this study is to gain structural insights into the genome replication of human enteroviruses (EVs), specifically Coxsackievirus B3 which is the leading cause of viral myocarditis. To this end, we are using the novel cryo-focused ion beam (cryo-FIB) milling technology combined with cryo-electron tomography (cryo-ET) of infected cells. Cryo-electron microscopy (cryo-EM) is limited to specimens of <~500 nm thickness, which has previously limited cryo-ET of adherent cells to peripheral regions of the cells. FIB milling can be used to trim away material and thus expose deeper regions of cells for subsequent analysis by cryo-ET, providing extraordinary in situ structural insights into biological processes that were before unreachable. The findings will clarify the long-standing question regarding the relation between membrane topology and the RNA synthesis machinery, by a direct visualization of viral proteins and RNA state, position and orientation with respect to the membranes.

Towards molecular architecture of the muscle Z-disk assembly

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The sarcomere is the minimal contractile unit in the cardiac and skeletal muscle, where actin and myosin filaments slide past each other to generate tension. This molecular machinery is supported by a subset of highly organised cytoskeletal proteins that fulfil architectural, mechanical and signalling functions. The ultra-structure of sarcomere is highly organized and delimited by Z-disks, which play a central role in the mechanical stabilization and force transmission.

In the Z-disks – the lateral boundaries of the sarcomere machinery – the protein α-actinin-2 cross-links antiparallel actin filaments from adjacent sarcomeres, and additionally serves as a binding platform for a number of other Z-disk proteins. In striated muscle cells, the Z-disk represents a highly organized three-dimensional assembly containing a large directory of proteins orchestrated in a multi-protein complex centred on its major component α-actinin, with still poorly understood hierarchy and three-dimensional interaction map. On the way to elucidate the molecular structural architecture of the Z-disk, the hierarchy of its assembly and structure-function relationships, we are studying binary and higher order sub-complexes of α-actinin using biophysical, structural and cell biological approaches.

I will present our recent data on interaction of muscle α-actinin with titin and adaptor proteins FATZ-1 and myotilin, forming dynamic fuzzy complexes, and discuss findings in view of muscle Z-disk architecture and assembly.

Mn/Fe ribonucleotide reductase by femtosecond crystallography

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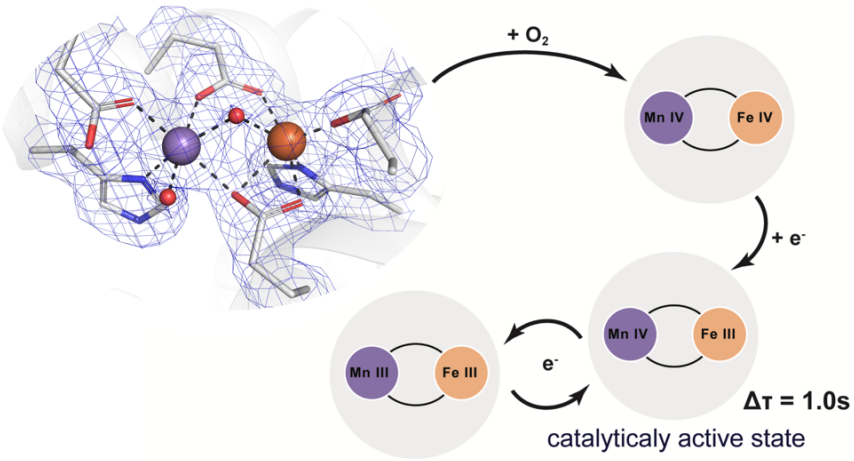
All domains of life require the essential enzyme ribonucleotide reductase (RNR), which represents the only pathway for *de novo* synthesis of deoxyribonucleotides from ribonucleotides. Class I RNR depends on a ferritin-like R2 subunit to produce a catalytic radical, subsequently shuttled via proton-coupled electron transfer to the R1 subunit performing the ribonucleotide reduction.

Except in the recently discovered metal-free subclass Ie[1], R2 utilises a dinuclear metal centre to generate the radical upon O_2 activation, via the formation of high-valent intermediates. Distinct subclasses of R2 proteins specifically rely on different dinuclear Fe and/or Mn cofactors. In subclass Ic R2 (R2c), a Mn^{II}/Fe^{II} heterodinuclear centre initially provides all four electrons required for complete O_2 reduction. It results in a Mn^{IV}/Fe^{IV} intermediate which undergoes a one-electron reduction to form the Mn^{IV}/Fe^{III} active state of the protein. Although still debated, the inorganic cores of R2c directly following O_2 exposure are to date believed to be $Mn^{IV}(\mu\text{-}O)_2Fe^{IV}$ and $Mn^{IV}(\mu\text{-}O)(\mu\text{-}OH)Fe^{III}$.

Here, we present our attempts to structurally characterise high-valent states of the R2c Mn/Fe cofactor using serial femtosecond X-ray crystallography and simultaneous X-ray emission spectroscopy (XES) at room temperature at X-ray free-electron laser (XFEL) sources. Using a drop-on-demand sample delivery protocol[2], we recently obtained high-resolution R2c XFEL crystal structures coupled with Mn and Fe XES data after different times of O_2-exposure. We will discuss how these new structural insights compare with the existing data from the literature.



The crystal structure of Mn/Fe R2c by XFEL in a reduced state (left), and subsequent steps of the O_2-activation proposed reaction pathway.

[1] Srinivas V, Lebrette H *et al*. (2018) *Nature* 563(7731):416-420.

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

Tracking Ca^{2+} ATPase intermediates in real-time by X-ray solution scattering

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Sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA) transporters regulate calcium signaling by active calcium ion reuptake to internal stores. Several, but not all, of the structural transitions associated with transport have been characterized by X-ray crystallography. We combined time-resolved X-ray solution scattering (TR-XSS) experiments and molecular dynamics (MD) simulations that enabled real-time tracking of concerted SERCA reaction-cycle dynamics in the native SR membrane at ambient temperature. The TR-XSS pre-pulse model differed in the nucleotide-binding (N) domain location compared to Ca_2E1 crystal structures. An intermediate with a 1.5 ms rise-time showed closure of the cytosolic domains typical of Ca^{2+}- and ATP-bound E1 states. A subsequent transient state with a 13 ms rise-time showed novel actuator (A) domain arrangement that resulted in exposure of the ADP-binding site. Hence, the obtained TR-XSS models determine the relative timing of so-far elusive domain rearrangements in a native environment.

MicroED: conception, practice and future opportunities

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My laboratory studies the structures of membrane proteins that are important in maintaining homeostasis in the brain. Understanding structure (and hence function) requires scientists to build an atomic resolution map of every atom in the protein of interest, that is, an atomic structural model of the protein of interest captured in various functional states. In 2013 we unveiled the method Microcrystal Electron Diffraction (MicroED) and demonstrated that it is feasible to determine high-resolution protein structures by electron crystallography of three-dimensional crystals in an electron cryo-microscope (CryoEM). The CryoEM is used in diffraction mode for structural analysis of proteins of interest using vanishingly small crystals. The crystals are often a billion times smaller in volume than what is normally used for other structural biology methods like x-ray crystallography. In this seminar I will describe the basics of this method, from concept to data collection, analysis and structure determination, and illustrate how samples that were previously unattainable can now be studied by MicroED. I will conclude by highlighting how this new method is helping us understand major brain diseases like Parkinson's disease; helping us discover and design new drugs; shedding new light on chemical synthesis and small molecule chemistry; and showing us unprecedented level of details with sub atomic resolutions.

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

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

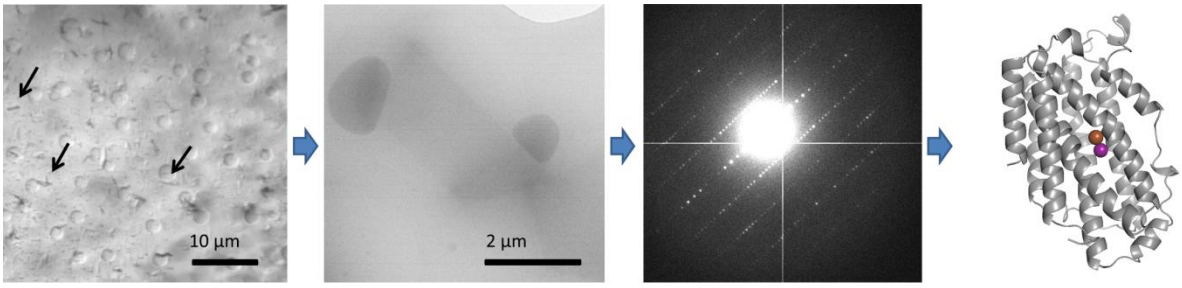

Figure 1 – Solving the structure of *Sa*2lox by MicroED

1. Shi, D., Nannenga, B. L., Iadanza, M. G. & Gonen, T. *eLife* **2**, (2013).
2. Nannenga, B. L., Shi, D., Leslie, A. G. W. & Gonen, T. *Nat. Methods* **11**, 927–930 (2014).
3. Yonekura, K., Kato, K., Ogasawara, M., Tomita, M. & Toyoshima, C. *Proc. Natl. Acad. Sci.* **112**, 3368–3373 (2015).
4. Clabbers, M. T. B. *et al. Acta Crystallogr. Sect. Struct. Biol.* **73**, 738–748 (2017).
5. Xu, H. *et al. Structure*, **26**, 667-675 (2018).
6. Khoshouei, M., Radjainia, M., Baumeister, W. & Danev, R. *Nat. Commun.* **8**, 16099 (2017).
7. Henderson, R. *Q. Rev. Biophys.* **28**, 171 (1995).
8. Xu, H. *et al.* (bioRxiv, 2019). doi:10.1101/600387

Single-tRNA tracking reveals the kinetics of erythromycin induced drug resistance in living bacterial cells

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The bacterial ribosome is one of the major targets for antibiotics, including for example the macrolide Erythromycin (ERY). During translation, the nascent peptide chain passes through the ribosomal exit tunnel which is also the binding site of ERY. As a result, drug binding can induce different effects depending on the amino acid composition of the nascent chain, including: peptidyl-tRNA drop off, nascent chain by-passing of the drug, and nascent chain-mediated translational arrest. Translational arrest is induced through specific interactions of the nascent chain with the drug and with the tunnel, causing conformational changes within the peptidyl transferase center (PTC). This sensing mechanism is used by the cell to regulate expression of ERY resistance genes.

Although ERY dependent translational arrest has been studied for decades in vitro, the dynamics of the regulation of ERY induced resistance, has been impossible to observe inside living cells. In order to study the effect of ERY on ongoing protein synthesis, , we use our recently developed single-tRNA tracking approach, where dye-labeled elongator $tRNA^{Phe}$ is electroporated and tracked inside *E. coli* cells. By following the binding events of tRNAPhe to ribosomes, in presence or absence of ERY, and during expression of specific Phe codon containing peptides, we study sequence-specific effects of ERY on ongoing translation, which helps us to decipher the mechanism behind the bacteriostatic effect of ERY and the regulation of the resistance against this drug.

In situ NMR spectroscopy of bacterial outer membrane protein OmpA

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Outer membranes of Gram-negative bacteria contain transmembrane proteins in a distinguished membrane environment of highly asymmetric architecture. Whereas the inner leaflet of outer membranes is composed of regular phospholipids, the outer leaflet consists almost exclusively of lipopolysaccharides (LPS). To date, no methods exist to experimentally mimic the complex native lipid environment of bacterial outer membranes. However, a growing number of studies indicate that the membrane composition directly impacts the structural and functional states of integral membrane proteins. Yet, studies characterizing the structure and function of outer membrane proteins rely on membrane-mimetic systems or artificially reconstituted bilayers, which lack characteristic features of cellular membranes such as lipid diversity, composition and bilayer asymmetry.

We recently developed a new and unique method utilizing outer membrane vesicles (OMVs) released from *Escherichia coli* to study outer membrane proteins in the native membrane environment. These vesicles are prepared to contain the outer membrane protein of interest at high density and circumvent the limitations of established methods[1,2]. Here, we elucidate the applicability of protein-enriched OMVs to characterize the structure of the major outer membrane protein OmpA from *E. coli* using high-resolution nuclear magnetic resonance spectroscopy, both solution state and solid state, for the first time in its native asymmetric lipid bilayer environment.

1. Thoma, J. *et al.* Protein-enriched outer membrane vesicles as a native platform for outer membrane protein studies. *Commun. Biol.* **1,** 23 (2018).
2. Thoma, J., Sun, Y., Ritzmann, N. & Müller, D. J. POTRA Domains, Extracellular Lid, and Membrane Composition Modulate the Conformational Stability of the β Barrel Assembly Factor BamA. *Structure* **26,** 987–996.e3 (2018).

Posters

Elucidating the functional details of the human mitochondrial HtrA2 serine protease

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Wallenberg Centre for Molecular and Translational Medicine

HtrA2 (High temperature requirement protein A2) or Omi is a mitochondrial serine protease of the HtrA family found in all kingdoms of life[1]. HtrA2 resides in the inner mitochondrial membrane where it exerts its role in protein quality control. Upon pro-apoptotic stimuli, HtrA2 is released from the membrane and into the cytosol through cleavage of its transmembrane domain, where it works as a pro-apoptotic factor by interaction with inhibitor of apoptosis (IAP) proteins such as XIAP[2]. HtrA2 has been implicated in a number of neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease as well as several different cancer types[1,3–6]. Due to its importance in protein quality control and its connection to several severe human diseases, HtrA2 is an important target of study. However, the functional cycle, interaction partners as well as the regulation of HtrA2 remain mostly elusive. To decipher the functional details of the HtrA2-protease we initiated a study of the structure and dynamics of human HtrA2 by using high-resolution nuclear magnetic resonance (NMR) spectroscopy techniques. To initially characterize the secondary structure elements in solution, we have created a mutant of HtrA2 rendering the functional active trimer monomeric. Further we have also isolated only the substrate binding PDZ domain of HtrA2. These constructs enable a first analysis of the structural adaptions and dynamics underlying HtrA2 function at the atomic levels. In addition, preliminary data will be presented on how HtrA2 interacts with different native substrates.

1. Vande Walle, L., Lamkanfi, M. & Vandenabeele, P. The mitochondrial serine protease HtrA2/Omi: An overview. *Cell Death Differ.* **15,** 453–460 (2008).
2. Suzuki, Y. *et al.* A serine protease, HtrA2, is released from the mitochondria and interacts with XIAP, inducing cell death. *Mol. Cell* **8,** 613–621 (2001).
3. Strauss, K. M. *et al.* Loss of function mutations in the gene encoding Omi/HtrA2 in Parkinson's disease. *Hum. Mol. Genet.* **14,** 2099–2111 (2005).
4. Park, H. J. *et al.* β-Amyloid precursor protein is a direct cleavage target of HtrA2 serine protease: Implications for the physiological function of HtrA2 in the mitochondria. *J. Biol. Chem.* **281,** 34277–34287 (2006).
5. Huttunen, H. J. *et al.* HtrA2 regulates β-amyloid precursor protein (APP) metabolism through endoplasmic reticulum-associated degradation. *J. Biol. Chem.* **282,** 28285–28295 (2007).
6. Narkiewicz, J. *et al.* Changes in mRNA and protein levels of human HtrA1, HtrA2 and HtrA3 in ovarian cancer. *Clin. Biochem.* **41,** 561–569 (2008).

With or without photoreduction - Radical generating subunits of ribonucleotide reductases under attack

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Metalloproteins are no strangers to the concept of X-ray induced radiation damage. One of the more recent developments in macromolecular crystallography to address this has been serial crystallography at which the single-crystal dataset per structure model is abandoned for multi-crystal datasets per structure model.

Method development surrounding serial crystallography has initially been strongly centred around femtosecond X-ray crystallography where X-ray free-electron lasers (XFELs) are used to supply X-ray pulses short enough for “diffraction before destruction” crystallography. The short pulses have made it possible to preserve structural data for metal sites in their oxidised state.

Synchrotron developments with serial crystallography have showed that even though radiation damage might not be possible to fully “outrun” on synchrotron timescales, the serial approach to data collection can distribute a lower dose per sample than for classical single-crystal approaches.

Results will be shown from serial crystallography at synchrotrons and XFELs as well as single-crystal radiation damage effects in a dinuclear metalloprotein.

Cryo-electron microscopy studies of a nucleosome-bound chromatin remodeler

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To fit inside the nucleus, eukaryotic DNA can be condensed into a highly compact structure called chromatin. The basic unit of chromatin is the nucleosome, which consists of about 150 DNA base pairs wrapped around a positively charged protein core made up of histones. This packaging into chromatin restricts access to the DNA - chromatin thus represents a barrier for all DNA-based processes. Therefore, the cell has evolved different ATP-dependent chromatin remodelers that can alter chromatin structure by evicting, sliding, inserting or replacing nucleosomes. Chromatin remodelers contain an ATPase subunit and typically additional subunits that play a role in regulation and specificity of the remodeling complexes. For example, ALC1 (Amplified in Lever Cancer 1) features, uniquely among chromatin remodelers, an additional macro domain that can bind to poly-ADP-ribose (PAR) chains. PAR represents an important post-translational modification observed following DNA damage, and the macro domain-mediated recruitment of ALC1 to PAR chains has been suggested to activate ALC1 remodeling activity. However, how the nucleosome is engaged by ALC1 in its active state is completely unknown. Here, we employ cryogenic electron microscopy (cryo-EM) to obtain structural information on ALC1 in its nucleosome-bound and remodeling-active state.

Construction and characterization of *de novo* metalloenzymes for production of solar fuels

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Inventing novel approaches to creating sustainable energy is an important cause in a world where the atmosphere temperature is rising, conflicts over fossil fuels are ongoing and options are sparse or inaccessible. The aim with this project is to develop biological catalysts that can sustainably and efficiently produce solar fuels such as hydrogen gas or methane through the oxidation of water or reduction of carbon dioxide. Using *de novo* proteins as a tool to formulate these types of catalysts is beneficial, as it is possible to create hyper-stable scaffolds which tolerate a range of pH-values, temperatures, solvents, atmospheres and structural perturbations, whereas natural proteins often are much more sensitive to variations in these factors. Transition metals are commonly found in natural enzymes, and so it is possible to model metal binding sites in *de novo* proteins, and using transition metals for this type of redox-chemistry has been an attractive target for some time.[1,2] It is our hopes that applying them to a biological scaffold will help overcome problems such as oxygen-sensitivity, solubility and stability, as well as being able to use cheaper, first row transition metals.

The α_3x-protein designed by Tommos et al. is currently the scaffold being used for this project, and several metal-binding sites have been planned.[3] Current work is ongoing to create binding sites containing four histidines, four cysteines and a mixture of the two using site directed mutagenesis (see figure 1 for an example). This scaffold will bind several of the interesting redox-active transition metals (Mn, Fe, Co, Ni, Cu). With this approach we can, with spectroscopic techniques, screen for what metal-protein complex will most readily form, and also perform redox chemistry most efficiently. The redox potentials and catalytic properties and mechanisms will be studied with protein film voltammetry and transient absorption laser spectroscopy. We aim to find a scaffold/metal system, which is active for both reducing and oxidizing chemistry, depending on, among other things, the substrate and the metal ion.

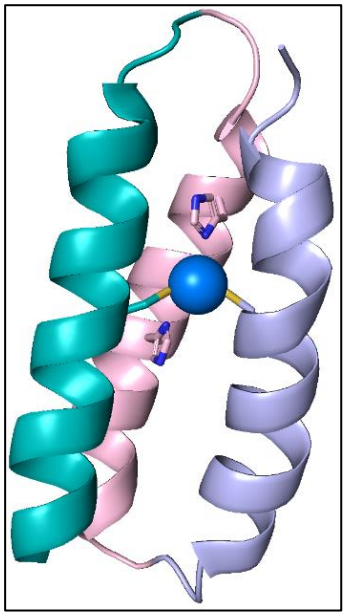

Figure 1: α_3x scaffold modified with two histidines and two cysteines, coordinating a metal ion (blue) (modelled mutations on NMR-ensemble (PDB: 1LQ7)).

It is our aim that this project lead to novel and applicable catalysts for solar fuel production, but also that they will act as a stepping stone for other solar-driven catalysis using pure proteins. This project will hopefully contribute to the bioinorganic chemistry-community as a whole with new insights on metal-protein interactions and the chemistry that can be achieved with this type of complexes.

1: Maret, W. *Int. J. Mol. Sci.* **2016**, 17(1): 66
2: Limburg, B. et al. *Coord. Chem. Rev.* **2012**, 256: 1451-14 67
3: Tommos, C. et al. *Biochemistry* **1999**, 38(29): 9495-507

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

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The galactomannan hemicelluloses are composed of a β-1,4-linked mannose backbone with α-1,6-galactose substitutions. They are commonly found as seed storage polysaccharides (1), and are used as food thickeners (2, 3). Thus they are a part of our diet and can be utilised by several different human gut bacteria (4). One of these bacteria, *Bacteroides ovatus*, contains a gene cluster, or polysaccharide utilisation locus (PUL) (5), encoding two glycoside hydrolase family 26 (GH26) β-mannanases, *Bo*Man26A and *Bo*Man26B. *Bo*Man26B generates a range of product lengths upon mannan based oligo- or polysaccharide hydrolysis, prefers longer substrates and is less restricted by galactose side-groups than the mannanase *Bo*Man26A, which primarily generates mannobiose from the same substrates (6, 7). The results suggested that *Bo*Man26B performs the initial attack on galactomannan, generating oligosaccharides that after transport to the periplasm are further hydrolysed by *Bo*Man26A. Crystal structures of these two enzymes reveal the structural basis for their biochemical differences. *Bo*Man26B, with galactosyl-mannotetraose bound in subsites –5 to –2, has an open and long active site cleft with Trp-112 in subsite –5 concluded to be involved in mannosyl interaction (7). Moreover, Lys-149 in the –4 subsite interacted with the galactosyl side-group of the ligand, which may indicate a preference in this subsite for substituted manno-oligosaccharides (7). *Bo*Man26A, on the other hand, revealed a narrow active site cleft that is restricted in one end by a loop, explaining its preference for generating shorter products (6).

References

1. Scheller, H. V. and P. Ulvskov Annu Rev Plant Biol (2010). **61**: 263-289.
2. Barak, S. and D. Mudgil Int J Biol Macromol (2014). **66**: 74-80.
3. Mudgil, D., S. Barak and B. S. Khatkar J Food Sci Technol (2014). **51**(3): 409-418.
4. Kulcinskaja, E., A. Rosengren, R. Ibrahim, K. Kolenova and H. Stalbrand Appl Environ Microbiol (2013). **79**(1): 133-140.
5. Martens, E. C., E. C. Lowe, H. Chiang, N. A. Pudlo, M. Wu, N. P. McNulty, D. W. Abbott, B. Henrissat, H. J. Gilbert, D. N. Bolam and J. I. Gordon PLoS Biol (2011). **9**(12): e1001221.
6. Bågenholm, V., S. K. Reddy, H. Bouraoui, J. Morrill, E. Kulcinskaja, C. M., Bahr, O. Aurelius, T. Rogers, Y.Xiao, D. T. Logan, E. C. Martens, N. M. Koropatkin and H. Stålbrand J Biol Chem (2017). **292**(1): 229-243
7. Bågenholm, V., M. Wiemann, S. K. Reddy, A. Bhattacharya, A. Rosengren, D. T. Logan and H. Stålbrand J Biol Chem (2019). (Accepted)

The Biological Laboratory at MAX IV

Yang Chen

MAX IV laboratory, Lund University

The biological laboratory at MAX IV is the central laboratory facility to support of life science related experiments at various beamlines and also a platform to carry out in-house life science research. The lab complements those beamline-attached sample preparation units and are designed and constructed to offer extended instrumentation access and top-notch supports with continuous upgrading possibility.

The Biolab is now available for users and in-house researchers to perform fundamental protein preparation, including limited protein analytics (UV-vis spectroscopy, gel electrophoresis, dynamic light scattering etc.). which allow a critical entry check for protein samples before the experiments at MAX IV. The lab is also equipped with a glovebox for sample preparation under anaerobic conditions, and storage of chemicals and protein samples. The lab's biosafety level is now classified as Biosafety Level 1 and restricted to work with well-characterized agents. The lab also has the capacity to be upgraded to Biosafety Level 2 in the future. The latest status of the MAX IV Biolab will be presented.

As more new beamlines enter the operation mode, the usage of the Biolab is also expected to correspondingly increase on account of the growing number of life-science users at MAX IV. The synergy between the Biolab and relevant beamlines will continuously improve ones' user experiences and cover the new needs.

BoNTbase: A Botulinum Neurotoxin Database and the Structural Characterisation of a BoNT Subtype

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To date, around 150 unique botulinum neurotoxin (BoNT) and BoNT-like peptide sequences have been discovered. The current established nomenclature categorizes BoNTs into serotypes, and then subtypes. This system has led to multiple similar sequences sharing the same subtype name; for example, there are 14 unique sequences identified as BoNT/B2. Such examples highlight the need for precise sequence information and characterisation. Small variation in protein sequence may have a consequential impact on the BoNT function. BoNTbase is an online, open-access database of all known BoNT sequences, which has been curated using both automated and manual methods. Each sequence within the database is linked to associated publications, bacterial strains, and useful biochemical information. A fast BLAST search is also provided, which may prove particularly useful for characterizing new BoNTs or comparing closely related sequences. We hope that BoNTbase will encourage the further characterisation of BoNTs and to this end we also present the high-resolution crystal structure of a BoNT subtype in complex with the GD1a ganglioside receptor. These data, along with biophysical characterisation of receptor interactions, provide a greater foundation on which to produce the next generation of therapeutic proteins.

Structural Characterization and dynamics of the C-terminal domain of spidroin in silk form

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The competitiveness of industry and well-being of our society have demanded the development of innovative and high-performance materials from distinct sources, in which proteins have attracted considerable attention. Among the varieties of biomaterials, spider silk surpasses ordinary standards, having a combination of strength and extensibility. Furthermore, spider silk fibers are biocompatible and biodegradable, which makes it an excellent material for applications ranging from textiles to medical uses. Major ampullate Spidroin 1 (MaSp1) from spider dragline silk is a large protein with poly-Alanine blocks and Glycine-rich repetitive motifs spaced between the non-repetitive domains (C-terminal and N-terminal). The highly conserved non-repetitive C-terminal domain plays a crucial role in the structural transition from soluble protein to insoluble silk form. Solid-state Nuclear Magnetic Resonance spectroscopy (ssNMR) has emerged as an important tool to characterize macromolecular complexes that cannot be crystallized, such as amyloid fibrils, oligomeric membrane proteins, and several protein assemblies. Here we use ssNMR to elucidate the structural characteristics of the C-terminal domain in silk fibers. The spider silk protein is recombinantly expressed in *E.coli*, purified, assembled into fibers and analyzed using ssNMR to characterize the molecular structure, specifically the transition to ß-sheets and the intermolecular interactions.

Structural Basis of Peptide-binding Specificity of MHC class I in songbirds

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The efficiency and specificity of the immune defence against a variety of pathogens in vertebrates is dependent on the presentation of foreign peptides (antigens) on the cell surface by major histocompatibility complex (MHC) molecules. Both, breadth of the peptide-binding repertoire of MHC molecules as well as their expression levels determine the occurrence of an immune response to non-self antigens.

MHC-I genes exhibit an extremely high level of polymorphism, which is thought to be maintained through balancing selection with pathogens being the major selective agent[i]. A greater number of MHC-I alleles as well as greater MHC-I diversity within a single individual increases the range of antigens that can be presented by MHC-I proteins. This has recently been shown to be of particular importance for bird species that reside in areas with high pathogen diversity, e.g. Africa, compared to areas with low pathogen diversity, e.g. Europe[ii]. However, the trade-off between the benefits of recognising and eliminating an increased range of pathogens and an elevated risk of immunopathology through self-reactive T cells, has to be adjusted to a reasonable extent. This leads to different levels of optimal MHC-I diversity between species colonising different latitudes.

Passerine birds (songbirds) such as the great reed warbler migrate seasonally between their breeding (Europe) and wintering (Africa) grounds and are hence exposed to low and high pathogen diversity, respectively, during the course of a year. Escaping from areas with high pathogen pressure during the breeding period seems to have resulted in a reduction in MHC-I diversity in migratory birds[ii]. However, during their wintering period, passerine birds have to cope with an increased diversity of pathogens.

Recently, the strategic evolution of two groups of MHC-I molecules (generalists and specialists) has been proposed with respect to different modes of pathogen resistance in chicken[iii]. Hence, we set out to explore the structural basis underlying peptide-binding specificity of MHC-I proteins in the great reed warbler as a model organism for passerine birds. Different peptides were chosen from a library which has been created based on their stabilising effect on the complex upon binding to MHC-I proteins. Here, we present the first structure of a great reed warbler MHC-I protein in complex with peptides and shed light on the question how passerine birds tolerate high pathogen diversity.

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[i] Westerdahl, H. et al. MHC class I typing in a songbird with numerous loci and high polymorphism using motif-specific PCR and DGGE. Heredity (Edinb). 2004;92(6): 534-542.
[ii] O'connor, E. et al. The evolution of immunity in relation to colonization and migration. Nat Ecol Evol . 2018;2(5): 841-849.
[iii] Chappell, P. et al. Expression levels of MHC class I molecules are inversely correlated with promiscuity of peptide binding. Elife. 2015;10(4).

Structure and function of the anticancer drug metabolizing enzyme β-ureidopropionase

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β-Ureidopropionase (βUP), naturally catalyzing the third step of the reductive pathway for degradation of uracil and thymine, also metabolizes the cytostatic drug 5-fluorouracil (5FU). β-UP deficiency is associated with an increased risk to develop severe toxicities during 5FU based chemotherapy. *(Maurer et. al., 2018)*

The enzyme is allosterically activated by its substrate and inhibited by the reaction product, respectively. Activation and inactivation are both mediated via shifts in oligomerization state of βUP. Allosteric regulation is observed only in a narrow window around physiological pH, at which the wild-type enzyme occurs as a mix of different oligomeric states in absence of ligands. At lower pH predominantly occurs as homooctamers (mimicking enzyme activation), whereas at higher pH the inactive homodimeric state predominates. That the effects of allosteric regulators can be mimicked by pH shifts indicates that changes in protonation states at intersubunit interfaces play a crucial role in the regulation mechanism. We perform site-directed mutagenesis followed by functional and structural studies to further elucidate this mechanism. *(Maurer et. al., 2018)*

Two residues were targeted by mutagenesis, the active site nucleophile C233 and H307 located in a functionally crucial loop region at a subunit interface. The oligomerization states of the created variants in absence and presence of allosteric effectors were determined using analytical size exclusion, and the enzymatic activity measured with a fluorimetric assay. The functional studies showed that all obtained variants (C233A, H307A, H307N) are inactive. However, whereas C233S is still responding to allosteric activators with subunit association, both H307 variants are non-responsive, indicating that this residue may play an important role in the regulation mechanism. Crystallization setups with the bUP variants yielded first crystals.

References

Maurer, D., Lohkamp, B., Krumpel, M., Widersten, M. and Dobritzsch, D. Crystal structure and pH-dependent allosteric regulation of human β-ureidopropionase, an enzyme involved in anticancer drug metabolism (2018), Biochemical Journal, 475(14) 2395-2416

Structural and dynamical details on the major yeast chaperone SSA1 by NMR Spectroscopy

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The ubiquitous Hsp70 chaperone family support cellular protein function in cells by mediating a broad range of folding processes. In concert with a variety of co-chaperones, Hsp70s constitute a highly efficient proteostasis machinery, capable of performing various tasks such as refolding of non-native proteins, co- and post-translational folding as well as disaggregation and solubilization of aggregated proteins.[1] They are highly conserved throughout the various domains of life and structurally, all Hsp70 homologs are characterized by two functional subdomains: a smaller carboxy-terminal domain of 25 kDa protein binding domain, often referred to as the substrate-binding domain (SBD), and a larger amino-terminal ATPase domain of 45 kDa, known as the nucleotide-binding domain (NBD). A hydrophobic interdomain linker flexibly link the two domains. Hsp70 activity is ATP dependent, cycling between two conformational states altering in their affinity to substrate proteins: an ATP-bound low-affinity state, and an ADP-bound high-affinity state.[2]

Within the framework of a larger investigation, where the elucidation of the interaction between SSA1 and the yeast peroxidase TSA1 is pursued, the main objective of the current project is to gain insight into the structural and dynamical details of the major yeast Hsp70 chaperone SSA1 from *S. cerevisiae*. The structure of SSA1 will be investigated by various biophysical techniques with a particular focus on nuclear magnetic resonance (NMR) spectroscopy. As classical solution state NMR is limited in terms of system size, the structural study of separate subdomains will be implemented. To facilitate the investigation by solution state NMR-spectroscopy, full-length SSA1 length as well as the individual NBD and SBD subdomains are expressed using specific isotope labelling patterns and purified for subsequent analysis. Expression and purification methods of the different constructs as well as findings in regard to the structural characterization along with biochemical studies of the activity of the expressed proteins will be presented.

1. M.P. Mayer & B. Bukau. 2005. Hsp70 chaperones: cellular function and molecular mechanism. *Cell. Mol. Life Sci.*. vol. 62 pp. 670–684.

2. A. Zhuravleva, E.M. Clerico & L.M. Gierasch. 2012. An interdomain energetic tug of war creates the allosterically active state in Hsp70 molecular chaperones. *Cell*. vol. 151. pp. 1296–1307.

Elucidating structural mechanisms of redox-linked proton pump in cytochrome c oxidase by time- resolved wide-angle X-ray scattering

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During cellular respiration, cytochrome c oxidase (CcO) participates as terminal acceptor of electrons in mitochondria and many bacteria. It receives electrons from upstream biological transporters that reduces molecular oxygen to water. The free energy stored in this process can then be used for transmembrane transport and ATP synthesis. As CcO plays an inevitable role in mitochondrial energetics and defects in CcO functionality are associated with mitochondrial dysfunction, the enzyme has been under intense investigation. A large amount of biochemical data and equilibrium-states structures of CcO are available to guide the proton translocation pathway of this enzyme, however, the mechanism and information on structural intermediates during this redox-linked proton translocation still remains elusive. Our project aims at elucidating the structural mechanisms that couple oxygen- reduction to proton-pumping and gating of proton channel in CcO. Using time-resolved spectroscopy and advanced X-ray methods like time-resolved wide-angle X-ray scattering (Tr-WAXS), we are exploring structural changes at ultrafast time-scales (microseconds to milliseconds) in *ba3-type* CcO from *Thermus thermophilus*. So far, ultrafast time-resolved structural studies have been performed on photo-active proteins. This is the first example where we structurally characterize transient intermediates of a non-light -sensitive enzyme during catalysis. We use photo-labile caged-oxygen that could be triggered photo-chemically in nano-seconds to initiate the reaction. Using these methods, our goal is to determine transient and catalytic intermediates of terminal oxidases during cellular respiration of organisms ranging from bacteria to mammals. Our research will provide the molecular framework for understanding electron and proton transfer pathways and mechanisms for addressing fundamental questions on redox reactions and energy conservation in cells. This study could also provide a guide to develop novel therapeutic agents targeting numerous human mitochondrial diseases.

References:

1) Andersson, R. *et al.* Serial femtosecond crystallography structure of cytochrome c oxidase at room temperature. *Sci Rep,* 7, 4518 (2017).
2) Cammarata, M. *et al.* Tracking the structural dynamics of proteins in solution using time-resolved wide-angle X-ray scattering. Nat. Methods 5, 881– 886 (2008).
3) Ludovici, C *et al*. Reaction of cytochrome bo3 oxidase with photochemically released dioxygen from a cobalt peroxo complex. Eur. J. Biochem. 269, 2630–2637 (2002).

Identification of novel interaction partners to human Aqp4

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Aquaporin 4 (Aqp4) is crucial for maintaining the water balance in the sensitive brain tissue and it is of great interest as a potential drug target for treatment of brain edema, cancer and neurological diseases. We aim to understand how protein-protein interactions regulate the function of Aqp4 and to get high-resolution structural insights into the regulatory process. Bimolecular Fluorescence Complementation (BiFC) will be used to identify novel interaction partners to Aqp4. The BiFC method relies on the complementation of two YFP fragments which are fused to the investigated proteins. Interaction leads to a reformation of the YFP molecule which brings the interaction partners together resulting in a detectable fluorescence signal. Yeast cells producing fluorescent BiFC complexes have previously been evaluated by fluorescence microscopy, but this method has a limited read out. In order to get a more efficient screening method, we are establishing a method based on Fluorescence-activated cell sorting (FACS), which helps us to achieve a high-throughput screening method to evaluate fluorescent membrane protein complexes. This method will be used to identify novel interaction partners of Aqp4 within a human brain cDNA library. For that, we will purify mRNA from total human brain RNA and construct a cDNA library in the Gateway donor vector pEXP-AD502. With the use of Gateway Technology, the cDNA will be cloned in Gateway destination vectors containing the YFP fragment to construct the fusion protein. Aqp4 is cloned into destination vectors containing the complementing YFP Fragment. We are planning to screen 5x10^9 clones for Aqp4 interaction via FACS analysis. This study delivers an efficient screening system and we are aiming to produce stable membrane:protein complexes in yeast anchored by BiFC, in yields suitable for structural determination. Structural information from Aqp4-protein complexes will give scientific insights into the molecular mechanisms of communication and recognition and will thereby help to understand life threatening cerebral conditions.

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

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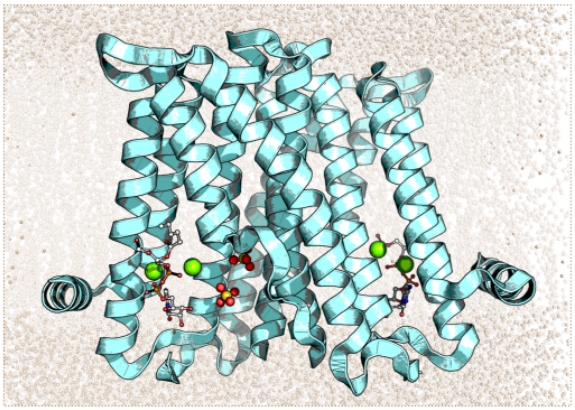
Mycobacterium tuberculosis is the most medically relevant pathogen of its genus, causing tuberculosis in humans and killing over one million people worldwide annually. Using X-ray crystallography we have characterised one of the *M. tuberculosis* membrane-embedded CDP-alcohol phosphotransferase family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



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

PgsA1 catalyses an essential step of inositol-derived phospholipid biosynthesis, producing structural precursors for biosynthesis 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 substrates (1.8 Å), and in complex with Mn^{2+} together with a serendipitously bound Mn-citrate complex (1.9 Å). The structures reveal atomic details of substrate binding and dynamics of the 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, the data suggest a refined general catalytic mechanism, including a substrate-induced carboxylate shift, for class I CDP-alcohol phosphotransferases.

Reference:

Structure of *Mycobacterium tuberculosis* phosphatidylinositol phosphate synthase reveals mechanism of substrate binding and metal catalysis. *Accepted manuscript.* (2019) *Commun. Biol*.

Screening of membrane components of the pCF10 Type IV Secretion System

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Type IV Secretion Systems (T4SS) are versatile systems used to transfer effector proteins as well as DNA via conjugation, a process that allows bacteria to share genetic information such as antibiotic resistance via horizontal gene transfer. Of the few T4SSs studied in G^+ bacteria, the one encoded by the conjugative plasmid pCF10 of *Enterococcus faecalis* is one of the best characterized. The pCF10 T4SS consists of about 14 proteins, which are all encoded by the 30kbp long P_Q operon on the plasmid. These genes can be divided into three main categories, encoding for (i) adhesion proteins, (ii) energetic proteins or (iii) channel proteins.

The aim of this research is to gain insight into the structure and function of the T4SS channel from pCF10. The channel consists of 7-8 proteins, but so far the information about these proteins is scarce. Here we present the initial studies of screening for optimal conditions for expression and purification of these proteins.

Structural and functional analysis of aquaporin 2 mutants in nephrogenic diabetes insipidus

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Aquaporins act as cellular water channels, allowing the passage of water molecules across the hydrophobic cellular membrane. One of them, aquaporin 2, regulates the reabsorption of water in the collecting duct of the kidney, thus allowing regulation of urine production. Point mutations (T125M, T126M, and A147T) in aquaporin 2 discovered in patients with nephrogenic diabetes insipidus were studied in order to investigate the effect of the aforementioned mutations upon the structure and function of the protein.

It was found via circular dichroism that all but T126M destabilised the protein to some degree, whereas T125M was shown via stopped-flow spectrometry to increase the speed at which the channel allows water to pass through. The structural consequences were studied via x-ray crystallography; however, the data requires further processing before drawing any conclusions.

Aquaporins act as water channels of cells, allowing water to pass across the hydrophobic cellular membrane. One of them, aquaporin 2, regulates the reabsorption of water in the collecting duct of the kidney, thus allowing regulation of urine production. Point mutations (T125M, T126M, and A147T) in aquaporin 2 discovered inpatients with nephrogenic diabetes insipidus are being studied in order to investigate the effect of the aforementioned mutations upon the structure and function of the protein. T126M was shown to be relatively unaffected structurally and retained the pore structure, as well as the stability of the wild-type protein. T125M and A147T were both noticeably destabilised, resulting in lower thermal stability and more disordered crystal formation, although A147T more so than T125M. So far, T125M has been shown through stopped-flow spectroscopy to be a less efficient water channel than the wild-type protein. T126M and A147T remain to be studied.

1. Goji, K.et al. Novel mutations in aquaporin-2 gene in female siblings with nephrogenic diabetes insipidus: Evidence of disrupted water channel function.Journal of Clinical Endocrinology and Metabolism 83, 3205–3209. issn:0021972X (1998).
2. Mulders, S. M. et al. New mutations in the AQP2 gene in nephrogenic diabetes insipidus resulting in functional but misrouted water channels. Journal of the American Society of Nephrology : JASN 8, 242–8. Issn: 1046-6673 (1997).
3. Frick, A. et al. X-ray structure of human aquaporin 2 and its implications for nephrogenic diabetes insipidus and trafficking. Proceedings of the National Academy of Sciences of the United States of America 111, 6305–10. issn: 1091-6490 (2014).

Cloning, Expression and Purification of the Mitochondrial Pyruvate Carrier Complex

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More than 400 million people in the world have type 2 diabetes, making it one of the most common national diseases according to the World Health Organization. Thiazolidinediones (TZDs) is used to treat type 2 diabetes since it increases the sensitivity of insulin in peripheral tissues. The knowledge of the mechanism of TZD has previously been limited which has resulted in patients getting serious side effects upon receiving treatment. The target of TZD has recently been identified as the mitochondrial pyruvate carrier complex (MPC). By determining the structure and function of the complex, designing a specific drug without side effects for patients receiving treatment for type 2 diabetes would be possible. Carriers are essential for transport of molecules in biological systems. MPC is a membrane protein, located in the inner mitochondrial membrane, that carries and transports pyruvate into the mitochondria. Studies have not yet provided detailed knowledge about the structure and functional mechanisms of the complex. The purpose of this study is to start an investigation of the MPC complex using a construct of MPC 4 from *Pinus patula* that is cloned and expressed in *E. coli* and different attempts of purification of the protein are performed.

A novel motif underlies the interactome of a chromatin remodelling enzyme

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Human ALC1 (Amplified in Liver Cancer 1), also called CHD1L (Chromodomain-helicase-DNA-binding protein 1-like), is an oncogene-encoded enzyme for chromatin remodelling. ALC1 belongs to the family of ATP-dependent remodelling enzymes, which plays an important role in the regulation of transcription, development, DNA repair, and cell cycle. ALC1 (897 residues) is composed of an N-terminal ATPase domain and a C-terminal macro domain, denoted as $ALC1^{macro}$.

Lehmann* *et al.* proposed a dual function of $ALC1^{macro}$. In the absence of DNA damage, $ALC1^{macro}$ auto-inhibits the ALC1 ATPase activity via electrostatic interaction. When damage to DNA is detected, PARylated PARP1 interacts with a conserved PAR binding site on $ALC1^{macro}$. PAR binding triggers an ALC1 conformational change and facilitates its binding to the DNA and nucleosome. Here, we apply proteomic peptide phage display using a human proteome library of disordered peptides in order to derive potential peptide motifs that mediate interactions with ALC1. Amplified peptides from phage selections were DNA sequenced by next generation sequencing and consequently translated into their corresponding peptide sequences. The frequencies in the NGS counts of DNA sequences with correct oligonucleotide length corresponding to individual peptides were high in the last cycle of selection, that reflects to the relative affinity of the binding within library. Based on the sum of identified peptides, we have determined a binding motif for $ALC1^{macro}$ interactions that likely mediates biologically relevant interactions with this remodelling enzyme.

*L. Lehmann, G. Hewitt, S. Aibara, A. Leitner, E. Marklund, S. Maslen, V. Maturi, Y. Chen, D. Spoel, J. Skehel, A. Moustakas, S. Boulton, and S. Deindl, **Molecular Cell**, **68**, 847-739, (2017)

Rearranging from 6- to 7- coordination initiates the catalytic activity – An EPR study on a Ru-bda water oxidation catalyst

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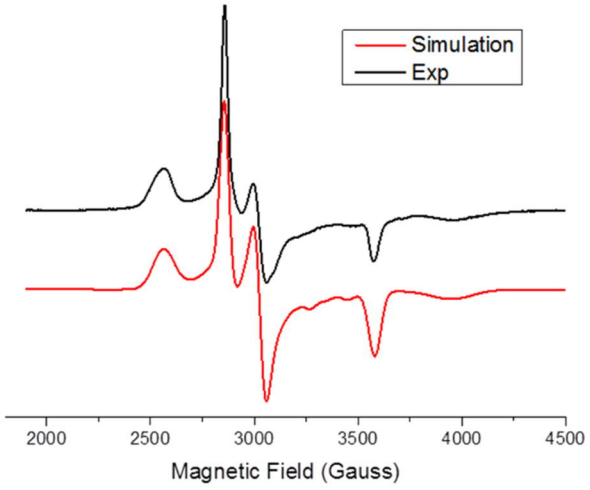
In 2009, Sun et al have reported an efficient catalyst based on ruthenium for water oxidation, called Ru-bda (bda: bipyridine dicarboxylic acid). Along the past years this type of catalyst have been improved, reaching a TOF over 1000s and a TON over 100 000. Interestingly this type of catalysts do water oxidation by a radical coupling mechanism between two Ru^{V}=O specie with 7 coordination. Even though this catalyst has been improved a lot over the years, few mechanistic studies have been done so far. In spite of the coordination of water as 7th ligand, at low valence state, has been suggested, no experimental data have been provided. We report, here, our study of Ru(III)-bda in which the water coordinate at the 7th position in pH1 solution is indicated by EPR.



Metal-free ribonucleotide reductase powered by a DOPA radical

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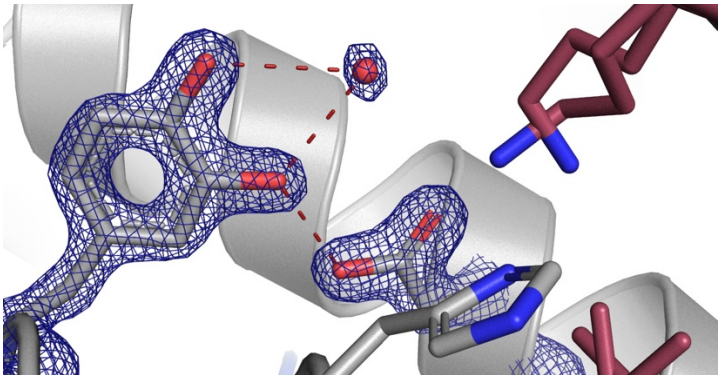
Ribonucleotide reductase (RNR) catalyzes production of all four deoxyribonucleotides required for DNA synthesis. Class I RNR:s employ protein R2 to generate and store an essential catalytic radical. Over half a century ago it was discovered that the R2 protein from *E. coli* required non-heme iron for function[1]. Since then it has been established that the radical is generated by a dinuclear metal site in an oxygen dependent reaction.

Notably, a number of variants of the metal site has been found in different organisms, likely because of adaptations to the metal availability in different growth environments. The metal site can be di-iron (class Ia), di-manganese (class Ib), or heterodinuclear Mn/Fe (class Ic)[2].

Here we describe the discovery of a new group of metal-free RNR proteins in Mollicutes, including Mycoplasma pathogens. In this group, the R2 protein initiates catalysis using a metal-independent DOPA radical residing on a post-translationally modified tyrosyl residue. The discovery overturns the presumed requirement of a dinuclear metal site in protein R2 and compels completely different mechanisms for radical generation and stabilization[3]. Organisms encoding this type of RNR are involved in diseases of the respiratory, urinary and genital tracts and it potentially developed in response nutritional immunity providing extreme metal restriction.



1. Brown, N. C., Eliasson, R., Reichard, P. & Thelander, L. Nonheme iron as a cofactor in ribonucleotide reductase from E. coli. *Biochem. Biophys. Res. Commun.* **30,** 522–527 (1968).
2. Hogbom, M. Metal use in ribonucleotide reductase R2, di-iron, di-manganese and heterodinuclear--an intricate bioinorganic workaround to use different metals for the same reaction. *Metallomics* **3,** 110–120 (2011).
3. Srinivas, V. *et al.* Metal-free ribonucleotide reduction powered by a DOPA radical in Mycoplasma pathogens. *Nature* **563,** 416–420 (2018).

Microcrystallization of the terminal respiratory chain enzyme *ba₃*-type cytochrome c oxidase for serial crystallography studies

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The terminal enzyme of the respiratory chain, cytochrome c oxidase, catalyzes the reduction of molecular oxygen to water, in which the free energy of the reduction is captured in an electrochemical gradient vital to all cellular life. The energy captured in this membrane gradient is conserved in the form of the high energy phosphoanhydride bond of the cells energy currency ATP, by action of ATP synthase. The mechanism by which this free energy from the reduction of molecular oxygen is utilized in the translocation of protons, which constitutes the electrochemical gradient, is still not completely understood. In contrast to the protein family to which mitochondrial cytochrome c oxidase belongs, *ba₃*-type cytochrome c oxidase from the thermophilic bacteria *Thermus thermophilus* only has one proton translocating channel. This oxidase is expressed under conditions of limited oxygen availability and has a higher affinity to oxygen, although the stoichiometry of the reaction is less favorable from an energy utilization perspective. Elucidation of this proton translocating mechanism can lead to a better understanding of the mechanisms behind the cell's energy utilization. A useful method for this purpose is serial crystallography, which is a method in which data is collected from many crystals which are hit sequentially by X-rays at room temperature. Radiation damage occurs when crystals are hit by X-rays, and thus deterioration of the sample as X-ray induced free radicals are allowed to propagate through the sample. In serial crystallography however, this is avoided as the same crystal is not hit repetitively. As the crystals are oriented in all different direction, thousands of images from microcrystals are collected in order to get a full data set. This collection of data from many crystals will also enable time resolved studies, which will require a triggering mechanism for the reaction initiation. In the case of cytochrome c oxidase, that trigger is oxygen. A way to deliver the oxygen is the use of a caged molecular oxygen donor, which releases the oxygen upon photolysis by a laser. The aim of the project is to achieve detergent-based microcrystals of *ba₃*-type that diffract to high resolution for time-resolved serial crystallography experiments with the caged oxygen donor.

References:

Chang H. Y., Hemp J., Chen Y., Fee J. A. and Gennis A. B. (2009) “The cytochrome *ba₃* oxygen reductase from *Thermus thermophilus* uses a single input channel for proton delivery to the active site and for proton pumping” PNAS 106(38), 16169-16173

Ludovici C., Fröhlich R., Voggt K., Mamat B. and Lübben M. (2002) ”Caged O2”European Journal of Biochemistry 269(10), 2630-2637

Standfuss J. and Spence J. (2017) “Serial crystallography at synchrotrons and X-ray lasers” IUCrJ. 4(Pt 2), 100-101

Optimization of crystallization conditions of metalloenzymes for serial crystallography and XFEL

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Developments in the recent years have made serial crystallography approaches more readily available to more users, both at synchrotrons and x-ray free electron lasers (XFEL). This allows design of novel experiments and new questions can be asked in the field of structural biology. By collecting serial crystallography datasets at synchrotron or XFEL it is possible to design time resolved experiments with induction of a reaction e.g. via a pump laser or oxygen-exposure. Room temperature experiments are possible and allow mixing of the mother liquor with substrate, so that different time points can be recorded. Especially XFELs with their high brilliance allow the usage of crystals as small as a few micrometres in size and make atomic resolution accessible to biological systems that grow no sufficiently large crystals. For systems that are prone to radiation damage in steady state synchrotron data collection, like metalloenzymes, serial crystallography is a great opportunity, since synchrotron or XFEL serial crystallography can reduce or even eliminate the problem of radiation damage altogether.

But serial crystallography also comes with its own set of challenges. In most cases large amounts of microcrystals are needed. They cannot be too fragile and have to survive at room temperature for a longer period of time. The crystallization condition, crystal size and concentration need to be considered for the specific experimental setup. The optimization of sample for a serial crystallography experiment differs therefore drastically from a single crystal synchrotron experiment.

Working with metalloenzymes additionally requires to control the occupancy and redox states of the metal cofactors involved in order to be able to start the experiment with the right conditions.

In this study experiences from sample preparation of metalloenzymes for serial crystallography will be presented and some challenges and their solutions will be discussed.

Investigating the structure and function of the conjugation channel of the pCF10 Type IV Secretion System

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Multidrug resistant bacteria are one of the most important current threats to public health and a serious problem in hospital acquired infections (HAIs). Most antibiotic resistance genes are acquired via conjugative gene transfer, facilitated by Type IV Secretion Systems (T4SSs). T4SSs are large, megadalton sized, complex protein machineries that transfer DNA and proteins in a contact-dependent manner from a bacterial donor into a recipient cell. This makes them important in the search of potential novel targets towards the development of new treatments. Of the few conjugation systems studied in G^+ bacteria, the T4SS encoded by the antibiotic resistance plasmid pCF10 of *Enterococcus faecalis* is one of the best characterized. pCF10 confers virulence and tetracycline resistance and has its T4SS situated within the 30 kbp large P_Q operon. The T4SS is built up by approximately 14 genes, all encoded on the P_Q operon. These genes can be divided into three main categories, encoding for (i) adhesion proteins, (ii) energetic proteins or (iii) channel proteins.

The actual T4SS mating channel consists of seven membrane proteins, named PrgD, PrgF, PrgH, PrgI, PrgK, PrgL, and PcfH. So far, only very limited information of these proteins is available, except for PrgD and PrgL that have been indicated to contain VirB8-like domains and PrgK, which possess murein hydrolase domains. Here we present our initial work on the expression, purification and crystallisation of the membrane proteins involved in forming the conjugation channel, with a focus on the VirB8-like protein PrgL.

Structural studies of aminoglycoside nucleotidyltransferases (ANTs)

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Aminoglycosides are broad-spectrum antibiotics that target the bacterial protein synthesis. Common resistance mechanisms involve chemical modification of the drugs. One type of resistance-mediating enzymes is aminoglycoside nucleotidyltransferases (ANTs). We previously characterized the structure and substrate binding of AadA from *Salmonella enterica* (1, 2). AadA is an ANT (3") 9' enzyme that confers resistance against the two aminoglycosides streptomycin and spectinomycin by adenylation of the 3'' and 9' hydroxyl positions of the two drugs. Complex structures with ATP, magnesium and streptomycin show details of streptomycin recognition. Bioinformatic characterization of the AadA family showed distinct patterns of conservation for enzymes active on both substrates or only on spectinomycin.

We now set out to clarify how these enzymes bind spectinomycin, a molecule with very limited similarity to streptomycin. For this reason, we recently solved a crystal structure of an ANT9 enzyme from *Enterococcus faecalis.* Structural and functional studies to explain spectinomycin recognition and modification are underway.

References:

1. Chen, Y., Näsvall, J., Wu, S., Andersson, D. I. & Selmer, M. Structure of AadA from Salmonella enterica: a monomeric aminoglycoside (3'')(9) adenyltransferase. Acta Crystallogr Sect D Biological Crystallogr 71, 2267–2277 (2015).
2. Stern AL , Van der Verren SE , Kanchugal PS , Näsvall J, Gutierrez de Teran H, Selmer M. Structural mechanism of AadA, a dual specificity aminoglycoside adenyl transferase from Salmonella enterica. J Biol Chem. 293(29) 11481–11490 (2018).

Structural characterisation of an ancient aquaporin

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Aquaporins are responsible for regulating osmosis in all kinds of cells based on their selectivity for water and compatible solutes. *B. improvisus* is an extraordinary successful invasive species that has the capacity to efficiently adapt to variations in osmolarity. Genetic studies of the bay barnacle indicate that it originates from Argentina. However, it is now found globally due to human-mediated dispersal with ships. Furthermore, the euryhaline bay barnacle can adapt and proliferate in a wide range of salinities. The barnacle aquaporin is attractive since it is evolutionarily distant from us humans, and its structure have the potential to illustrate interesting aspects of aquaporin evolution. In this study, through X-ray crystallography and hanging drop vapour diffusion, we aim at determining the three-dimensional structure of the aquaporin-1 from *Balanus improvisus*.

By previous studies, we have shown that *Pichia pastoris* can produce high yields of functional membrane proteins, especially aquaporins, being suitable for crystallisation and structural determination. For that reason, we chose *P. pastoris* for heterologous production of the Barnacle Aquaporin-1 protein.

This far, the crystallisation of *B. improvisus* aquaporin-1 has been successful in two conditions. However, the crystals diffracted to about 7 Å, as tested at the BIOMAX beamline at MaxIV facility in Lund, Sweden. The two conditions will be further explored and optimised to produce better-diffracting crystals, aiming at a structure with a resolution of 3 Å, or higher.

LP3 and DEMAX

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Proteins are diverse molecules and of enormous importance to life on earth. They have a multitude of different functions in all organisms and can work as enzymes, gene regulators, structural components, transporters, and receptors. In disease, most drugs act on proteins. It is therefore expected that the structures and mechanisms of proteins are prominent topics in life science research.

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

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

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

1 Koruza, K., Lafumat, B., Nyblom, M., Knecht, W. & Fisher, Z. From initial hit to crystal optimization with microseeding of human Carbonic anhydrase IX—A case study for neutron protein crystallography. *Crystals* **8**, 434 (2018).

2 Koruza, K., Lafumat, B., Vegvari, A., Knecht, W. & Fisher, S. Z. Deuteration of human carbonic anhydrase for neutron crystallography: Cell culture media, protein thermostability, and crystallization behavior. *Archives of biochemistry and biophysics* **645**, 26-33, doi:10.1016/j.abb.2018.03.008 (2018).

3 Koruza, K. *et al.* Using neutron crystallography to elucidate the basis of selective inhibition of carbonic anhydrase by saccharin and a derivative. *Journal of structural biology*, doi:10.1016/j.jsb.2018.12.009 (2019).

4 Kelpsas, V., Lafumat, B., Blakeley, M.P., Coquelle, N., Oksanen, E. & von Wachenfeldt, C. Perdeuteration, large crystal growth and neutron data collection of *Leishmania mexicana* triose-phosphate isomerase E65Q variant *Acta Cryst. F*, (2019) (accepted manuscript)

Crystal structure of the *Weissela oryzae* Botulinum toxin-like catalytic domain.

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Botulinum neurotoxins (BoNTs) are, to this date, the most potent toxins known to man. All eight identified serotypes (A-G, X) have been shown to act as zinc dependent endopeptidases targeting mammals. Recently, several botulinum toxin-like proteins were identified outside the clostridium genome. The first of them was discovered during bioinformatical analysis of the genome of *Weissela oryzae*, a non-spore forming anaerobic bacteria isolated from fermented rice grains, and designated botulinum toxin-like protein from *Weissela oryzae* (BoNT/Wo) [1]. The natural targets of BoNT/Wo are yet to be determined, however, the toxin was shown to cleave VAMP2 at a unique cleavage site [2]. Here we report the 1.6 Å crystal structure of the BoNT/Wo catalytic domain. LC/Wo shares the core fold common to all other BoNTs, demonstrating that LC/Wo is a bona fide member of BoNT-LCs. The catalytic pocket of LC/Wo is negatively charged and resembles the one of LC/B but interestingly it is even wider and more accessible. Close to the active site a calcium ion with potential importance for substrate binding was observed. However, we cannot exclude the possibility that the ion is a crystallization artefact. Another unique feature present in the LC/Wo structure is a twisted beta-hairpin that could play a role in substrate recognition and binding. The observed structural information provides the basis for establishing the substrate profile of BoNT/Wo with regards to novel target species and helps our understanding of BoNT evolution [3].

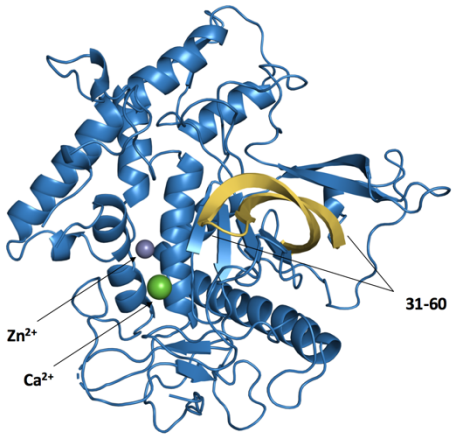

Figure 1: Crystal structure of LC/Wo (blue). Zinc ion and calcium ion are shown as grey and green sphere, respectively. Unique twisted beta-hairpin is highlighted in yellow.

1. Mansfield, M. J., Adams, J. B. & Doxey, A. C. (2015) Botulinum neurotoxin homologs in non-Clostridium species, FEBS Lett. 589, 342-8.
2. Zornetta, I., Azarnia Tehran, D., Arrigoni, G., Anniballi, F., Bano, L., Leka, O., Zanotti, G., Binz, T. & Montecucco, C. (2016) The first non Clostridial botulinum-like toxin cleaves VAMP within the juxtamembrane domain, Sci Rep. 6, 30257.
3. Košenina, S., Masuyer, G., Zhang, S., Dong, M. & Stenmark, P. (2019) Crystal structure of the Weissela oryzae Botulinum toxin-like catalytic domain, manuscript.

The role of phosphorylation in calmodulin-mediated gating of human AQP0

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Aquaporin-0 (AQP0) is the main water channel in the mammalian lens and is involved in accommodation and maintaining lens transparency. AQP0 binds the Ca^{2+}-sensing protein calmodulin (CaM) and this interaction is believed to gate its water permeability by closing the water-conducting pore. In this study we express recombinant and functional human AQP0 in *P. pastoris* and investigate how phosphorylation affects the interaction with CaM *in vitro* as well as the CaM-dependent water permeability of AQP0 in proteoliposomes. Using microscale thermophoresis (MST) we show that wild type full-length AQP0 interacts with CaM with positive cooperativity. In contrast, the introduction of the single phospho-mimicking mutations S229D and S235D in AQP0 abolishes its binding to CaM. CaM interacts with S231D with similar affinity as wild type, but without the positive cooperativity, suggesting difference in the complex compared to wild type. Permeability studies of wild type AQP0 in proteoliposomes showed that the water conductance was significantly reduced by CaM in a Ca^{2+}-dependent manner, whereas AQP0 S229D, S231D and S235D were all locked in an open state, insensitive to CaM. We propose a model where AQP0 can have three states in relation to its phosphorylation status and to CaM: (1) CaM interaction with non-phosphorylated AQP0 causes pore closure. (2) Phosphorylation of S229 or S235 abolishes binding (the pore remains open) and (3) phosphorylation of S231 results in CaM-binding without causing pore closure, the functional role of which remains to be elucidated. Since the level of S235 phosphorylation increases towards the lens inner cortex, our results suggest that AQP0 may become insensitive to CaM-dependent gating along this axis.

Lipid flippases of *Plasmodium* parasites: from heterologous production to functional characterization

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Malaria is a devastating disease caused by a parasite of the genus *Plasmodium*. Recently, gene-deletion studies in malaria mouse models have shown that *Plasmodium* P4-ATPases (the P4 subfamily of P-type ATPases[1], or lipid flippases) are essential for the parasite survival[2]. In eukaryotes, the phospholipid translocation activity of P4-ATPases is needed to maintain the distribution and composition of membranes, a key element in many essential processes like vesicle budding or apoptosis. To understand the functional role of these still putative transporters during malaria infection we need to study their transport mechanism and identify their substrate(s). We have conducted the heterologous expression in *Saccharomyces cerevisiae* [3] of PcATP2, the *P. chabaudi* P4-ATPase present as well in all *Plasmodium* species. Lipid flippases form heteromeric complexes with members of the Cdc50 protein family, also found in the genomes of *Plasmodium* parasites. Using heterologous co-expression of PcATP2 and the putative PcCdc50 proteins followed by immune precipitation and FSEC, we have managed to identify the β-subunit of PcATP2. Currently, we are purifying the complex PcATP2/CPc50.1 using immobilized nanobodies that recognize the GFP fused at the C-terminal end of PcATP2.

Refences:

1-López-Marqués R *et al*. "Pumping lipids with P4-ATPases". Biological Chemistry. (2011)

2-Bushell E *et al.* "Functional profiling of a Plasmodium genome reveals an abundance of essential genes". Cell. (2017)

3- Azouaoui H *et al*. "A high-yield co-expression system for the purification of an intact Drs2p-Cdc50p lipid flippase complex, critically dependent on and stabilized by phosphatidylinositol-4-phosphate." PLoS One. (2014)

PrgU, a repressor of conjugation in *Enterococcus faecalis* pCF10

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Antibiotic resistance is a major problem that is on the rise, especially in hospital acquired infections. One of the most clinically relevant issues is transferable resistance linked to conjugative plasmids, which transfers DNA laterally through Type 4 Secretion Systems (T4SS). The main focus of this project is on the regulation of the T4SS in the pCF10 plasmid from *Enterococcus faecalis*, a gram-positive bacterium. All of the genes encoding for the T4SS in *E. faecalis* pCF10 are under the regulation of one promotor named P_Q. Conjugation in Gram-positive bacteria in plasmid-containing donors are often induced by peptide sex pheromones produced by recipient cells (1). A few years ago, a new protein involved in the regulation of pCF10 was found: PrgU. This protein inhibits the transcription from the P_Q promotor 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). It is believed that PrgU binds to the intergenic region (IGR) that can be found after the first gene under the P_Q promotor. The IGR is predicted to have a rRNA structural fold (3). *In vivo* data also points towards PrgU interacting with another regulator on the same operon, PrgR.

Here we have purified PrgU and initiated the *in vitro* characterization of the protein. *In vitro* data shows that PrgU does bind DNA in a sequence unspecific manner but with a weak affinity. *In vivo* data indicates that PrgU indeed binds to the IGR RNA, and we are currently conducting *in vitro* EMSAs to quantify the interaction. To get a better understanding of the underlying mechanism of RNA binding we are also conducting PrgU:RNA crystallization experiments.

References:

1. Dunny GM, Berntsson RPA. Enterococcal sex pheromones: Evolutionary pathways to complex, two-signal systems. J Bacteriol. 2016;198(11):1556–62.
2. Bhatty M, Camacho MI, Gonzalez-Rivera C, Frank KL, Dale JL, Manias DA, et al. PrgU: a suppressor of sex pheromone toxicity in Enterococcus faecalis. Mol Microbiol. 2017;103(3):398–412.
3. Bensing BA, Dunny GM. Pheromone-inducible expression of an aggregation protein in Enterococcus faecalis requires interaction of a plasmid-encoded RNA with components of the ribosome. Mol Microbiol. 1997;24(2):295–308.

Autoinhibition and Activation of the Oncogenic Chromatin Remodeler ALC1

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Human ALC1 is an oncogene-encoded chromatin-remodeling enzyme required for DNA repair that possesses a poly(ADP-ribose) (PAR)-binding macro domain. Its engagement with PARylated PARP1 activates ALC1 at sites of DNA damage, but the underlying mechanism remains unclear. Here, we establish a dual role for the macro domain in auto-inhibition of ALC1 ATPase activity and coupling to nucleosome mobilization. In the absence of DNA damage, an inactive conformation of the ATPase is maintained by juxtaposition of the macro domain against predominantly the C-terminal ATPase lobe through conserved electrostatic interactions. Mutations within this interface displace the macro domain, constitutively activate the ALC1 ATPase independent of PARylated PARP1, and alter the dynamics of ALC1 recruitment at DNA damage sites. Upon DNA damage, binding of PARylated PARP1 by the macro domain induces a conformational change that relieves auto-inhibitory interactions with the ATPase motor, which selectively activates ALC1 remodeling upon recruitment to sites of DNA damage.

Rapid Structure Solution of Small Organic Molecules from Nanocrystals of a Combination Medicine by 3D Electron Diffraction

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Determining the crystal structure of a pharmaceutical compound is essential for understanding both its physical and physiological properties. NMR and other spectroscopic techniques are often used in combination to identify small organic molecules. Yet, the structures of the vast majority of small organic molecules are identified through X-ray crystallography. However, this technique is still limited to micrometre-sized crystals, even using the most advanced synchrotron sources, and several thousand active pharmaceutical ingredients (APIs) are only available as sub-microcrystalline powders[1].

Rapid, atomic resolution structure determination of nanometre-sized small organic molecules has been demonstrated using rotation electron diffraction (RED) [1,2] or MicroED[3,4]. This technique has shown potential to become a powerful tool for chemical synthesis and drug characterisation and discovery[5,6]. Here we report the use of MicroED to provide the structures of individual small organic compounds from a combination medicine, Symbicort®. This medicine, which is available as a dry powder inhaler, is used in the management of asthma and chronic obstructive pulmonary disease (COPD) and contains both budesonide and formoterol as the APIs. The sample was prepared by simply squirting the fine powder from the inhaler straight onto a holey carbon copper grid, with each dose containing 160 mg budesonide and 4.5 mg formoterol fumerate dehydrate. From this simple powder mixture and very minimal sample preparation, we could collect high quality MicroED data from nanocrystals (~100 x 100 x 100 nm) and identify each of the species within minutes based on the diffraction patterns and unit cell parameters. Using the data collected and only standard X-ray crystallography software, we could gain atomic resolution (< 1 Å) crystal structures of each API within an afternoon.

These findings further illustrate that electron crystallography has the potential to become a powerful tool for the characterization of small organic molecules, creating not only new opportunities within drug discovery but for the field of organic chemistry as a whole.

1. Zhang, D.; Oleynikov, P.; Hovmöller, S.; Zou, X. *Z. Kristallogr.* **2010**, *225*, 94–102.
2. Wan, W.; Sun, J.; Su, J.; Hovmöller S.; Zou, X. *J. Appl. Crystallogr.* **2013**, *46*, 1863–1873.
3. Shi, D.; Nannenga, B. L.; Iadanza, M. G.; Gonen, T. *eLife* **2013**, *2*, e01345.
4. Nannenga, B. L.; Shi, D.; Leslie, A. G. W.; Gonen, T. *Nat. Methods* **2014**, *11*, 927–930.
5. Gruene, T. *et al. Angew. Chem. Int. Ed*. **2018**, *57*, 16313–16317
6. Jones, C. G. *et al. ACS Cent. Sci.* **2018**, *4*, 1587–1592.

Scavenging of superoxide by a membrane-bound superoxide oxidase

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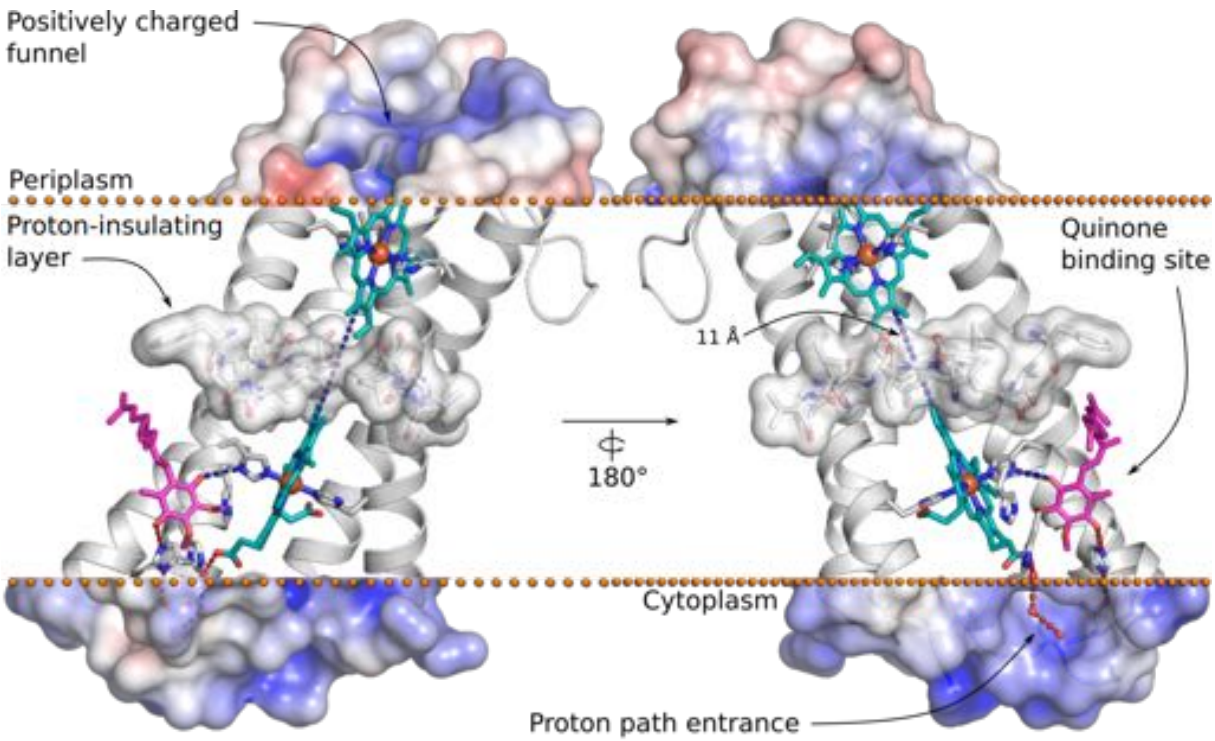
E. coli Cytochrome b561 (CybB) is a heme-containing integral membrane protein, previously proposed to play a role in the respiratory chain. We have solved the crystal structure of CybB to 2.0 Å resolution and have found that CybB reacts with superoxide at rates close to the diffusion-limit in a similar fashion to other superoxide scavengers, such as superoxide dismutase. We propose that this previously undescribed family of enzymes should be denoted superoxide oxidase (SOO).

The structure reveals a four-helix bundle with two non-covalently bound *b*-type hemes. The bundle opens up in a funnel, exposing the porphyrin ring of one heme to the periplasmic space. The distance between the two hemes suggest electron transfer rates in the nanosecond range, and the space between them is shielded by a layer of hydrophobic amino acids, preventing proton leakage.

We propose that SOO rescues electrons "lost" to superoxide and recycles them back into the respiratory chain, releasing oxygen. At the same time SOO contributes to the proton motive force by uptake of protons from the cytoplasmic side of the membrane.



Camilla A. K. Lundgren*, Dan Sjöstrand*†, Olivier Biner*, Matthew Bennett, Axel Rudling, Ann-Louise Johansson, Peter Brzezinski, Jens Carlsson, Christoph von Ballmoos † and Martin Högbom †. Scavenging of superoxide by a membrane-bound superoxide oxidase. Nature Chemical Biology (2018) 14:788–793. *contributed equally †corresponding author

Identification of lipid interactions in the transmembrane regions of human Na^+, K^+-ATPase

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The function of ion-transporting Na^+,K^+-ATPase (NKA) depends on the surrounding lipid environment in the plasma membrane [1]. Certain lipid types activate different confirmations of NKA and are known to stabilize the structural conformation induced by the interaction of lipids at different sites (named as site A, B and C) within the transmembrane domains [2]. An atomic-scale molecular dynamics (MD) simulation approach was used to identify the interacting lipids within the transmembrane (TM) region and their specific sites of interaction.

Homology models of Na^+ and K^+ binding NKA states were embedded into a complex plasma membrane comprising of different phospholipids, sphingolipid, glycolipids and cholesterol thus mimicking native-like conditions. This system was simulated on a microsecond level to elucidate the dynamics of interacting lipids.

The resulting lipid-interaction pattern showed abundance of high-occupancy cholesterol and POPS lipids in site A, while site B contained mostly POPE and POPC lipids. The site C, which is a hotspot for disease-causing mutants at the lipid-protein interface, was observed to host mostly POPE lipids. This study identifies important lipid-interaction sites and their dynamics, which underlie the stability of NKA within the plasma membrane.

References

[1] Cornelius, F., Habeck, M., Kanai, R., Toyoshima, C., & Karlish, S. J. D. (2015). General and specific lipid–protein interactions in Na, K-ATPase. Biochimica et Biophysica Acta (BBA)-Biomembranes, 1848(9), 1729-1743.

[2] Habeck, M., Kapri-Pardes, E., Sharon, M., & Karlish, S. J. (2017). Specific phospholipid binding to Na, K-ATPase at two distinct sites. Proceedings of the National Academy of Sciences, 114(11), 2904-2909.

The iron-dependent regulator IdeR from *Saccharopolyspora erythraea* controls the expression of a gene presumably involved in tryptophan production

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One of the biggest challenges bacteria face in their environment is keeping metal homeostasis. Iron is an essential metal needed as a cofactor for multiple enzymatic reactions. However, iron bioavailability is severely limited due to the oxidation of the soluble Fe^{2+} to the insoluble Fe^{3+} in aerobic environments. Bacteria have therefore developed a diverse set of uptake mechanisms to ensure adequate iron supply. On the other hand, an excess of iron is toxic due to the production of reactive oxygen species through Fenton reactions and the displacement of other metals with lower affinity from protein cofactors. The iron-dependent regulator IdeR is a DNA-binding repressor preventing the expression of iron uptake genes when there is enough iron in the cell, ensuring that the iron concentration does not reach toxic levels.

The analysis of *S. erythraea* IdeR revealed the presence of a putative IdeR binding site upstream of at least 20 gene clusters, most of them harbouring genes involved in metal homeostasis. A specific DNA region drew our attention, as it contained two IdeR binding sites controlling the expression of two divergent genetic clusters. One of these clusters is predicted to be involved in siderophore production (an iron-chelating compound that captures extracellular iron for its incorporation into the cell). The other cluster (containing the gene SACE_2689) encodes an anthranilate synthase component I, presumably involved in tryptophan production.

Electrophoretic mobility shift assays (EMSAs) performed with the predicted IdeR DNA recognition sequence closest to the SACE_2689 gene revealed that IdeR is only able to bind to the sequence in the presence of transition metal ions. Furthermore, we found that Fe^{2+} and Co^{2+} induce DNA binding of IdeR equally efficiently, while Mn^{2+} does so with lower efficiency.

These results show that IdeR binds to the SACE_2689 promoter sequence in the presence of iron, most likely repressing its expression. To our knowledge there is only one reported case of iron-dependent regulation of tryptophan production in bacteria, where it is related to the pathogenic behaviour of the species (Pokorzynski *et al.*, 2019). As *S. erythraea* is not pathogenic and does not share a similar habitat, this points to a new correlation between iron and tryptophan levels in bacteria, maybe similar to the one found in animals where iron can lead to tryptophan toxicity (Johnson, 2001).

- Pokorzynski, N.D., Brinkworth, A.J., and Carabeo, R.A. (2019) A bipartite iron-dependent transcriptional regulation of the tryptophan salvage pathway in *Chlamydia trachomatis*. Elife. pii: e42295. doi: 10.7554/eLife.42295.

- Johnson, S. (2001) The possible crucial role of iron accumulation combined with low tryptophan, zinc and manganese in carcinogenesis. Med Hypotheses. 57(5): 539-543.

A rationally designed and highly versatile epitope tag for nanobody-based purification, detection and manipulation of proteins

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Specialized epitope tags are widely used for detecting, manipulating or purifying proteins, but often their versatility is limited. Here, we introduce the ALFA-tag, a novel, rationally designed epitope tag that serves an exceptionally broad spectrum of applications in life sciences while outperforming established tags like the HA, FLAG or myc tags. The ALFA-tag forms a small and stable α-helix that is functional irrespective of its position on the target protein in prokaryotic and eukaryotic hosts. We developed a nanobody (NbALFA) binding ALFA-tagged proteins from native or fixed specimen with extremely high affinity. It is ideally suited for super-resolution microscopy, immunoprecipitations and Western blotting, and also allows *in-vivo* detection of proteins. By solving the crystal structure of the complex we were able to design a nanobody mutant ($NbALFA^{PE}$) that permits efficient one-step purifications of native ALFA-tagged proteins, complexes and even entire living cells using peptide elution under physiological conditions.

Pursuing the catalytic cycle of semi-artificial hydrogenases under in vivo conditions

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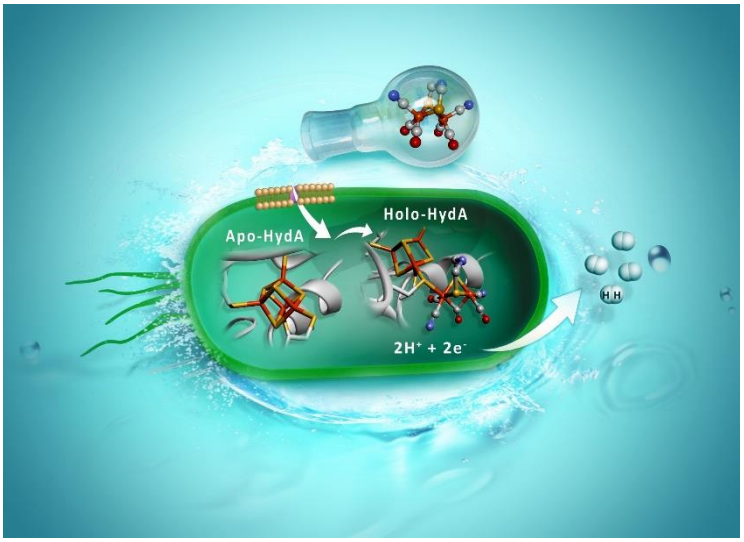
[FeFe] hydrogenases are metalloenzymes that catalyse the interconversion between H_2 and protons with remarkable efficiency. The reaction occurs at the H-cluster featuring an organometallic dinuclear [2Fe] subsite. Previously we could show that synthetic complexes mimicking the composition of the [2Fe] subsite can be introduced into the enzyme under *in vitro* conditions.[1,2] This provides a direct link between biomimetic chemistry and biology, and allows us to manipulate the enzyme using synthetic chemistry.
Here I will present how the concept of artificial maturation can be extended to *in vivo* conditions and the apo-enzyme activated using synthetic cofactors inside living cells.[3,4] I will provide an overview how the catalytic cycle of semi-artificial hydrogenases can be followed and modified inside the living cells,[5] giving us a novel tool for studies of [FeFe] hydrogenases.



1 Berggren, G. et al. Biomimetic assembly and activation of [FeFe]-hydrogenases. *Nature* **499**, 66-69 (2013).
2 Esselborn, J. et al. Spontaneous activation of [FeFe]-hydrogenases by an inorganic [2Fe] active site mimic. *Nat. Chem. Biol.* **9**, 607-609 (2013).
3 Khanna, N. et al. In vivo activation of an [FeFe] hydrogenase using synthetic cofactors. *Energy & Environmental Science* **10**, 1563-1567 (2017).
4 Meszaros, L. S. et al. In vivo EPR characterization of semi-synthetic [FeFe] hydrogenases. *Angew Chem Int Ed Engl* **57**, 2596-2599 (2018).
5 Meszaros, L. S. et al. Pursuing the catalytic cycle of [FeFe] hydrogenases under *in vivo* conditions. *Mauscript*.

Metamorphic Proteins in Action: Activation of the Bacterial Transformer Protein RfaH

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RfaH is a bacterial paralogue of NusG, an essential transcription elongation factor comprising the only transcription factor family that is ubiquitous to all domains of life[1]. Unlike NusG, RfaH is non-essential and controls expression of specific subsets of genes, to which it is recruited by *cis*-acting DNA sequence elements, named *ops* (*o*peron *p*olarity *s*uppressor)[2,3]. Both, RfaH and NusG are two-domain proteins and share conformationally identical N-terminal domains (NTD), which interact with the RNA polymerase (RNAP) to ensure transcription elongation[4–6]. Contrary, the C-terminal domains (CTD) adapt distinct conformations: the CTD of NusG displays an all-beta fold and is independent of the NTD, whereas for RfaH the CTD folds as alpha-hairpin and makes close contacts with the NTD, thereby masking the RNAP binding site[4,7,8]. The release of the CTD from the NTD, i.e. RfaH activation, initiates a domain switch from alpha-helix to beta-barrel enabling the CTD to recruit the translation machinery[8]. Although several important interdomain-interface-residues have been identified, the actual trigger and mechanism of domain opening still remain elusive. We therefore are applying solution nuclear magnetic resonance (NMR) spectroscopy to address the structural basis and the dynamics of this activation process using the well-described RfaH protein of *Escherichia coli*. These dynamic and structural studies are further complemented by biochemical and biophysical studies of several mutants altering the activation mechanism. Further, to test the validity of our findings to other organisms, we initiated the structural analysis of the homologous protein from *Vibrio cholerae***.**

1. Werner, F. & Grohmann, D., *Nat. Rev. Microbiol.* **9,** 85–98 (2011).
2. Bailey, M. J. et al., *Mol. Microbiol.* **26,** 845–851 (1997).
3. Belogurov, G. A. et al., *EMBO J.* **28,** 112–122 (2009).
4. Belogurov, G. A. *et al.*, *Mol. Cell* **26,** 117–129 (2007).
5. Mooney, R. A. *et al., J. Mol. Biol.* **391,** 341–358 (2009).
6. Sevostyanova, A. *et al*., *Mol. Cell* **43,** 253–262 (2011).
7. Burmann, B. M. *et al*., *Biochem. J.* **435,** 783 LP-789 (2011).
8. Burmann, B. M. *et al.*, *Cell* **150,** 291–303 (2012)

MX-PReSTO 2019

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In 2013, Protein Science Facility (PSF) from Karolinska Institutet in Stockholm and National Supercomputer Centre (NSC) in Linköping, started a pilot project to evaluate performance of Macromolecular X-ray crystallography (MX) applications running in an HPC environment. In 2015, the Swedish light source MAX IV decided to fund a pilot extension called PReSTO (https://www.nsc.liu.se/support/presto/), that aim to support integrated structural biology calculations including MX, Nuclear Magnetic Resonance (NMR) and cryo-electron microscopy (cryo-EM). During 2017-2018 the PReSTO HPC-MX installation was made using easybuild (https://easybuild.readthedocs.io/en/latest/) with several advantages to a standard HPC installation such as A) software environments can be send to compute nodes B) software dependencies are visible in easyconfigs. In 2018, the Swedish Research Council granted funds (dnr. 2018-06479) to a collaborative extension of MX-PReSTO towards Cryo-EM/SciLifeLab and NMR/Swedish NMR Centre. Now in 2019, the PReSTO installation will be placed under version control and in September 2019, we want to share easybuild daily maintenance and operations with local HPC administrators at the MAX IV site. Swedish researchers visiting BioMAX receive some MX-HPC training during beamtime. Regular after beamtime training sessions with users own data/questions will be available at weekly drop-in sessions at protein science facility, Karolinska Institutet. Startup PReSTO seminar or workshop can also be requested by all Swedish Universities by contacting project PI (Martin Moche) as done by UU in 2015 and SLU in 2018.

Access to the PReSTO installation is via Swedish National Infrastructure of Computing (SNIC) funded by the Swedish Research Council. Thinlinc is a remote desktop server from Cendio https://www.cendio.com/thinlinc/what-is-thinlinc that supports the integrated structural biology workflow by enabling graphic applications i.e. coot/chimera/ccp4mg/pymol to run smoothly from a remote computer. The homepage (https://www.nsc.liu.se/support/presto/) is written for HPC-MX newcomers showing how to interact with the HPC setup by loading modules, request compute time, core and nodes, writing sbatch scripts, schedule and monitor jobs, describe how to use PHENIX with slurm scheduling, and pointing towards many MX software developer manuals. To adapt new users to the MX-HPC workflow we also developed a helpful PReSTO menu that A) launch MX software at login or compute node with graphics support B) enable user to select number of cores and runtime at compute nodes C) enable user to select output directory for software such as hkl2map.

PReSTO for MX is now available at NSC Tetralith, LUNARC Aurora and the MAX IV cluster. Please acknowledge SNIC (https://www.snic.se/allocations/apply4access/) when using its resources.

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. 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 contain a bHLHzip DNA-binding motif (basic helix-loop-helix and leucine zipper motif). These motifs play an important role in Myc interactions with DNA and other transcription factors. Our research 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. We are focusing on investigating the structure envelope of DNA-bound complexes of Max-Max and Max-Myc dimers using small angle neutron scattering (SANS) method. As a first step, we aim to target the SANS 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.

In vitro phosphorylation of C-Myc (1-88) by different serine-threonine kinases

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Background
The transcription factor c-Myc is a master gene regulator associated with several normal cellular processes, but its overexpression in many human cancers is often associated with unlimited cell growth and poor prognosis[1]. Tight regulation of Myc at multiple levels, be it transcriptional, translational and/or post-transitional levels, is therefore essential to prevent the formation of tumour cells. Phosphorylation of specific residues in myc is thought to trigger the recognition event of the ubiquitin proteasome pathway, which plays a key role in regulating Myc cellular stability[2]. Phosphorylation of the conserved residues Thr-58 and Ser-62 in the intrinsically disordered N-terminus (TAD) of c-Myc is particularly interesting because of their location in the mutational hotspot region in Burkitt's lymphoma[3].

Methods
To get sufficient amounts of protein to analyze the structural effects of Myc phosphorylation, such proteins must be produced *in vitro*. As a step towards reaching this goal, in this project we investigated several phosphorylation strategies using some serine-threonine kinases to phosphorylated c-Myc (1-88) TAD region *in vitro* in two specific sites (Thr-58 and Ser-62). The phosphorylation was confirmed by both mass spectrometry (MS) and Nuclear magnetic resonance spectroscopy (NMR).

Results
Here, we report that the *in vitro* phosphorylation of Thr-58 is dependent on the pre-phosphorylation of Ser-62[4], and that several kinases can phosphorylate different site in c-Myc (1-88) TAD region. The kinase ERK2 alone have the ability to phosphorylated c-Myc (1-88) TAD region at several sites but will only phosphorylate S62 and S71 in a sequential multistep phosphorylation with CDK2/cyclin A. The kinases CDK2/cyclin A and GSK3ß can phosphorylate TAD c-Myc (1-88) domain at Thr-58 and Ser-62 *in vitro*.

Conclusions
The current result will pave the way for further studies of the potential *in vitro* effects of phosphorylation on the structure and dynamic of c-Myc TAD region, and how this will affect its interaction to other proteins.

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[1] D'Cruz CM, Net Med 2001
[2] Yada M, *EMBO J* 2004
[3] Bahram F, Blood 2000
[4] Lutterbach B, *Mol. Cell. Biol.* 1994

Structural insights into membrane protein targeting to multivesicular bodies – characterization of the interaction between LIP5 and AQP2

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The multivesicular sorting machinery is a crucial mechanism for targeting membrane proteins for recycling or degradation. The lysosomal trafficking regulator-interacting protein 5 (LIP5) which coordinates the action of this machinery is also known to bind directly to the membrane protein cargo. In case of aquaporin 2 (AQP2) the binding of LIP5 during the endocytic pathway in kidney collecting duct cells ensures an effective regulation of urine volume [1].

In our group, we have previously studied the role of AQP2 phosphorylation in AQP2-LIP5 interaction [2]. Currently we are focusing on elucidating the structural details of the complex in order to better understand how membrane proteins are delivered to the multivesicular bodies. We have constructed alanine mutants of single residues in the proposed binding sites of both AQP2 and LIP5. Studying the binding affinity of these mutants using microscale thermophoresis and fluorescence anisotropy helps us understand which residues are directly involved in the binding. We have also determined the stoichiometry of the interaction – that one molecule of LIP5 binds to one AQP2 tetramer.

Further, AQP2 was successfully incorporated into MSP-based nanodiscs and negative stain electron microscopy confirmed homogeneous state of the particles. This sample will be used to further study the structure of the complex using cryoEM.

[1] B. W. M. Van Balkom, M. Boone, G. Hendriks, E. Kamsteeg, J. H. Robben, H. C. Stronks, A. Van Der Voorde, and F. Van Herp, “LIP5 Interacts with Aquaporin 2 and Facilitates Its Lysosomal Degradation,” pp. 990–1001, 2009.
[2] J. V. Roche, S. Survery, S. Kreida, V. Nesverova, H. Ampah-Korsah, M. Gourdon, P. M. T. Deen, and S. Törnroth-Horsefield, “Phosphorylation of human aquaporin 2 (AQP2) allosterically controls its interaction with the lysosomal trafficking protein LIP5,” J. Biol. Chem., vol. 292, no. 35, pp. 14636–14648, 2017.

Ribonucleotide reductase in *Francisella tularensis*

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Francisella tularensis is a highly virulent pathogen causing tularemia and is classified as a potential bio-weapon. The enzyme ribonucleotide reductase (RNR), the producer of DNA building blocks – deoxyribonucleotides - in the cell, is a potential efficient target to interrupt the replication pathway in this organism.

RNR in *F. tularensis* belongs to the aerobic class I, which is formed by two subunits: alpha (denoted NrdA) and beta (denoted NrdB). Using a metal cofactor, NrdB generates a radical that is shuttled via a long-range pathway to the catalytic subunit NrdA. Both the radical and the metal factor can vary in class I RNRs, resulting in different subclasses.

F. tularensis NrdB belongs to the phylogenetic subclass Ik, with yet unstudied type of cofactor and radical type. This NrdB carries a rare N-terminal fusion of a glutaredoxin (Grx) domain, a known RNR reductant usually encoded independently from the RNR operon. This Grx is a *grxC* belonging to the domain family (COG0695). Interestingly, a free-standing glutaredoxin gene, of the *grxA* family (PRK11200) is also present in the RNR operon.

We have biochemically characterized the *F. tularensis* RNR to reveal its mode of action. We show that NrdB is a dimeric protein forming a stable tyrosyl radical and binding iron (Fe) as a cofactor, suggesting that it belongs to the class Ia RNRs. Modelling of the *F. tularensis* NrdB amino acid sequence on known three-dimensional structures of NrdB proteins indicate that three tyrosine residues are located close to the metal site and may potentially harbor the radical. To identify which of the tyrosines is the radical-harboring one, we mutated each of the tyrosine to phenylalanine to test their activity. We will also study the role of the two glutaredoxins, using a *grxC* deletion construct of NrdB and the purified GrxA protein.

Protein Science Facility
Karolinska Institutet/SciLifeLab

Ida Johansson, Martin Moche, Elin Dunevall, Tomas Nyman, Helena Berglund

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http://ki.se/psf

PSF offers established know-how and expertise to provide academic users with subsidized cloning, expression screening and protein production services, as well as user training and access to instrumentation for high throughput crystallography and biophysical protein characterization. PSF also offers service within macromolecular crystallography and biophysical characterization of proteins.

The Protein Science Facility (PSF) started in 2011, and was approved as a national facility within SWEDSTRUCT 2012 and also within SciLifeLab 2013. Since the start PSF has worked with more than 170 different groups from Swedish academia (KI, SU, KTH, GU, UU, SLU, LiU, LU, UmU, ÖrU), industry, and foreign academia. During the years 2012-2019, access to the PSF platforms have contributed to over 130 publications.

Towards new design strategies for ring-fused 2-pyridones as anti-virulence drugs against *Listeria monocytogenes*

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According to the World Health Organization the development of antibiotic resistances in bacteria is one of the biggest threats to global health and our extensive use of antibiotics accelerates this process even more. Antibiotics target the essential processes of survival and growth in bacteria and therefore put a high selective pressure on the bacteria to develop antibiotic resistances. However, the ability to infect or damage a host, the virulence, is less essential for bacteria. Thus, targeting the virulence is supposed to put a lower selective pressure on the bacteria and this alternative mode-of-action of a drug can help to decelerate the development of antibiotic resistances.

The virulence in the bacterium *Listeria monocytogenes*, which causes the disease listeriosis, is regulated by the transcription factor PrfA. It was shown, that inhibition of PrfA is sufficient to reduce the bacterial virulence [1] and therefore PrfA is a target for anti-virulence drugs.

Several small molecules, with a ring-fused 2-pyridone as scaffold, were identified to inhibit PrfA [2]. Although the current compounds aren't promising drug candidates for pre-clinical and clinical testing, they serve as starting point for the design of improved compounds.

All of the reported ring-fused 2-pyridones have functional groups at positions 3, 7 and 8 of the core scaffold, but only at positions 7 and 8 different groups were introduced and their influence on the activity of PrfA were evaluated [3]. The influence of the functional group at position 3 has not been studied yet as it was kept the same in all compounds.

To evaluate the influence of the group at position 3 we extended the carboxyl group at this position by introducing a methylene group while keeping the most promising groups from the previous study at positions 7 and 8. As the ring-fused 2-pyridones have a chiral center at position 3, the new compound, MK383, was obtained as a racemic mixture.

MK383 reduces the infection rate of Listeria monocytogenes more than the previous reported compounds. Furthermore, the crystal structure shows PrfA in its inactive state and MK383 binds at the same position as the previous compounds. However, the other enantiomer binds to PrfA, which allows new interactions of the extended carboxyl group with residues K122 and C229. This finding enables us to develop completely new design strategies for position 3 of the ring-fused 2-pyridones.

From the current results, it cannot be definitely concluded if only one or both enantiomers of MK383 are active. Therefore, the pure enantiomers are currently synthesized and evaluated.

[1] Chakraborty, T et al. “Coordinate regulation of virulence genes in Listeria monocytogenes requires the product of the prfA gene” *Journal of bacteriology* 174,2 (1992): 568-74

[2] Good, J A D et al. “Attenuating Listeria monocytogenes Virulence by Targeting the Regulatory Protein PrfA” *Cell chemical biology* 23,3 (2016): 404-14

[3] Kulén, M et al. “Structure-Based Design of Inhibitors Targeting PrfA, the Master Virulence Regulator of Listeria monocytogenes” *Journal of Medicinal Chemistry* 61,9 (2018): 4165-4175

Acquired functions on capsid of metazoan totivirus-like dsRNA viruses

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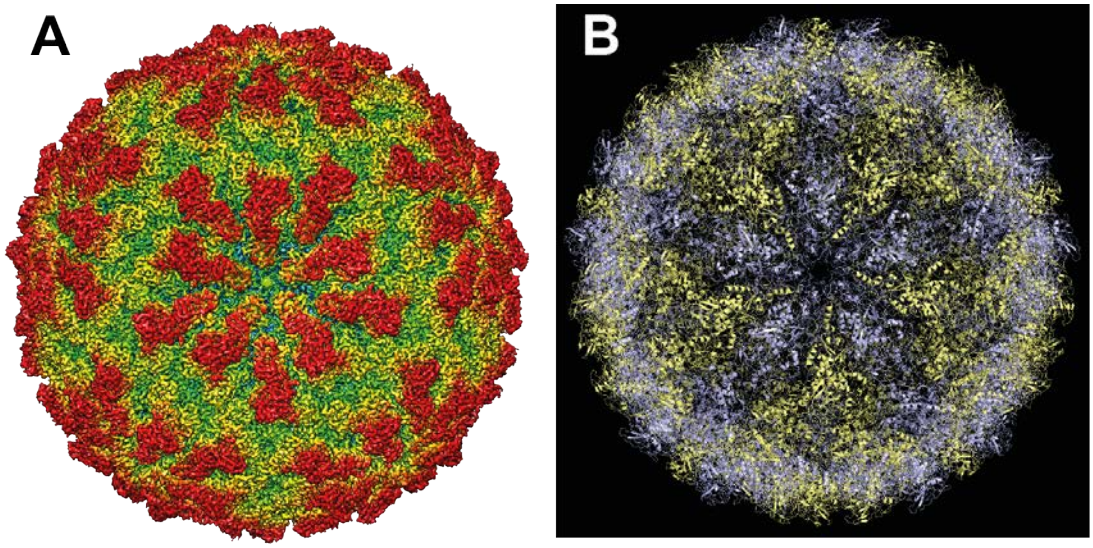
*The Laboratory of Molecular Biophysics, Department of Cell and Molecular biology, Uppsala University, Sweden ** The laboratory of Protein Synthesis and Expression, Institute for Protein Research, Osaka University

Non-enveloped icosahedral dsRNA viruses that infect unicellular hosts are transmitted intracellularly. However, phylogenetically closely-related viruses found in multicellular hosts (metazoa) have acquired surface traits to facilitate an extracellular lifestyle. We aimed to determine the acquired metazoan dsRNA virus structures for the extracellular lifestyle, which are dominant factors in their host tropism, virulence, and immune responses within their hosts. We focused on a representative case of the icosahedral dsRNA virus; protozoan/fungal *Totiviridae* and metazoan totivirus-like virus, Omono River virus (OmRV).

The first structure of a metazoan dsRNA totivirus-like OmRV was determined by electron cryo-microscopy (cryo-EM) single particle analysis (SPA) (Figure A). We have obtained the 3D map to a resolution of 3.3 Å (overall, FSC-cutoff 0.143) and built the first *de novo* atomic model of the OmRV capsid (Figure B). We compared the atomic 3D model of the metazoan OmRV particle with that of a fungal dsRNA virus ScV-LA (PDB ID: 1M1C). We found three new structural features (surface loops, obstructed pores, and interlocking domains) in the metazoan totivirus-like virus OmRV. The dsRNA viruses require at least one pore in their virions to take up nucleoside triphosphates (NTPs) and to release the synthesized genomes (intra-particle genome synthesis). Protozoan/fungal dsRNA viruses have pores, however the metazoan dsRNA virus pores are obstructed. Further structural analysis of the obstructed pore suggests the presence of a new mechanism for switching between the closed and open states of the pore in the metazoan totivirus-like virus OmRV.



Towards solving the structure of loosenin-like protein from *Phanerochaete carnosa*

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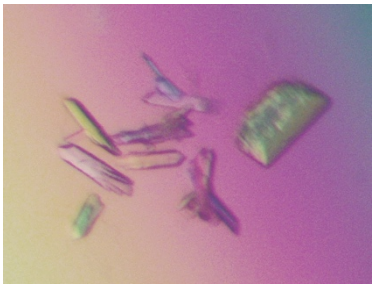

Figure 1. LOOL12 crystals.

Loosenins (LOOS) and loosenin-like (LOOL) proteins are homologous to the N-terminal domain of expansins, which are ubiquitous in plants and responsible for the cell wall loosening process during cell growth. The LOOS and LOOL proteins lack a C-terminal Ig-like domain that is present in expansins, and they consist of a single double-Ψ β-barrel domain that is structurally related to family-45 glycosyl hydrolases (GH45). Despite having structural similarity, loosenin-like proteins lack the enzymatic activity of most GH45's, which in turn often lack the wall extension activity of expansin-like proteins (Payne et al., 2015). The action mechanism of cell wall loosening by expansins remains unknown, but is speculated to be directed towards selectively unravelling cellulose fibrils by hydrolysing certain load-bearing structures in the cell wall, rather than cleaving other easily accessible β-1,4 linkages in the cell wall. Loosenin-like genes are wide-spread among cellulolytic fungi. One loosenin from a wood-degrading fungus has been shown to cause swelling of cotton fibers and to boost the activity of cellulases on recalcitrant plant biomass material (Quiroz-Castaneda et al., 2011).

To date, no structure is available of any LOOL. In our pursuit to understand the action mechanism of expansin-like proteins, we are now preparing a range of LOOL proteins for crystallization towards structure determination. So far, crystals that look promising have been obtained of one protein, LOOL12 from the basidiomycete fungus *Phanerochaete carnosa*, expressed in *Komagataella pastoris* (formerly known as *Pichia pastoris*). Few crystals have been tested at the BioMAX beamline at MAX IV (Lund, Sweden), but they did not give sufficient diffraction for data collection. We continue looking for conditions which produce LOOL12 crystals with a detectable diffraction pattern, and at the same time other LOOLs are employed for crystallization. Thus, we anticipate to be able to obtain structures in the near future of loosenin-like protein(s).

References:

Quiroz-Castañeda R.E., Martínez-Anaya C., Cuervo-Soto L.I., Segovia L. and Folch-Mallol J.L. (2011). Loosenin, a novel protein with cellulose-disrupting activity from *Bjerkandera adusta*. *Microbial Cell Factories*, 10, 8.

Payne C. M., Knott B. C., Mayes H. B., Hansson H., Himmel M. E., Sandgren M., Ståhlberg J., Beckham G. T. (2015). Fungal Cellulases. *Chemical reviews*, 115 (3): 1308-1448.

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.

1. Ehrenfeld, E., Domingo, E., & Roos, R. P. (Eds.). (2010). *The Picornaviruses*. Washington, DC: ASM Press.
2. Hughes, P. J., & Stanway, G. (2000). The 2A proteins of three diverse picornaviruses are related to each other and to the H-rev107 family of proteins involved in the control of cell proliferation. *Journal of General Virology*, *81*(1), 201–207.
3. Staring, J., Von Castelmur, E., Blomen, V. A., Van Den Hengel, L. G., Brockmann, M., Baggen, J., … Brummelkamp, T. R. (2017). PLA2G16 represents a switch between entry and clearance of Picornaviridae. *Nature*, *541*(7637), 412–416

Structural and functional characterization of Ribonucleotide reductase from the hyperthermophile *Aquifex aeolicus*

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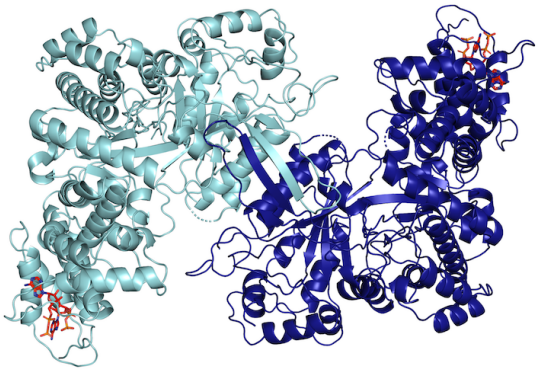
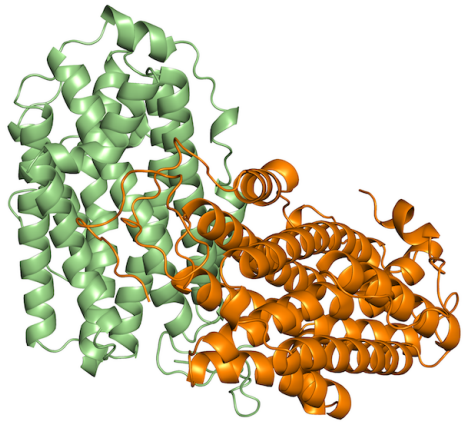
Ribonucleotide reductases (RNRs) are critically important enzymes, which are found in all domains of life. These enzymes substitute the 2'-OH of ribonucleotides with a hydrogen atom, thereby converting them into deoxyribonucleotides. The protein consists of two different subunits. The R2 subunit generates a stable radical that is used in the catalytic reaction, which takes place in the R1 subunit [1]. The R1 subunit binds the four different substrates and uses the radical from the R2 to carry out the reaction. It also binds allosteric effectors that modulate the substrate affinity of the enzyme, depending on which effector is bound. This complex mechanism of allosteric regulation ensures that there is a balanced supply of deoxyribonucleotides in the cell [2]. In this investigation, we studied the RNR from the organism *Aquifex aeolicus,* which is a hyperthermophile that thrives in water at temperatures between 85-95 °C and is typically found around submarine volcanoes and hot springs. The DNA sequence of the R2 subunit in this organism contains an intein that cleaves itself out post-translationally via protein splicing. We present the structures of the R2 subunit as well as structures of the R1 subunit bound to activity regulators. The radical in the R2 subunit was studied using EPR. In addition, we studied the activity regulation by measuring the activity in the presence of different effector/substrate combinations. We determined that the overall activity is regulated by binding of ATP/dATP to an allosteric domain ATP-cone and that the enzymatically optimal temperature is 79 °C.



R1 subunit



R2 subunit

1. Nordlund, Pär, and Hans Eklund. 1993. "Structure and Function of the Escherichia Coli Ribonucleotide Reductase Protein R2." *Journal of Molecular Biology* 232 (1): 123–64.
2. Larsson, Karl-Magnus, Albert Jordan, Rolf Eliasson, Peter Reichard, Derek T Logan, and Pär Nordlund. 2004. "Structural Mechanism of Allosteric Substrate Specificity Regulation in a Ribonucleotide Reductase." *Nature Structural & Molecular Biology* 11 (11): 1142–49.

Structural studies of the Pannexin 1 membrane channel

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KTH Royal Institute of Technology, Stockholm, Sweden , * Department of Physiology and Biophysics, University of Miami, Miami, USA ** University of California San Diego, San Diego, USA

Pannexins play an important role in various physiological and pathological events, and are involved in the control of oxygen delivery, control of ciliary beat in airway epithelia, pathogen sensing by immune cells, propagation of calcium waves in various tissues including astrocytes, and possibly in several sensory mechanisms. They are also involved in pathological settings including ischemia, inflammation, myocardial infarction and secondary cell death. ATP release is a common denominator in all of these functions of Panx1. However, structural information is still scarce limiting the understanding of its molecular mechanisms.

To move towards a better structural understanding and shed light on the mechanisms involved in its functions, the aim of this project is to determine the Panx1 channels structure using cryo electron microscopy (cryo-EM).

For the protein production a Sf9 expression system has been established using baculoviruses. The expressed protein was purified by affinity and size exclusion chromatographies, reaching high purity. The sample was used for grid preparation in two states, encased in a detergent micelle and reconstituted in protein lipid-nanodiscs. The corresponding grids were used for cryoEM experiments and the collected data analysed by current methods. The data collection from the protein encased in detergent micelle generated particles that allowed us to reconstruct pannexin to an intermediate resolution. However, we observed a high background which resulted in a low resolution structure. As a step towards higher resolution, we were successful in obtaining a homogeneous population from the protein reconstituted in nanodiscs, and were able to obtain classes showing domains similar to other pannexin related channels. The data showed particles with a preferred orientation but enable us to generate a preliminary reconstruction. The 3D reconstruction and the classification were calculated without imposing any symmetry and despite these limitations we can clearly observe secondary structure elements. As future perspective, the sample and data collection strategies are being optimized in order to increase the total amount of particles and circumvent the preferred orientation limitations, enabling us to reach higher resolutions.

A Cation-π interaction, the key to conformational activation of adenylate kinase

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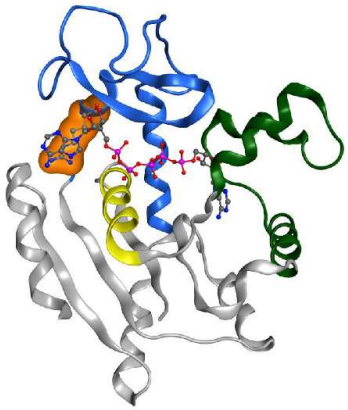
Department of Chemistry, Umeå University, Integrated Structural Biology (@ISB_UmU)

Upon binding to its substrate, adenosine tri-phosphate (ATP), the metabolic enzyme adenylate kinase (Adk) undergoes a conformational transformation from the inactive open state to the active, closed state. ATP binding is mainly mediated through an extensive hydrogen bond network, however, we have through an integrated approach, involving biochemical activity assays, NMR, and theoretical calculations, been able to show the importance of a cation-π interaction between the π-system of the adenine-moiety of ATP and the positively charged side-chain of arginine 119. In the closed conformation, this side-chain packs against the adenosine moiety of ATP. This stacking of an arginine side-chain and the adenine is a common feature among ATP binding proteins

We demonstrated that a variant of *E. coli* Adk where the arginine in position 119 (orange in figure) was replaced with an alanine (R119A) only showed residual activity. By using paramagnetic NMR we could further show that the R119A was not able to change into its active closed form, by closing the ATPlid (blue in figure), upon ATP binding. DFT calculations showed that there is an attractive force between the arginine side chain and the adenine even in the open conformation. This would suggest that the conformational exchange is nucleated by this interaction.

When combined with our previous study about the selectivity ATP/GTP selectivity* of Adk when have obtained a complete picture of the requirements for an ATP binding protein with regards to selectivity and conformational activation.



Structure of *E. coli* Adk, closed state, in complex with the inhibitor Ap5A (pdb: 1AKE (Muller1992)). The three domains are shown in blue: the ATPlid, in green: the AMP binding domain, and in grey: the core. The crucial arginine 119 is shown in orange.

*Rogne P, et al. (2018) Molecular mechanism of ATP versus GTP selectivity of adenylate kinase. Proc Nat Acad Sci USA 115(12):3012-3017.

Ribonucleotide reductase class Id ($Mn^{IV}Mn^{III}$) from *Facklamia ignava*

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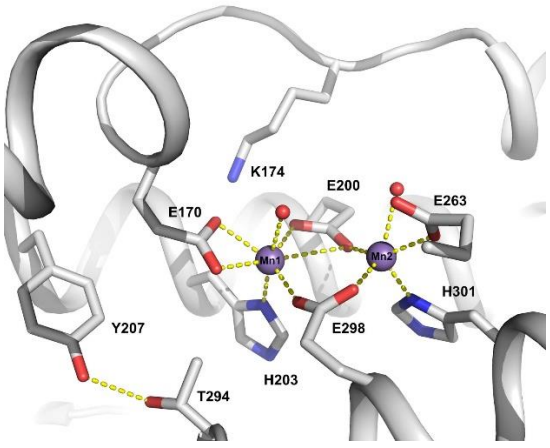
Class I ribonucleotide reductase (RNR) consists of a catalytic subunit (NrdA) and a radical-generating subunit (NrdB) that together catalyze reduction of ribonucleotides to their corresponding deoxyribonucleotides, the building blocks of DNA. NrdB from the firmicute *Facklamia ignava* is a unique fusion protein with N-terminal add-ons of a glutaredoxin (Grx) domain followed by an ATP-binding domain, the ATP-cone. Grx, usually encoded separately from the RNR operon, is a known RNR reductant. We showed that the fused Grx domain functions as an efficient reductant of the *F. ignava* class I RNR via the common dithiol mechanism and, interestingly, also via a monothiol mechanism, although less efficiently.

The ATP-cone is in most RNRs an N-terminal domain of the catalytic subunit. It is an allosteric on/off switch promoting ribonucleotide reduction when ATP is bound and inhibiting RNR activity when dATP is bound. We found that the ATP-cone of *F. ignava* NrdB is a functional allosteric domain. ATP induces enzymatically competent dimers, whereas dATP induces non-productive tetramers, resulting in different holoenzymes.

F. ignava NrdB belongs to the NrdBi subclass of RNRs. Its mixed valent $Mn^{IV}Mn^{III}$ metal site has a distinct EPR signal in the temperature range of 5-15K, with no other Mn related EPR signals at 30K and no trace of a tyrosyl radical. The observations presented underscore the catalytic relevance of the $Mn^{IV}Mn^{III}$ site, and support the notion that the NrdBi proteins represent a new subclass of class I RNRs, denoted subclass Id. The X-ray structure of NrdBi from the flavobacterium *Leeuwenhoekiella blandensis* shows the environment of the class Id $Mn^{IV}Mn^{III}$ metal center.



Metal center of *Leeuwenhoekiella blandensis* NrdB

HTS Approach for Identification of Broad Spectrum FabG Inhibitors Targetting ESKAPE Pathogens

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Paul Ehrlich coined term magic bullets in 1909, referring to agents selectively targeting microbes causing disease without effecting host. The discovery of penicillin, aminoglycosides, sulfonamides in 20th century marked the beginning of golden era of antibiotics (1), but the irresponsible use led to emergence of multidrug resistant bacteria within a short time in evolutionary perspective. ESKAPE pathogens are major source of hospital-born infections and leading cause of death worldwide (2), and these pathogens often appear as multi-drug resistant superbugs complicating many medical interventions which in turn affect global health (3).

Fatty acid biosynthesis in bacteria has been identified as an attractive drug target and several antibiotics (e.g. triclosan, isoniazid) inhibiting this pathway are in use. FabG is a validated target for antibiotics in *Pseudomonas aeruginosa*. In many Gram-negative pathogens and *Mycobacterium tuberculosis* it appears to be essential (4,5). FabG catalyses the reduction of 3-oxoacyl-ACP to 3-hydroxyacyl-ACP in the fatty acid biosynthetic pathway. Earlier we have reported the discovery of FabG inhibitors specific to *Pseudomonas aeruginosa* FabG showing nM-ar affinity binding to an allosteric site (6). None of these inhibitors had good inhibitory potential against FabG from other Gram-negative pathogens.

Our present work focuses on development of novel FabG inhibitors targeting the protein in several pathogens from the ESKAPE family. HTS was employed to screen for inhibitors on *Salmonella t*. and *Acinetobacter b*. enzymes. 36 hit molecules were tested on 8 different FabGs and 12 hits were characterized which inhibit the enzyme from several species. The crystal structure of the *Acinetobacter b.* FabG in complex with this compound was solved to investigate the mechanism of action of this wide spectrum FabG inhibitor.

References-

1. Gould K J. Antimicrob. Chemother. 71.3 (2016): 572-575.
2. Costa D. M. et al. Lett. Appl. Microbiol. 68 (2019): 269-276.
3. Shahper N. K, Asad U. K, Front Microbiol. 7(2016): 174.
4. Richard J. H, Charles O. R, Curr Opin Investig Drugs. 5.2 (2004): 146-153
5. Dutta D. Front Microbiol 9 (2018).
6. Cukier, C. D., et al. ACS Chem Biol 8.11 (2013): 2518-2527.

Insights in structural determinants of β2-Microglobulin stability

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KTH Royal Instutute of Technology, * Department of Biotechnology and Biosciences, University of Milano-Bicocca, ** Department of Bioscience, Università degli Studi di Milano

β2-Microglobulin (β2m), the light chain of the major histocompatibility class-I complex (MHC-I), can assemble into amyloid fibrils in vitro and in vivo. It deposits in joints and bones of patients receiving haemodialysis leading to dialysis-related amyloidosis (DRA) (1). Another amyloid-related disorder, hereditary and systemic, is linked to the natural variant D76N β2m (2). This mutation decreases the thermodynamic stability of the protein that fibrillates in vitro even under physiological conditions.

Previous studies on β2m mutants show that Trp60 plays a crucial role for β2m stability, in particular, W60G β2m has an increased conformational stability and a reduced amyloidogenicity than the wild type protein.

Through the integration of solution NMR spectroscopy and molecular dynamics (MD) simulations we compared the native state dynamics of WT b2m and W60G b2m to identified new residues crucial for the stability and amyloidogenicity of the protein. Starting from MD simulation three β2m variants were designed. All mutants displayed the expected structure stability and aggregation propensity, allowing a fine analysis of the structural features that differentiate the stability of wt b2m and W60G b2m (3).

To assess the feasibility of employing crystalline solid state NMR spectroscopy to study dynamic and stability of β2m, I investigated through WT, W60G and D76N b2m variants whether its thermodynamic stability in solution correlates with the dynamic stability in the crystalline form. The changes in secondary structure content of the crystalline pellet compared to the protein in solution were assessed at increasing temperature by Fourier transform infrared spectroscopy. Each variant shows a good correlation between the stability in solution and in crystals

References

1. Gejyo, F. (1985) Biochem Biophys Res Commun 129, 701-706.
2. Valleix, S. (2012) N. Engl. J. Med. 366, 2276–2283.
3. Camilloni, C (2016) Sci. Rep. 6, 25559.

The mitochondrial Lon protease and its essential role in mitochondrial maintenance

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Wallenberg Centre for Molecular and Translational Medicine

The Human Lon protein complex is an ATP-dependent protease residing in the mitochondrial matrix.(1) Lon protein is a product of the nuclear gene *LONP1* and plays a crucial role in maintenance and repair of mitochondrial DNA as well as being an essential regulator of the mitochondrial metabolism. Lon is ATP-stimulated going from being bound to the inner parts of the mitochondrial membrane (mitochondrial genome) to released form in the matrix where degradation of malfunctioning proteins and maintenance of the DNA is performed.(2) Three functional roles have been elucidated about Lon function: degrading of oxidized dysfunctional proteins, regulation of vital enzymes such as the TFAM transcription factor (3) as well as co-chaperoning functions with other proteins such as Hsp60-mtHsp70 complex.(4) Lon plays also an important role in different diseases due to its involvement in oxidative stress of cells caused by diverse syndromes. Even though the importance of Lon is highlighted, the mechanism and its mechano-chemical principles are not thoroughly known. To unravel these properties, we have started to study the structure of Lon as well as its underlying dynamic properties by using nuclear magnetic resonance (NMR) techniques. Due to its large size, we employ a divide-and-conquer approach, of splitting the protein into its smaller functional units facilitating the characterization by NMR. Lon can be divided into three subparts, one N-terminal domain (proposed to function in substrate recognition and binding), AAA^+ domain (ATP-binding site) and protease domain (proteolytic active site). We have started working on the protease domain and will proceed with the remaining domains and the protein as a whole with interactive studies to known substrates and other molecules of interest.

1. García-Nafría J, Ondrovicová G, Blagova E, Levdikov VM, Bauer JA, Suzuki CK, et al. Structure of the catalytic domain of the human mitochondrial Lon protease: proposed relation of oligomer formation and activity. Protein Sci Publ Protein Soc. 2010 May;19(5):987–99.

2. Bota DA, Davies KJA. Mitochondrial Lon protease in human disease and aging: Including an etiologic classification of Lon-related diseases and disorders. Free Radic Biol Med. 2016;100:188–98.

3. Matsushima Y, Goto Y, Kaguni LS. Mitochondrial Lon protease regulates mitochondrial DNA copy number and transcription by selective degradation of mitochondrial transcription factor A (TFAM). Proc Natl Acad Sci U S A. 2010 Oct 26;107(43):18410–5.

4. Kao T-Y, Chiu Y-C, Fang W-C, Cheng C-W, Kuo C-Y, Juan H-F, et al. Mitochondrial Lon regulates apoptosis through the association with Hsp60-mtHsp70 complex. Cell Death Dis. 2015 Feb 12;6:e1642.

Structural Basis for Selective Targeting of Proteasome Deubiquitinases by Enone-containing Compounds

Johannes Salomonsson[1], Alexandra Ahlner[1], Vivian Morad[1], Eleonore von Castelmur[1], Padraig D'arcy[2,3], Stig Linder[2,3], Maria Sunnerhagen[1]

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The ubiquitin-proteasome system (UPS) regulates cell functions by conjugation of ubiquitin to proteins which are transported to the proteasome for degradation and is therefore essential to most cellular processes. The UPS is a promising area for drug development where targets such as E1/E2 ubiquitin ligases and deubiquitinases (DUBs) contain functional cysteines. One such DUB is Ubiquitin-specific protease 14 which is a two-domain protein consists of a ubiquitin-like (Ubl) domain that is responsible for association to the proteasome and a catalytic core domain which holds a ubiquitin binding site and the catalytic triad. In collaboration with our pharmacology collaborators, we addressed this question by screening a library of ~5000 enone-containing compounds for inhibition of proteasome processing. By a range of biophysical, biochemical and cellular methods, we characterized 10 different active drugs and found evidence of selective inhibition of USP14 and proteasome DUB activity. The results suggested that all compounds can bind to a USP14 crevice close to the active site and some compounds also bind covalently, presumably to the active cysteine. We finally demonstrate limited developmental toxicity and significant antineoplastic activity of enone DUB inhibitors in zebrafish embryos. From small angle x-ray scattering we were able to obtain an overall shape of USP14 to get an idea how the two domains are related to each other and by using NMR experiments we assigned the Ubl domain and performed relaxation experiments in ps-ns and ms time frame to investigate differences of relaxation rates between the isolated Ubl domain and the Ubl domain in full-length USP14.

The 8-oxo-dGTP hydrolase NUDT1 from *Arabidopsis thaliana* also utilizes non-nucleotide monoterpene substrates

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Arabidopsis thaliana NUDT1 (AtNUDT1) belongs to the Nudix family of proteins, which have a diverse range of substrates, including oxidized nucleotides (1). The hydrolysis of oxidized dNTPs is highly important as it prevents their incorporation into DNA, thus preventing mutations (2). AtNUDT1 is the sole Nudix enzyme from *A. thaliana* shown to have activity against 8-oxo-dGTP, a common oxidized nucleotide (3). The structure of AtNUDT1 in complex with 8-oxo-dGTP was solved *via* X-ray crystallography. Structural comparison with homologues from bacteria and humans revealed a conserved overall fold. Analysis of the 8-oxo-dGTP binding mode indicated that the residues Asn76 and Ser89 interact with the O8 group of the substrate, a feature not observed in related structures solved to date. Kinetic analysis of wild-type and mutant AtNUDT1 confirmed these active site residues to have an influence on 8-oxo-dGTP hydrolysis. In addition to nucleotide substrates, a recent study showed AtNUDT1 is also able to hydrolyse monoterpene compounds. In this study, the kinetic parameters for the monoterpene GPP and several precursors were determined, which indicated that AtNUDT1 actually hydrolyses monoterpene substrates much more efficiently than 8-oxo-dGTP. The diversity of reactions catalysed by AtNUDT1 suggests that this Nudix enzyme from higher plants has evolved in a manner distinct to its homologues from both humans and bacteria.

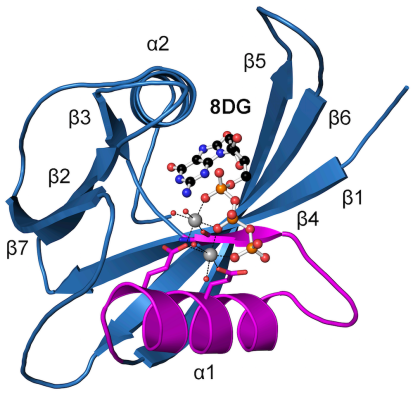

Figure 1. Crystal structure of the AtNUDT1 8-oxo-dGTP complex. Ribbon representation of the AtNUDT1 monomer. The secondary structure elements, α-helices (1-2) and β-strands (1-7) are labelled. The highly conserved Nudix motif is coloured magenta. The 8-oxo-dGTP (8DG) ligand is depicted as a ball-and-stick model. Magnesium ions involved in ligand coordination are shown as grey spheres.

References:

1. Bessman, M. J., Frick, D. N., and O'Handley, S. F. (1996) The MutT proteins or "Nudix" hydrolases, a family of versatile, widely distributed, "housecleaning" enzymes. *J Biol Chem* **271**, 25059-25062
2. Haghdoost, S., Sjolander, L., Czene, S., and Harms-Ringdahl, M. (2006) The nucleotide pool is a significant target for oxidative stress. *Free Radic Biol Med* **41**, 620-626
3. Ogawa, T., Ueda, Y., Yoshimura, K., and Shigeoka, S. (2005) Comprehensive analysis of cytosolic nudix hydrolases in *Arabidopsis thaliana*. *J Biol Chem* **280**, 25277-2528

Structural and functional characterization of Gram+ T4SS adhesion components

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

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

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

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

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

Characterization of aquaporin-driven hydrogen peroxide transport

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Aquaporins are membrane-intrinsic proteins initially defined as water (H_2O) transporting channels in all organisms and subsequently found to have many other substrate specificities, such as hydrogen peroxide (H_2O_2). H_2O_2 is a signaling molecule that partakes in immune responses where its transport is mediated by aquaporins. To shed further light on the molecular basis of the aquaporin function in H_2O_2 transport, we have characterized an *Arabidopsis thaliana* aquaporin, AtPIP2;4, recombinantly produced to high yields in *Pichia pastoris*. Here, we present a newly established assay that allows detection of H_2O_2 transport by purified aquaporins reconstituted into liposomes, enabling us to compare aquaporin homologues with respect to substrate specificity. To get additional insight into the structural determinants for aquaporin-mediated H_2O_2 transport, we solved the 3D-structure of AtPIP2;4 to 3,7 Å resolution and found structural identity to that of SoPIP2;1. The transport specificities of the two plant aquaporins were compared to a human homologue, AQP1. Overall, we conclude that AtPIP2;4, SoPIP2;1 and hAQP1 are all transporters of both H_2O and H_2O_2, but have different efficiencies for various specificities. Notably, all three homologues expedite H_2O transport equally well while the plant aquaporins are more permeable to H_2O_2 than hAQP1. Comparison of the structures indicates that the observed variations in H_2O and H_2O_2 transport cannot be explained by differences in the monomeric pore. Presumably, the determinants for transport specificities reside in the flexible domains outside the membrane core of these channels.

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

Kajsa Sigfridsson Clauss, Ann Terry, Dörthe Haase, Yang Chen, Ross Friel* and Laurent Barbe**

MAX IV Laboratory Lund University, *Halmstad Högskola, ** Uppsala University

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

The project team and expert partners have together competence in bio-X-ray techniques, in microfluidic chip manufacturing (Customized Microfluidics facility, Uppsala University), lab-on-a-chip integration, protein production, microfluidic device usage and time-resolved studies to execute the three year project. Year-one goal, the delivery of integrated microfluidic device for XAS, following up year two with add-on UV-vis spectroscopy lab-on-chip AdaptoCell-XAS and AdaptoCell-SAXS for time-resolved data collection, and year three AdaptoCell-SSX. We expect to release devices to Swedish academic and industry users for sample delivery after each finished development stage.

Project status will be presented with the first test results from Balder.

Activity and asymmetry in the permanent respiratory supercomplex

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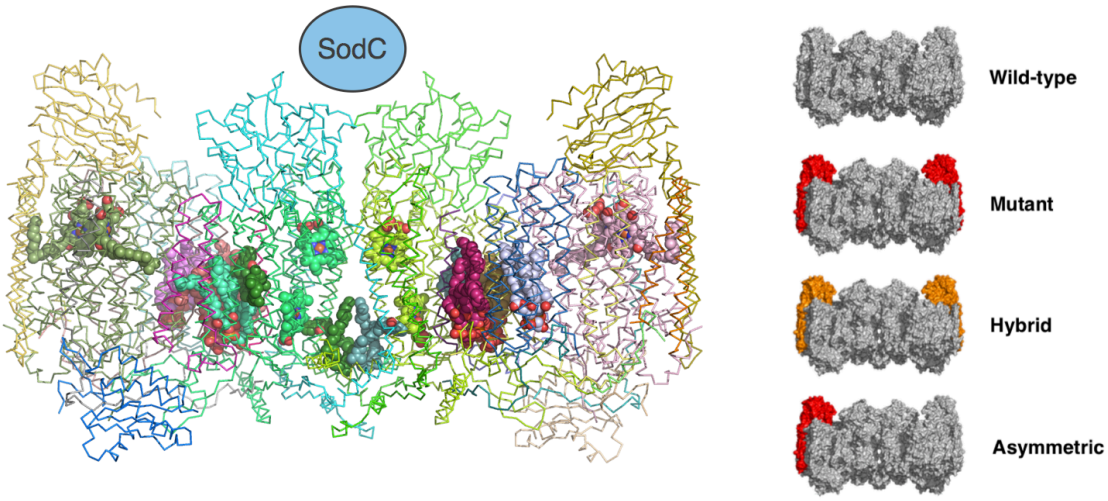
Cellular respiration provides energy for life's chemical reactions, and the respiratory chain complexes are at the heart of this process. It was recently found that they assemble into even larger *supercomplexes*, which appear to be permanent in many species of *Actinobacteria*. We recently determined the structure of one such permanent supercomplex, from *Mycobacterium smegmatis,* which reveals a completely new, asymmetric architecture with novel subunits and features. The membrane-bound Cytochrome c-like protein *QcrC* adopts radically different conformations in the two halves of the supercomplex, potentially acting as an electrical switch. At least two hitherto unknown subunits are located close to a novel potential proton pathway on the cytoplasmic side, possibly regulating the activity of the complex. Intriguingly, a periplasmic superoxide dismutase molecule is also attached to the complex, for reasons unknown.

This complex encompasses the entire respiratory process of electron flow from quinones to oxygen and proton translocation across the membrane. We are currently studying the details of this process, to render a more complete picture of how respiratory supercomplexes work and why they are permanently assembled in *Actinobacteria*. We will use asymmetric mutants to investigate how asymmetry affects activity *in vitro* and *in vivo*, and the functions of the novel subunits are being explored using mutant complexes and knockout strains. Moreover, we are trying to find out if the attached SodC is important for ROS protection or energy conservation (e.g. by the recycling of ROS electrons). We will also make homology models of supercomplexes from related species – including *M. tuberculosis* – and study the function of these homologues using hybrid complexes (with the *M. smegmatis* supercomplex as a scaffold).



Wiseman B, Nitharwal RG, Fedotovskaya O, Schäfer J, Guo H, Kuang Q, Benlekbir S, Sjöstrand D, Ädelroth P, Rubinstein JL, Brzezinski P, Högbom M.; Nat Struct Mol Biol. 2018 Dec;25(12):1128-1136.

Crystalisation of rhomboid membrane protease GlpG in lipidic cubic phase

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Rhomboid intramembrane protease GlpG from *E. coli* is the main model for structural and mechanistic studies of all rhomboids. This enzyme comprises 6 transmembrane helices (TM) and a soluble N-terminal domain. The catalytic residues S201 and H254 are located on TM4 and TM6, respectively, and the active site is fully emerged in the membrane [1]. All structural information about GlpG is based on X-ray structures obtained in micellar detergents. Therefore, a GlpG structure providing insight into the enzyme-substrate interaction mechanism in a more native lipidic environment is highly demanded.

Biological membranes are a complex environment which, by lipid composition, can dramatically affect protein properties like oligomeric state, enzymatic activity, or stability, which is crucial for successful protein crystallization. We have investigated the effect of several different lipids on GlpG stability using an engineered thermal-shift screen [2] (Figure 1) and identified lipids with stabilization potency to GlpG. We have implemented this information in lipidic cubic phase crystallization trials and obtained GlpG crystals (Figure 2) as a starting point for crystallization optimization and structure determination

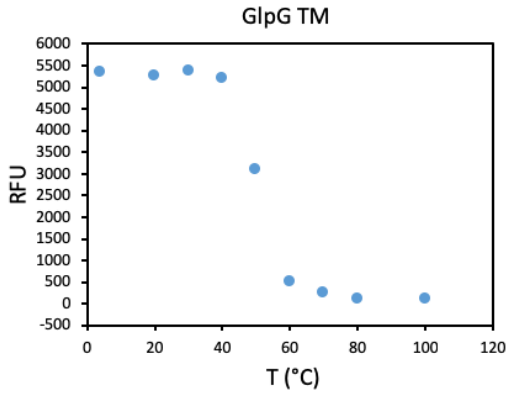

Figure 1: Determination of melting temperature (TM) of GlpG

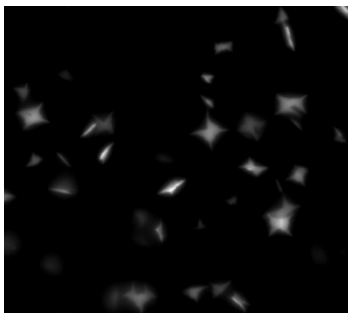

Figure 2: GlpG crystals in LCP visualized by UV light

References

1. Zoll et al., Substrate binding and specificity of rhomboid intramembrane protease revealed by substrate-peptide complex structures, EMBO J. 2014

2. Nji et al., An engineered thermal-shift screen reveals specific lipid preferences of eukaryotic and prokaryotic membrane proteins, Nature Communications 2018

Acknowledgement: The work of Jan Skerle was supported from ERDF/ESF project "UOCHB Mobility" (No. CZ.02.2.69/0.0/0.0/16_027/0008477) granted to the Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences.

Structural studies of human PAICS

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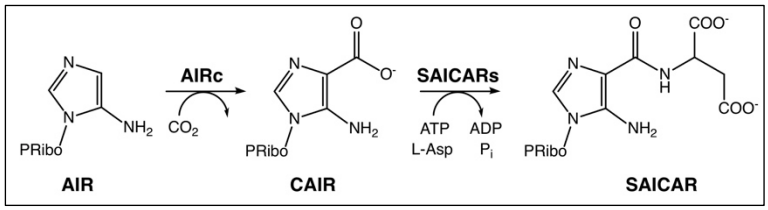
PAICS catalyzes two essential steps in *de novo* purine biosynthesis and is a promising target for the development of cancer therapeutics [1]. We have solved crystal structures of the octameric human enzyme PAICS in complexes with its native ligands.

The bifunctional human enzyme PAICS catalyzes two reactions: The carboxylation of 5-aminoimidazole ribonucleotide (AIR) and the subsequent ATP-dependent conversion of 4-carboxy-5-aminoimidazole ribonucleotide (CAIR) and L-aspartate to 4-(*N*-succinylcarboxamide)-5-aminoimidazole ribonucleotide (SAICAR) [2]. Currently, structural information on this enzyme is limited. Only a single structure of PAICS has been published so far, without any ligand bound [3]. We have determined structures of PAICS with CAIR bound in both active sites (Fig. 1), and with SAICAR and the ATP analogue, AMP-PNP, occupying the SAICAR synthetase active site. These structures provide insight into substrate and/or product binding and the architecture of the two active sites of PAICS and this structural information will assist future rational design of inhibitors.



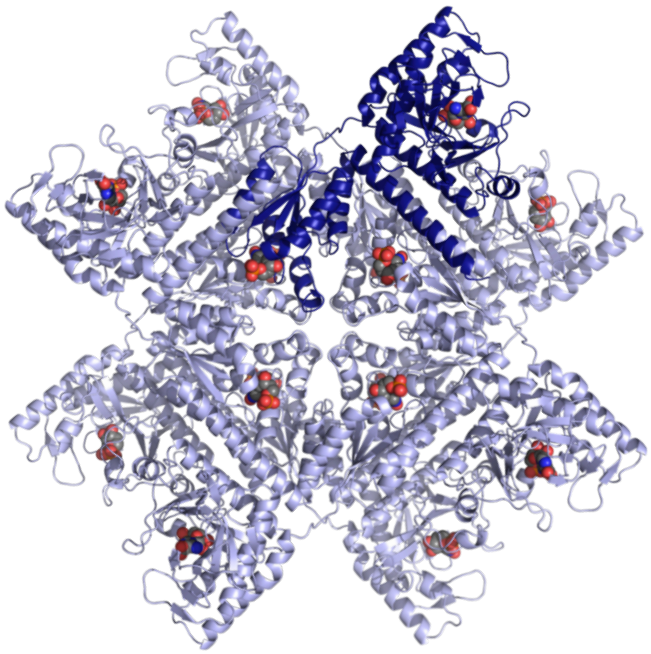


Figure 1: Human PAICS octamer in complex with CAIR.

References:
1. Yin *et al.*, Front. Immunol. (2018) 9, 1697.
2. Lukens and Buchanan, J. Biol. Chem. (1959) 234, 1799-1805.
3. Li *et al.*, J. Mol. Biol. (2007) 366, 1603-1614.

Acknowledgements:
This work was supported by grants from the Swedish Research Council and the Swedish Cancer Society to P.S. The work of J.S. was supported from ERDF/ESF project "UOCHB Mobility" (No. CZ.02.2.69/0.0/0.0/16_027/0008477) granted to the Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences.

Interplay between Conformational Entropy and Solvation Entropy in Protein−Ligand Binding

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Understanding the driving forces underlying molecular recognition is of fundamental importance in chemistry and biology. The challenge is to unravel the binding thermodynamics into separate contributions and to interpret these in molecular terms. Entropic contributions to the free energy of binding are particularly difficult to assess in this regard. Here we pinpoint the molecular determinants underlying differences in ligand affinity to the carbohydrate recognition domain of galectin- 3, using a combination of isothermal titration calorimetry, X-ray crystallography, NMR relaxation, and molecular dynamics simulations followed by conformational entropy and grid inhomogeneous solvation theory (GIST) analyses. Using a pair of diastereomeric ligands that have essentially identical chemical potential in the unbound state, we reduced the problem of dissecting the thermodynamics to a comparison of the two protein−ligand complexes. While the free energies of binding are nearly equal for the R and S diastereomers, greater differences are observed for the enthalpy and entropy, which consequently exhibit compensatory behavior, $\Delta\Delta H°(R - S) = -5 \pm 1$ kJ/mol and $-T\Delta\Delta S°(R - S) = 3 \pm 1$ kJ/mol. NMR relaxation experiments and molecular dynamics simulations indicate that the protein in complex with the S-stereoisomer has greater conformational entropy than in the R-complex. GIST calculations reveal additional, but smaller, contributions from solvation entropy, again in favor of the S-complex. Thus, conformational entropy apparently dominates over solvation entropy in dictating the difference in the overall entropy of binding. This case highlights an interplay between conformational entropy and solvation entropy, pointing to both opportunities and challenges in drug design.

Dynamics governing DegP protease-chaperone function

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Protein quality control is an essential function in every living cell and it is mainly executed by a large array of proteases and molecular chaperones. Dysregulation of these important cellular components may lead to cell death and is often associated with diseases such as cancer, age-related disorders or neurodegenerative diseases like Parkinson's or Alzheimer's diseases [1]. The HtrA protein family (high temperature requirement A) is a group of heat-shock inducible serine proteases, which are widely spread across all kingdoms of life [2]. One of the bacterial HtrA protein family members is the homo-oligomeric DegP-protease, playing a crucial role in the biogenesis and degradation of β-barrel outer-membrane proteins within the periplasmic space [3]. Although the role of DegP under heat shock conditions is well characterized on a genetic and biochemical level [4], its functional, structural as well as dynamical details remain poorly understood.

The aim of our study is to delineate the origin of molecular changes of different DegP oligomeric states at the atomic level by using advanced solution NMR techniques to obtain a detailed insight of this protein function. This increased knowledge may enable us to understand DegPs role in the assembly and maintenance of bacterial integral outer membrane proteins. Since DegPs size can vary from 50 kDa to 1 MDa, sophisticated isotope labeling schemes are used to obtain high-resolution NMR spectra and derive structural and dynamical information.

Different DegP variants were successfully expressed, purified and investigated by solution NMR spectroscopy. Based on the initial results, it was possible study the substrate binding PDZ domains of DegP within the active hexameric variant. Current efforts are ongoing to understand the dynamical properties of these domains and how they influence the oligomeric assembly as well as the substrate transfer to the peptidase domain. To correlate our investigation to the physiological context, initial results of our efforts to study the DegP protein in its native habitat, the periplasmic space, by employing bacterial outer membrane vesicles will be presented.

[1] F. U. Hartl, A. Bracher, and M. Hayer-Hartl, "Molecular chaperones in protein folding and proteostasis," *Nature*, vol. 475, no. 7356, pp. 324–332, 2011.

[2] T. Clausen, M. Kaiser, R. Huber, and M. Ehrmann, "HTRA proteases: regulated proteolysis in protein quality control," *Nat. Rev. Mol. Cell Biol.*, vol. 12, no. 3, pp. 152–162, 2011.

[3] J. G. Sklar, T. Wu, D. Kahne, and T. J. Silhavy, "Defining the roles of the periplasmic chaperones SurA, Skp, and DegP in Escherichia coli," *Genes Dev.*, vol. 21, no. 19, pp. 2473–2484, 2007.

[4] S. Kim and R. T. Sauer, "Distinct regulatory mechanisms balance DegP proteolysis to maintain cellular fitness during heat stress," *Genes Dev.*, vol. 28, no. 8, pp. 902–911, 2014.

Structural characterisation of the catalytic domain of botulinum neurotoxin F5

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Botulinum neurotoxins (BoNTs) are highly potent neurotoxins, produced by members of the bacterial class *Clostridia*. There are eight established serotypes (BoNT/A-G, X) that are composed of more than 40 subtypes. BoNT/F, a serotype that rarely causes botulism (<1% of cases), was first described in 1960. Recently, based on phylogenetic analysis, seven subtypes (F1-F7) of BoNT/F were identified. All BoNTs share a similar architecture that allow them to block neurotransmission at the neuromuscular junction, through their proteolytic activity on one of the intracellular SNARE proteins. BoNT/F was shown to display distinct enzymatic cleavage sites between its subtypes. More precisely, subtype F5 cleaves VAMP2 between residues L54-E55, which differs from all other subtypes that cleave VAMP2 between residues Q58-K59 [1]. The cleavage site of BoNT/F5 is the same as that of BoNT/FA (also known as BoNT/H or BoNT/HA), which is a recently described mosaic neurotoxin that displays 81% sequence identity to the catalytic domain of BoNT/F5 [2], [3]. Here, we report the 2.0 Å crystal structure of the BoNT/F5 catalytic domain, a zinc-peptidase. Despite the high sequence identity with LC/FA, LC/F5 presents unique features that suggest variation in the mechanism of substrate recognition. The structural information presented here will help our understanding of substrate specificity between BoNTs, and the evolution of these toxins.

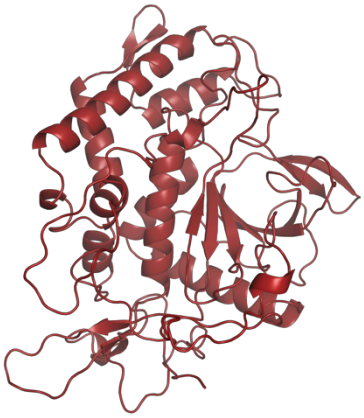

Figure 1 – 2 Å crystal structure of the BoNT/F5 light chain.

[1] J. Guo, E. W. C. Chan, and S. Chen, "Mechanism of substrate recognition by the novel Botulinum Neurotoxin subtype F5," *Sci. Rep.*, vol. 6, no. December 2015, pp. 1–9, 2016.

[2] G. Hackett *et al.*, "Purification and characterization of recombinant botulinum neurotoxin serotype FA, also known as serotype H," *Toxins (Basel).*, vol. 10, no. 5, 2018.

[3] K. H. Lam *et al.*, "Structural and biochemical characterization of the protease domain of the mosaic botulinum neurotoxin type HA," *Pathog. Dis.*, vol. 76, no. 4, pp. 1–10, 2018.

Secretagogin interactome conveys calcium signaling of insulin release, β-cell identity and survival

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Calcium-binding proteins (CBPs) are essential mediators of intracellular calcium (Ca^{2+}) concentration-dependent cell signalling. Extracellular stimuli driven transient Ca^{2+} spikes allow precise modulation of CBPs, so called Ca^{2+} sensors. Upon binding, they undergo conformational changes enabling formation of protein-protein interactions which assure rapid, dynamic decoding of short-lived intracellular Ca^{2+} signals.

Secretagogin (SCGN), a hexa EF-hand Ca^{2+} sensor, has been shown to be predominantly expressed in the central nervous system and in endocrine glands playing crucial role in hormone and neuropeptide secretion. It is one of the most abundant proteins of endocrine pancreatic β-cells and suggested to regulate insulin release via interaction with cytoskeletal proteins and components of vesicle-mediated trafficking. Accordingly, SCGN loss-of-function correlates with decreased insulin secretion and leads to diabetic phenotype.

Ca^{2+}-dependent SCGN interaction network in pancreas was investigated to provide a more comprehensive picture on SCGN-mediated signal transduction. In INS-1E cells the analysis revealed 13 proteins involved in protein folding including members of the chaperonin containing T complex. Moreover, we detected enzymes of deubiquitination such as ubiquitin carboxyl-terminal hydrolase USP9X and USP7. These findings pinpoint abnormal protein folding and degradation with subsequent β-cell loss that may explain impaired insulin secretion in SCGN knock downs (1). In a second study, we described SCGN interacting proteins in mouse embryonic pancreas and identified subunits of the 26S proteasome complex. Ca^{2+}-dependent interaction of SCGN with the 26S proteasome modulates proteasome activity which determines availability of transcription factors defining β-cell identity (2).

Taken together, our results indicate SCGN to be a pivotal molecular hub in many fundamental cellular processes conveying Ca^{2+} signals in the endocrine pancreas. Furthermore, we address that detailed one on one analysis of putative partner proteins has the potential to depict molecular background and functional consequences of these interactions.

References:

1. Malenczyk et al. EMBO J. 2017 Jul 14;36(14):2107-2125.
2. Malenczyk et al. Mol Metab. 2018 Aug;14:108-120.

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

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

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

References:

Smith, B. O. H. and Yanofsky, C. (1962) 'Enzymes Involved in the Biosynthesis of Tryptophan', Methods Enzymol., 5, pp. 794–806.

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

Structure-function characterization of phage-encoded SAM hydrolase enzymes

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S-adenosyl methionine (SAM) acts as a co-substrate in a great number of metabolic reactions, including the methylation of nucleic acids, histones, phospholipids, small molecules and synthesis of polyamines.

We are interested in a phage-encoded enzyme family that can hydrolyse SAM into S-methyl-5-thioadenosine (MTA) and homoserine (EC 3.3.1.2). The first SAM hydrolase, from phage T3, was discovered 50 years ago and only partly characterized (Hausmann, 1967; Spoerel & Herrlich, 1979). The enzyme was proposed to have a role in the protection of the phage against the type I restriction modification (RM) system by hydrolysing the essential cofactor needed by restriction endonucleases.

Recently, a set of three phage-encoded enzymes, that in an un-predicted manner could rescue an *Escherichia coli ilvA* knock-out un-able to synthesize isoleucine, were discovered (Jerlström Hultqvist et al., 2018). The mechanism of rescue by SAM hydrolases Svi3-3, Svi3-7 and Orf1 was found to be an up-regulation of the genes involved in methionine biosynthesis when SAM was degraded. This allowed a promiscuous activity of MetB (Cystathionine gamma-synthase) to provide the cells with the missing essential metabolite.

Enzymes Svi3-3, Svi3-7 and Orf1 showed very low sequence similarity to the T3 SAM hydrolase as well as to each other. To characterize the kinetics of Svi3-3, a discontinuous assay was developed where substrate (SAM) and product (MTA) were separated on a cation exchange column and quantified at 260 nm.

We now aim to further characterize the structure, function and mechanism of this enzyme family. Recent progress in expression and purification of toxic SAM-degrading proteins and in their kinetic characterization will be presented.

References

Hausmann, R. (1967). Synthesis of an S-Adenosylmethionine-cleaving Enzyme in T3-infected Escherichia coli and Its Disturbance by Co-infection with Enzymatically Incompetent Bacteriophage. *Journal of Virology*, *1*(1), 57–63.

Jerlström Hultqvist, J., Warsi, O., Söderholm, A., Knopp, M., Eckhard, U., Vorontsov, E., … Andersson, D. I. (2018). A bacteriophage enzyme induces bacterial metabolic perturbation that confers a novel promiscuous function. *Nature Ecology and Evolution*, *2*(8), 1321–1330. https://doi.org/10.1038/s41559-018-0568-5

Spoerel, N., & Herrlich, P. (1979). Colivirus-T3-Coded S-Adenosylmethionine Hydrolase. *European Journal of Biochemistry*, *95*(2), 227–233. https://doi.org/10.1111/j.1432-1033.1979.tb12957.x

News from the BioMAX beamline

Johan Unge, Jie Nan, Mikel Eguiraun, Ana Gonzalez, Roberto Appio, Mirko Milas, Ross Friel, Chris Ward, Robert Lizatovic, Gustavo Lima, Anastasya Shilova, Johan Eckdahl, Thomas Ursby and Uwe Müller.

MAX IV, Lund University, Lund Sweden.

BioMAX is still in commissioning phase and at the same time accepting users on a regular but limited basis. Basic functionalities have during the last year stabilised and collecting single crystal data from visible crystals ideally from 100 um down to 5 um at energies between 6 and 20 keV is now routine. The design as a high-throughput beamline includes the sample changer which takes samples in Unipuck using SPINE standard pins only, and samples is annotated in ISPyB, which organises both samples and reduced data for the online user. Automatic data processing is an integrated service for high-throughput data collection for which we can access the MAX IV cluster with more than 1200 cores.

The state-of-the-art construction including an Eiger 16M detector running in shutterless mode and MD3 diffractometer on a fourth generation synchrotron X-ray source is an excellent setup for providing data with outstanding quality with all parameters fine-tuned. Radiation damage has to be properly dealt with however during data collection and currently efforts are spent on analysing both electron beam and X-ray beam stabilities in order to find optimal parameters for data collection.

Several development projects are ongoing including remote operation, grid scans, flourescence energy scans, in-line humidifier and more with the aim of providing these to the user community shortly. Also beamtime accessibility is currently revised with the aim of providing more beamtime in the near future to both the academic and industrial communities. Work has started and first experiments has been initiated in order to launch a fragment screening facility in the near future in collaboration with the proteins production platform LP3.

Although BioMAX is designed for high-througput experiments, the beam properties also makes it suitable for serial crystallography experiments and first experiments have been performed with the aim of applying the technique for MicroMAX within a few years, which is currently being designed.

Expanding The Domain Of Serial Crystallography: Membrane Protein & In Cellulo Crystallization

Owens Uwangue[1], Gisela Brändén[1], Richard Neutze[1], Lars Redecke[2] Matthias Wilmanns[3], Thomas R. Schneider[3], Thomas Ursby[4], Alke Meents[5], and Adrian P. Mancuso[6]

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Structural biology aims to elucidate at atomic resolution the three-dimensional structure of biological macromolecules such as proteins, RNA and DNA. X-ray structures of macromolecules have made a pivotal contribution in understanding enzyme catalysis, substrate selectivity, transcription and translation, energy transduction, protein-protein interactions and signal propagation. Protein structures are also of the utmost importance for rational drug design and almost all pharmaceutical and biotech companies use macromolecular structures to facilitate their research. The throughput and impact of macromolecular crystallography has grown over the last three decades in close collaboration with the increasing brilliance of synchrotron radiation. The emergence of X-ray free electron lasers (XFELs) a decade ago, along with the development of serial femtosecond crystallography (SFX), has allowed for useful diffraction data to be collected from a continuous stream of microcrystals that would usually be too small for data collection using standard synchrotron-based methods. SFX has opened up a broad range of new scientific opportunities such as time-resolved serial crystallography, data collection from in cellulo grown micro-crystals, and has lowered barriers to very challenging problems such as the structural biology of G protein-coupled receptors. An unavoidable caveat is that all serial crystallography approaches rely upon the growth of microcrystals and their efficient delivery across an X-ray beam. With a collaborative effort with 5 other institutions across europe, we aim to strengthen this rapidly advancing sub-field of structural biology research by: Developing advanced approaches for the growth of microcrystals from a variety of samples well suited for serial crystallography studies, streamlining efficient delivery of microcrystals for diffraction studies at state-of-the-art X-ray sources within the Röntgen-Ångstrom Cluster (RÅC) region, and training the scientific community to take advantage of these new scientific opportunities created by these powerful tools at the large-scale instruments supported by RÅC.

Superantigen binding to human cytokine receptor

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Lund University

Superantigens are bacterial toxins that are known to bind to the T cell receptor and cause massive T cell activation and cytokine production, that may lead to food poisoning and toxic shock syndrome. Recently, we discovered that superantigens also can bind directly to the signalling domain of human cytokine receptors, called gp130 (1). The biological outcome of this interaction is yet to be discovered.

To resolve the structural details of the supernatigen-gp130 complex, we have performed mass spectrometry cross-linking (MS-XL) analysis to identify crucial residues for the complex formation and we have investigated the binding affinity applying surface plasmon resonance (SPR).

Our SPR-results show that the binding is specific to the human gp130, as we cannot detect binding of superantigen to the mouse and rat variant of gp130. The affinity of the complex is approximately 0.6 µM. We also show that the superantigen use another binding site than the well-known gp130 activator, human leukaemia inhibitory factor (LIF), as LIF and the superantigen do not compete for binding to gp130. Our preliminary MS-XL results show that the superantigen binds to the end of domain 3 of the human cytokine receptor, while LIF binds between domain 2 and domain 3 (2). Based on these results we have generated a computational model of the complex between superantigen and gp130.

1. Banke E, Rodstrom K, Ekelund M, Dalla-Riva J, Lagerstedt JO, Nilsson S, et al. Superantigen activates the gp130 receptor on adipocytes resulting in altered adipocyte metabolism. Metabolism: clinical and experimental. 2014;63(6):831-40.
2. Boulanger MJ, Bankovich AJ, Kortemme T, Baker D, Garcia KC. Convergent mechanisms for recognition of divergent cytokines by the shared signaling receptor gp130. Mol Cell. 2003;12(3):577-89.

UrdA: structural analysis of a novel enzyme

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

UrdA protein construct consisting of a FAD binding and a mobile domain was successfully expressed, purified and crystallized. Two high-resolution X-ray structures were obtained, one with no substrate but with ADP bound, and one in complex with urocanate and FAD. The data reveals the overall structural arrangement of the enzyme as well as the substrate binding mode.

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

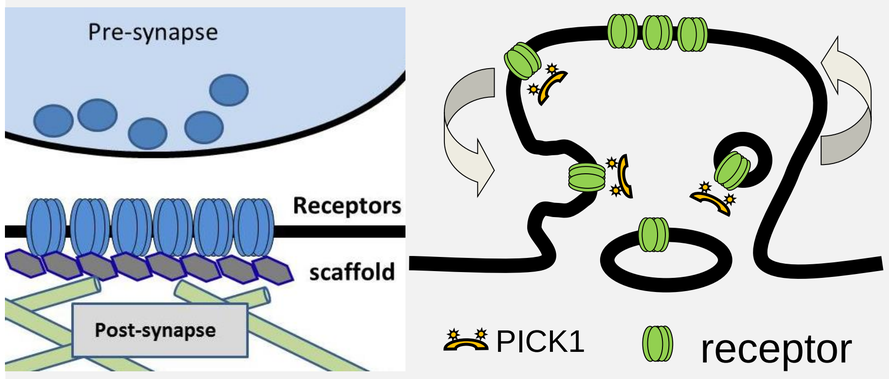
References

1. Bogachev, A. V. et al. (2012), Urocanate Reductase: Identification of a Novel Anaerobic Respiratory Pathway in *Shewanella oneidensis MR-1*. *Molecular Microbiology*, 86: 1452-1463.
2. Koh A. et al. (2018) Microbially Produced Imidazole Propionate Impairs Insulin Signaling through mTORC1. *Cell* 175: 947-961.

Visualizing of PICK1 complexes at the membrane interface by Cryo-electron microscopy

Zuoneng Wang Andreea Scacioc Carsten Mim*

Overview



Chemical synapse are the most specific structures to transfer and receive information from one cell to the next in neural system, during this process, scaffold proteins have a crucial role in trafficking synaptic receptors in and out of membrane.

PICK1(Protein interacting with C kinase1) is a modular scaffolding protein containing a PDZ domain, a BAR domain, and an acidic C-terminal tail (ACT). it regulates the synaptic targeting and surface expression of many important neuronal proteins, for example, the GluA2 subunit of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPAR). Disruption of this precise regulation will lead to a range of neurological diseases such as Alzheimer's, Huntington's, and brain ischemia.

Numerous studies show that PICK1 interacts with diverse proteins through its PDZ while the BAR domain is mainly responsible for curvature sensing and the ACT tail for the negative regulation of binding. However, the mechanism by which PICK1 regulates protein trafficking and how its governing the assembly of complex by different modular on membrane interface remains unclear.

Cryo-electron microscopy has emerged as a powerful tool to visualize membrane proteins, their scaffolds and their interactions with the bilayer, in this study we will focus on the complex formed by PICK1 and its binding partners and trying to illuminate the regulate mechanism at atomic range.

Purpose

- Measure PICK1 PDZ domain bind affinity on lipid bilayer with different components by Biacore or other biology techniques.
- Detect BAR domain curvature sensing property and regulating function of the ACT tail when PICK1 interact with ligands or lipid membranes.
- Investigate the mechanism of synaptic trafficking in vitro by visualizing complexes of PICK1 (with and without binding partners) on the lipid bilayer using Cryo-electron microscopy.

Method

Sample preparation:

Recombinant proteins were expressed and purified in BL21 (DE3)Gold Escherichia coli cells with an N-terminal fused GST tag or without tag but stabilized by detergent, followed a size-exclusion chromatography was implemented.

Biochemical assay:

Surface plasmon resonance technique (Biacore) was used to detect the binding preference and affinity of PDZ domain on liposome with varies of lipid components. the binding cooperation of PICK1 with different binding ligands on lipids was also been studied.

Biophysical test

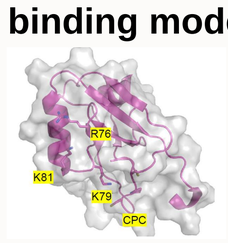
For PICK1 BAR domain curvature sensing property and the regulating function of ACT tail, we utilized a series of sedimentation, tabulation and KcSA pull down experiments to detect those properties, in the meanwhile we screened and optimized the component of lipid biolayer for further structure study.

Structure study

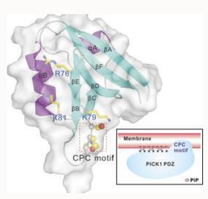
Negative staining and cryo-EM imaging of PICK1 complex on lipid membrane and then process the structure information data to improve the current understanding of PICK1 in vitro.

Result

PDZ domain binding modelling



PDB 2lui



Pan L et al, EMBO J, 2007

The membrane binding of PDZ domain is mediated by a conserved hydrophobic Cys-Pro-Cys motif (CPC loop).

Paralleling with the CPC loop, existing a patch of positively charged residues (Arg76, Lys79, and Lys81) at the local region which mainly responsible for the ligands binding.

Generally, three classes of PDZ ligands are all adopting a distinguish interacting pattern.

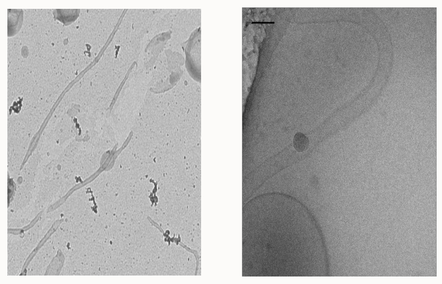
The PDZ domain also prefer to bind to PIP containing lipids by insert the CPC loop into the membrane.

Two membrane-interacting motifs located at distinct sides of the peptide-binding grove which were not structurally overlap with the peptide binding sites.

Curvature sensing and Sedimentation assay

A: Negative stain image of lipid nano tubes decorated with Triton X-100 stabilized PICK1
B: PICK1 co-sedimented with the nanotubes (arrow) when and tested by SDS-PAGE

Tubulation assay



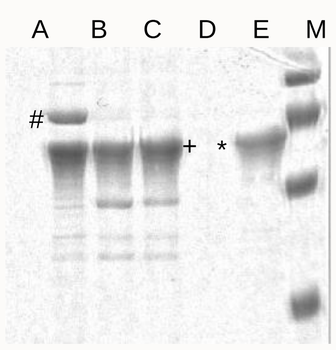
Left:

Negative stain micrograph of membrane tubules formed by GST-PICK1 fusion protein

Right:

The same tubules were imaged by cryo-EM (Scale bar 600nm (left) and 100nm)

PICK1 pull down test



Pull down of PICK1 by a KcSA fusion protein that contains the PDZ motif from the dopamine transporter (KcSA-DAT):
The proteins were characterised by Coomassie Blue stained SDS-PAGE. KcSA is known to be resistant to SDS and runs in this gel as a tetramer (*). We tested the binding of Triton X-100 stabilized PICK1 wt (+) and GST-PICK1 (#). All samples contain metal affinity resin: Lanes E: Only KcSA-DAT; D: only PICK1; C+B: PICK1 + KcSA; A: GST-PICK1+KcSA-DAT. The pull down shows that both PICK1 constructs co-sediment with the membrane protein

Outlook

- Focus on the structure of PICK1 membrane complex, we will use optimized liposomes or lipid nanotubes to assemble the protein on the membrane.
- Focus on the structure of a complex of pick1 with a membrane protein, the purified potassium channel KcSA, which has be fused with a short binding motif for PICK1 PDZ domain, is our simplified binding partner, the complex between PICK1 and KcSA will be reconstituted in different membrane systems.

References

Herlo et al., Cell Reports,2018, 23, 2056–2069
Karlsen et al., Structure 2015,23,1258–1270
W. Jin, et al, J Neurosci. 2006,26,2380-2390
Egea AL et al, Nature Communications, 2016

Acknowledgments

The project is supporting from the Human Frontier Science Program (CM). The China Scholarship Council (CSC)

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Structural and Functional Characterization of PLA2G16

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The Picornavirus family is a large group of non-enveloped RNA viruses, which can cause a wide range of biomedically important human and animal diseases, such as polio or the common cold. During infection, the picornaviruses depend upon cellular proteins, so called host factors, in order to replicate and complete their viral life cycle. Due to the high mutation rates and rapid evolution of viral proteins, host factors, which are non-essential for the cell, are attractive as alternative targets in the development of novel antiviral therapies.

PLA2G16 is a small, cytosolic phospholipase, which recently was identified as a host factor required for viral infection of certain enteroviruses.[1] PLA2G16 is involved early in the viral life cycle and facilitates genome delivery to the cytoplasm. Its role as a host factor is reliant on its catalytic activity. Therefore, cells lacking the catalytic function of PLA2G16 (either through mutation or inhibition) should be resistant against these certain viral infections.

PLA2G16 is composed of an N-terminal catalytic domain, followed by a C-terminal hydrophobic region. Prior structural studies have revealed that the N-terminal globular domain houses a conserved catalytic triad in an arrangement compatible with catalysis.[2,3,4] The C-terminal hydrophobic region does not appear to be constitutively membrane associated, but rather is involved in the recruitment of PLA2G16 to sites of membrane damage. This function of the C-terminus appears to be crucial for the catalytic function of the enzyme.[1,2]

In this study, we aim to clarify the role of the C-terminal region. To gain a better understanding of PLA2G16'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 activity assays to probe the catalytic function of PLA2G16.

References:

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

2. Pang X., Cao J., Addington L., Lovell S., Battaile K. P., Zhang N., Rao J. L. U. M., Dennis E. A., Moise A.R. *Structure/Function Relationships of Adipose Phospholipase A2 Containing a Cys-His-His Catalytic Triad* Journal of Biological Chemistry, **287(42)**, 35260-74 (2012)

3. Golczak M., Kiser P. D., Sears A. E., Lodowski D. T., Blaner W. S., Palczewski K. *Structural basis for the acyltransferase activity of lecithin: retinol acyltransferase-like proteins.* Journal of Biological Chemistry, ***287(28)***, 23790-807 (2012)

4. Ren X., Lin J., Jin C., Xia B. *Solution structure of the N-terminal catalytic domain of human H-REV107 – A novel circular permutated NlpC/P60 domain* FEBS Letters, **584(19)** 4222-26 (2010)

Structure of a functional obligate complex III_2IV_2 respiratory supercomplex from *Mycobacterium smegmatis*

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In the mycobacterial electron-transport chain, respiratory complex III passes electrons from menaquinol to complex IV, which in turn reduces oxygen, the terminal acceptor. Electron transfer is coupled to transmembrane proton translocation, thus establishing the electrochemical proton gradient that drives ATP synthesis. We isolated, biochemically characterized, and determined the structure of the obligate III_2IV_2 supercomplex from *Mycobacterium smegmatis*, a model for *Mycobacterium tuberculosis*. The supercomplex has quinol:O_2 oxidoreductase activity without exogenous cytochrome c and includes a superoxide dismutase subunit that may detoxify reactive oxygen species produced during respiration. We found menaquinone bound in both the Q_o and Q_i sites of complex III. The complex III-intrinsic diheme cytochrome cc subunit, which functionally replaces both cytochrome c_1 and soluble cytochrome c in canonical electron-transport chains, displays two conformations: one in which it provides a direct electronic link to complex IV and another in which it serves as an electrical switch interrupting the connection.

Dual Roles of Residues in Enzymatic Substrate Selectivity

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Enzymes bridges the time-scale gap between chemistry and biology and are as such essential for cellular viability. A fundamental property of enzymatic function inside the complex and crowded cellular milieu is recognition of correct over in-correct substrates. This is particularly challenging when chemically related substrates such as ATP and GTP should be discriminated. Specificity can, in principle, be accomplished with positive interference with the correct substrate and negative interference with in-correct substrates. Here we present the atomic mechanism for ATP versus GTP selectivity of the small kinase Adenylate kinase. The finding was enabled through a combination of protein NMR spectroscopy, x-ray crystallography and synthetic organic chemistry. We discovered that while ATP activates the enzyme by triggering a massive conformational change, the enzyme is arrested in an inactive conformation in its complex with GTP. Unexpectedly, we found that a set of residues that are vital for ATP dependent activation also are responsible for stabilizing the inactive GTP-complex. Hence, this set of residues has evolved to accomplish positive interference with ATP and at the same time negative interference with GTP. From a structure-function analysis of a key hydrogen bond formed between ATP and the enzyme, we discovered that this single hydrogen bond is essential for substrate selectivity. This particular aspect of ATP recognition is conserved throughout the entire family of eukaryotic protein kinases thus highlighting its importance.

Rogne et. al, (2018), PNAS, **115**, 3012-3017

Towards time-resolved serial femtosecond crystallography (TR-SFX) of cytochrome c oxidase – a molecular movie

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Cytochrome c oxidase catalyses the reduction of molecular oxygen to water while the energy released in this process is used to pump protons across a biological membrane. Even though many members of the CcO superfamily have been structurally characterized in detail, there is no detailed structural understanding of how unidirectional proton translocation takes place. To understand the catalytic steps carried out by oxygen-dependant enzymes from a structural standpoint, kinetic crystallography methods need to allow the formation of intermediates along the reaction pathway and solve their structures. A billion-fold jump in the peak X-ray brilliance delivered by X-ray free electron laser (XFEL) and the development of serial femtosecond crystallography (SFX) allowed the determination of protein structures at room temperature, thus opening up opportunities for time-resolved (TR) experiments in measuring ultrafast reactions in proteins. With the development of mixingjet technologies that introduce a substrate to initiate a chemical reaction within microcrystals, XFEL-based TR-SFX has the potential to move beyond the study of only light-driven reactions. For the first time, we aim to employ TR-SFX to determine a whole series of structural snapshots of cytochrome c oxidase in action. By introducing molecular oxygen to the active site of crystalline ba3-type cytochrome c oxidase via photolysis of the cobalt-based caged oxygen molecule, we want to track structural changes that occur during oxygen reduction and proton translocation in real time.

References

1. Andersson, R., Safari, C., Dods, R., Nango, E., Tanaka, R., Yamashita, A., Nakane, T., Tono, K., Joti, Y., Båth, P., Dunevall, E., Bosman, R., Nureki, O., Iwata, S., Neutze, R., Brändén, G. (2017). Serial femtosecond crystallography structure of cytochrome c oxidase at room temperature. Scientific Reports. 7. 10.1038/s41598-017-04817-z.

2. Howard-Jones, A., Adam, V., Cowley, A., E Baldwin, J., Bourgeois, D. (2009). Cryophotolysis of a caged oxygen compound for use in low temperature biological studies. Photochemical & photobiological sciences : Official journal of the European Photochemistry Association and the European Society for Photobiology. 8. 1150-6. 10.1039/b821516b.

3. Mizohata, E., Nakane, T., Fukuda, Y., Nango, E., Iwata, S. (2017). Serial femtosecond crystallography at the SACLA: breakthrough to dynamic structural biology. Biophysical Reviews. 10. 10.1007/s12551-017-0344-9.

4. Standfuss, J. (2019). Membrane protein dynamics studied by X-ray lasers – or why only time will tell. Current Opinion in Structural Biology. 57:63–71. 10.1016/j.sbi.2019.02.001.

5. Wickstrand, C., Nogly, P., Nango, E., Iwata, S., Standfuss, J., Neutze, R. (2019). Bacteriorhodopsin: Structural Insights Revealed Using X-Ray Lasers and Synchrotron Radiation. Annual Review of Biochemistry. 88. 10.1146/annurev-biochem-013118-111327.

Inhibition of glucose uptake in cancer cells to sensitize them for chemotherapy

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Cancer cells are in demand of elevated uptake of glucose for their increased proliferation rate. This makes glucose transporters (GLUTs) a promising target for cancer therapy. A set of novel inhibitors has been evaluated for glucose uptake inhibition as well as for their effect on leukaemia cells.

To evaluate the effect of the GLUT inhibitors two acute myeloid leukemia (AML) cell lines, with either low or high expression of GLUT1, were studied. Two inhibitors were shown to actively block the uptake of glucose by GLUT1 in an *in vitro* assay. These inhibitors were further studied in an ATP-based colorimetric cell viability assay to evaluate the effect by GLUT inhibitors alone or in co-treatment with a well-known chemo agent, Cytarabine.

Preliminary data from the cell viability test show synergistic effect of both the tested GLUT1 inhibitors in combination with Cytarabine, in the cell line overexpressing GLUT1. These results indicate that these inhibitors could be a good complement to Cytarabine in the treatment of AML.

Bestowing Hydrolytic Activity to the B1 Domain of Protein G: A Joint Theoretical and Experimental Investigation

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Mimicking the catalytic activity of metallohydrolases opens many appealing applications in biotechnology and basic science. Using computational redesign, we have reprogrammed a zinc binding mini-protein, the pentamutant of the B1 domain of Streptococcal protein G (GB1) whose metal coordination sphere have until now remained elusive. Mixed quantum mechanical/molecular mechanical (QM/MM) molecular dynamics simulations reveal that this metallo-GB1 possesses a His_2CysO type zinc binding site. On the basis of this finding, the 56-residue protein was repurposed to create a hydrolytically competent His_3O type metal binding site resulting in a newly designed nona-mutant (2Q/3F/15V/16H/18H/25E/30H/33T/39I). We report the hydrolytic activity of this reengineered construct with p-nitrophenyl acetate as a substrate and describe the structure as well as the thermal and organic solvent stability of this mini-protein. Our results demonstrate the strength of small protein domains as flexible scaffolds for the introduction of tailored biomimetic catalytic functions and herald other promising applications in biocatalysis with metallo-GB1 variants.