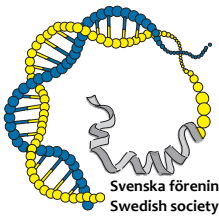
20th Swedish Conference on Protein Structure and Function

Tällberg, 17-20 June 2016

SBNet



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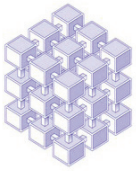

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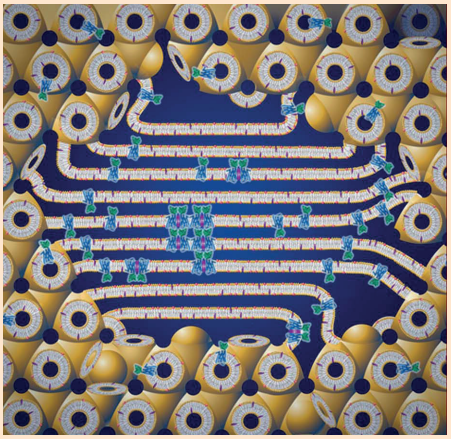
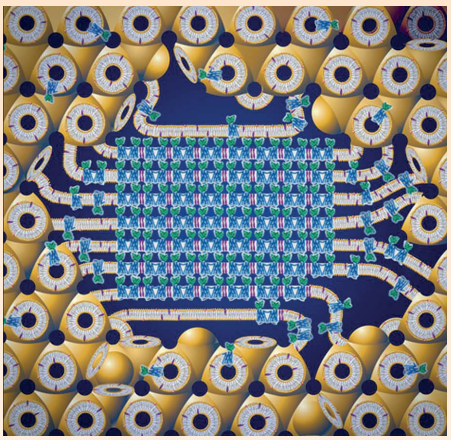
References:

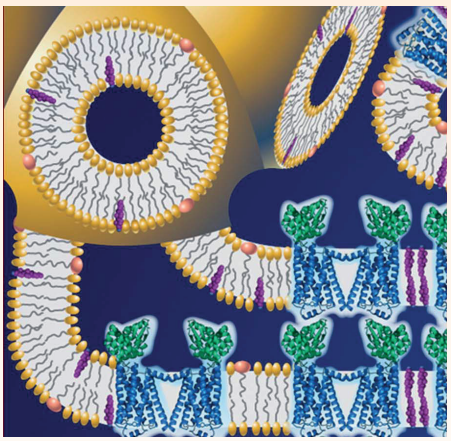
[1] Caffrey (2015) A comprehensive review of the lipid cubic phase or in meso method for crystallizing membrane and soluble proteins and complexes. *Acta Cryst.* F **71**:3.

[2] Caffrey and Cherezov (2009) Crystallizing Membrane Proteins Using Lipidic Mesophases. *Nat. Protoc.* **4(5)**:706.

[3] Landau and Rosenbusch (1996) Lipidic cubic phases: a novel concept for the crystallization of membrane proteins. *PNAS* **93**:14532.







Cartoon representation of the events proposed to take place during the crystallization of an integral membrane protein from the lipid cubic mesophase. Image from [1], used by courtesy of Prof. Martin Caffrey, Trinity College Dublin, Ireland.

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SBNet

20th Swedish Conference on Protein Structure and Function

Tällberg, 17-20 June 2016

Friday 17th June

18:00 – 19:30	Arrival & Dinner	
	Session I: 20 years of SBNet & presentation of the Hugo Theorell Prize.	Chair: Mats Hansson
20:00 – 20:30	**Göran Karlsson (Swedish NMR centre):** *20 years of SBNet.*	
20:30 – 21:15	*Presentation from the Hugo Theorell Prize in Biophysics Awardee.*	
21:20 – 22:00	PI meeting	

Saturday 18th June

07:00 – 09:00	Breakfast	
	Session II: SWEDSTRUCT Integrated Structural Biology	Chair: Helena Käck
09:00 – 09:45	**Patrick Cramer (Max Planck Institute Göttingen):** *Integrated structural biology of gene transcription*	
09:55 – 10:10	**Maarten Schledorn (ETH Zurich):** *Monomer structure of a human somatostatin-14 amyloid.*	
10:15 – 10:30	**Johanna Höög (University of Gothenburg):** *Human spermatozoa with a twist.*	
10:30 – 11:00	Coffee	
11:00 – 11:10	**Christin Reuter (Jena Bioscience):** *Protein Crystallization: Simply grab the needle from the haystack.*	
11:10 – 11:20	**Thom Leiding (Probation Lab AB):** *ProbeDrum: The Titrating Multimode Spectrometer for Research and Life Science.*	
11:20 – 11:35	**Roland Bergdahl (Umeå University):** *Structural insights of the assembly and repair of Photosystem II complex.*	
11:40 – 11:55	**Marjolein Thunnissen (Max IV Laboratory):** *BioMAX – A state-of-the-art beamline for protein crystallography.*	
12:00 – 13:30	Lunch	
	Session III: Metabolomics & Intrinsically Disordered Proteins.	Chair: Göran Karlsson
13:30 – 14:15	**Elaine Holmes (Imperial College London):** *Molecular Phenomics: A window on human health*	
14:25 – 14:40	**Emma Åberg (Uppsala University):** *Emergence and evolution of an interaction between intrinsically disordered proteins.*	
14:45– 15:00	**Axel Abelein (Karolinska Institute):** *Modulation of amyloid beta peptide aggregation by metal ions.*	
15:00 – 15:30	Coffee	
15:30 – 15:40	**Martin Fisher (Formulatrix):** *Multiple Fluorescence Imaging – A Complementary Imaging Tool.*	
15:40 – 15:50	**Bruker Biospin**	
15:50 – 16:05	**Oskar Berntsson (University of Gothenburg):** *Signal Transduction in Photosensory Proteins.*	
16:10 – 16:25	**Henryk Korza (Stockholm University):** *Tetraspanins in control of epithelial-to-mesenchymal transition.*	
16:30 – 17:15	**Robert Konrat (University of Vienna):** *Finding Order in Disordered Proteins.*	
18:00 – 19:30	Dinner	
20:00 – 21:30	Evening Poster Session I	

Sunday 19th June

07:00 – 08:45	Breakfast	
	Session IV: Structural Biology & Disease	Chair: Rosmarie Friemann
08:45 – 09:30	**Christopher Dobson (University of Cambridge):** *The Amyloid State of Proteins and its Significance in Biology and Medicine.*	
09:40 – 09:55	**Oanh Ho (Umeå University):** *Pathogenic effector secretion in Yersinia is coupled to the dissociation of the key protein YscU.*	
10:00 – 10:15	**Ana Laura Stern (Uppsala University):** *Structural mechanism of substrate recognition by the antibiotic resistance enzyme AadA.*	
10:15 – 10:45	Coffee	
10:45 – 10:55	**Christian Hansson (Bruker AXS):** *The 2nd Generation D8 VENTURE diffractometer – Crystallography without Compromises.*	
10:55 – 11:10	**Mohit Narwal (Stockholm University):** *Hypoxic Signalling and the Cellular Redox Tumour Environment Determine Sensitivity to MTH1 inhibition.*	
11:15 – 12:00	**Sheena Radford (University of Leeds):** *Sticking together: A portrait of a protein aggregation disease.*	
12:00 – 13:30	Lunch	
	Session V: Molecular Interactions	Chair: Gisela Brändén
13:30 – 14:15	**John Kuriyan (UC Berkley):** *Control of catalytic activity in the EGF receptor*	
14:20 – 14:35	**Harriet Nilsson (Karolinska Institute):** *The supramolecular packing of the gel-forming MUC5B and MUC2 mucins and its importance for cystic fibrosis.*	
14:40 – 14:55	**Olof Stenström (Lund University):** *Changes in dynamics and conformational entropy of the Galectin-3 carbohydrate recognition domain upon binding two designed ligands.*	
15:00 – 15:10	**Ewa Pol (GE Healthcare):** *Biacore™ systems for protein-protein interactions.*	
15:15 – 15:45	Coffee	
16:00 – 18:00	Annual football match.	
18:00 – 19:30	Dinner	
20:00 – 21:30	Evening Poster Session II	

Monday 20th June

07:00 – 08:45	Breakfast	
	Session VI – Membrane proteins & Protein Complexes	Chair: Richard Neutze
08:45 – 09:30	**Susan Buchanan (NIDDK, Bethesda):** *Structural insight into biogenesis of beta barrel membrane proteins.*	
09:40 – 09:55	**Mathieu Coincon (Stockholm University):** *Crystal structures reveal the molecular basis of ion translocation in sodium/proton antiporters.*	
10:00 – 10:15	**Rob Dods (University of Gothenburg):** *Time-resolved Serial Femtosecond Crystallography of Photosynthetic Reaction Centre.*	
10:15 – 10:45	Coffee	
10:45 – 11:00	**Jennifer Roche (Lund University):** *AQP2 interaction with lysosomal sorting protein LIP5.*	
11: 05 – 11:50	**Gregers Rom Andersen (Aarhus University):** *Structural insight into the mechanism of complement activation through the lectin pathway.*	
12:00 – 13:30	Lunch & Departure	

Modulation of amyloid β peptide aggregation by metal ions

Axel Abelein[1,2], Astrid Gräslund[1], Jens Danielsson[1]

[1]Department of Biochemistry and Biophysics, Stockholm University, Svante Arrhenius väg 16, 106 91 Stockholm

[2]Department of Neurobiology, Care Sciences and Society (NVS), Division of Neurogeriatrics, Karolinska Institutet, 14157 Huddinge, Stockholm

Metal ions, in particular zinc and copper ions, have emerged to play a key role in the aggregation process of amyloid β (Aβ) peptide that is closely related to the pathogenesis of Alzheimer's disease. A detailed understanding of the underlying mechanistic process of peptide-metal interactions, however, has been challenging to obtain. By applying a combination of NMR techniques and fluorescence kinetics methods we have investigated quantitatively the thermodynamic Aβ-metal binding features as well as how metal ions modulate the nucleation mechanism of the aggregation process. Our results show that, under near-physiological conditions, sub-stoichiometric amounts of Zn^{2+} effectively retard the generation of amyloid fibrils. The inhibition process shows an exponential dependence of aggregation half times on the Zn^{2+} concentration. A global kinetic profile analysis reveals that in the absence of Zn^{2+} Aβ40 aggregation is driven by a monomer-dependent secondary nucleation process in addition to fibril-end elongation. In presence of Zn^{2+}, the elongation rate is reduced, resulting in reduction of the aggregation rate, but not a complete inhibition of amyloid formation. We show that Zn^{2+} transiently binds to the N-terminus of the monomeric peptide. A thermodynamic analysis supports a model where the N-terminus is folded around the Zn^{2+} ion, forming a marginally stable, short-lived folded Aβ40 species. This conformation is highly dynamic and only a few percent of the peptide molecules adopt this structure at any given time point. Our findings suggest that zinc ions effectively prevent Aβ40 monomers to be involved in fibril elongation. In this conceptual framework we suggest that zinc adopts the role of a minimal anti-aggregation chaperone for Aβ40.

Reference:

Abelein A, Gräslund A, Danielsson J, The zinc ion as chaperone-mimicking agent for retardation of amyloid β peptide fibril formation, 2015, *Proceedings of the National Academy of Sciences of the United States of America*, 112(17), 5407-12

Structural insights of the assembly and repair of Photosystem II complex

Roland Bergdahl[1], Cecilia Persson[2], Christin Grundström[1], Per-Gustaf Norman[1], Gunter Stier[3], Wolfgang Schröder[1], Göran Karlsson[2] & Uwe H. Sauer[1]

1) Dept. of Chemistry, Umeå University, 2) Swedish NMR Centre, Gothenburg, 3) Heidelberg University, Biochemistry Center

Our aim is to gain structural understandings into the mechanisms underlying assembly and repair of the Photosystem II (PSII) complex in the thylakoid membrane, which is a poorly understood process. For example, one of the central subunits of PSII, the D1 protein, must be replaced roughly every 30 minutes[1,2] due to light and ROS damage during the water-splitting reaction. So far, only a few thylakoid lumen helper proteins have been identified[3,4], but no structural information is available.

In the current work we have determined the three-dimensional X-ray structure of two key *A. thaliana* assembly factors, the "High Chlorophyll Fluorescence 136" protein (HCF136) at 1.7 Å resolution and the "Low PSII Accumulation 19" protein (LPA19) at 1.3 Å resolution, both using MAD phasing. HCF136 has been reported to be essential for assembly of the PS II reaction center in *A.thaliana*[3]. LPA19, also called Psb27-H2, takes part in biogenesis and stabilization of the PSII complex by interacting with the soluble C-terminal region of precursor and mature D1 protein in the thylakoid lumen[5]. Using X-ray Crystallography, SPR, ITC, NMR and mass-spectrometry methods, we are trying to characterize these interactions and also with other thylakoid proteins.

In collaboration with the Swedish NMR Centre in Gothenburg have determined the solution structure of the highly flexible *A. thaliana* thylakoid lumen protein 16 (TL16) which is a potential interaction partner of HCF136.

1. Meurer J. et al. (1998) EMBO J. 17, 5286-5297.
2. Mattoo et al. (1984) PNAS 81, 1380-1384
3. Plücken H. et al. (2002) FEBS Lett. 532:(1-2), 85-90
4. Wei L, et al. (2010) J Biol Chem. 285:28, 21391-8
5. Chen et al. (2006) Plant Mol. Biol. 61, 567-575.

Signal Transduction in Photosensory Proteins

Oskar Berntsson, Alexander Björling, Sebastian Westenhoff

University of Gothenburg, Department of Chemistry and Molecular Biology

Light is a ubiquitous environmental stimulus of great importance to any organism on the face of the earth. To be able to sense and respond to light organisms, ranging from bacteria to man, make use of dedicated photosensory proteins. These proteins are regulating processes such as phototropism, seed germination, light dependent virulence or the circadian rhythm. Typically these proteins hold a chromophore, responsible for the absorption of incoming light. The absorption of a photon by the chromophore will cause conformational rearrangements in the protein matrix, in order to relay the signal to downstream processes. We have used time-resolved X-ray solution scattering to study conformational changes related to the signal transduction process in the red light sensing phytochrome photosensor from *Deinococcus radiodurans* and in the blue light sensing histidine kinase YF1. In phytochrome photosensors we are able to connect the chromophore photocycle with global conformational changes, showing how a certain step in the photocycle is related to substantial rearrangement of the protein structure around as well as several nanometers away from the chromophore. In YF1 we have been able to reveal the light induced conformational changes in the sole photosensory domain, identifying a mechanism by which the signal can be relayed further through the protein. We have also been able to show that global conformational changes occur simultaneous to the light induced adduct formation between the flavin chromophore and a proximal cysteine residue.

Crystal structures reveal the molecular basis of ion translocation in sodium/proton antiporters

Mathieu Coincon, Povilas Uzdavinys, Emmanuel Nji, David L Dotson, Iven Winkelmann, Saba Abdul-Hussein, Alexander D Cameron, Oliver Beckstein & David Drew

Sodium/proton exchangers of the SLC9 family mediate the transport of protons in exchange for sodium to help regulate intracellular pH, sodium levels and cell volume. In humans, their dysfunction has been linked to a number of diseases such as hypertension and cardiac pathologies.

To fully understand the transport mechanism of Na+/H+ exchangers, it is necessary to clearly establish the global rearrangements required to facilitate ion translocation. Currently, two different transport models have been proposed. Some reports have suggested that structural isomerization is achieved through large elevator-like rearrangements similar to those seen in the structurally unrelated sodium-coupled glutamate-transporter homolog GltPh. Others have proposed that only small domain movements are required for ion exchange, and a conventional rocking-bundle model has been proposed instead. Here, to resolve these differences, we report atomic-resolution structures of the same Na+/H+ antiporter (NapA from Thermus thermophilus) in both outward- and inward-facing conformations. These data combined with cross-linking, molecular dynamics simulations and isothermal calorimetry suggest that Na+/H+ antiporters provide alternating access to the ion-binding site by using elevator-like structural transitions.
Nat. Struct. Mol. Biol. 23, 248–255 (2016).

The Amyloid State of Proteins and its Significance in Biology and Medicine

Christopher M. Dobson

University of Cambridge, Department of Chemistry,

Lensfield Road, Cambridge CB2 1EW, United Kingdom

Abstract

Interest in the phenomenon of amyloid formation by peptides and proteins has developed with extraordinary rapidity in recent years, such that is now a major topic of research across a wide range of disciplines. The reasons for this surge of interest arise primarily from the links between amyloid formation and a range of rapidly proliferating medical disorders such as Alzheimer's disease and type-2 diabetes, and also from the insights that studies of the amyloid state can provide about the nature of the biologically functional forms of peptides and proteins and the means of the maintenance of protein homeostasis within healthy living systems. Recent progress in understanding the factors affecting the stability of the amyloid state relative to that of the native state of a protein, along with the development of methods for defining the mechanism of the conversion between the different states, has led to a much more detailed understanding of the links between protein aggregation, amyloid formation and human disease. This talk will give an overview of recent advances in this field of study and discuss recent progress from our own laboratory towards understanding the structural and physical properties of the amyloid state, the kinetics and mechanism of its formation, and the nature and origins of its links with disease. In addition, the talk will discuss the ways in which protein aggregation and amyloid formation may be inhibited or suppressed, both to understand the nature of protein homeostasis in naturally functioning organisms and also to address the development of therapeutic strategies through which to combat the loss of homeostasis and the onset and progression of disease.

Time-resolved Serial Femtosecond Crystallography of Photosynthetic Reaction Center

Robert Dods, Petra Båth, David Arnlund, Gisela Brändén, Anton Barty*, Daniel Deponte**, Sebastien Boutet** and Richard Neutze

University of Gothenburg, * University of Hamburg, ** SLAC National Accelerator Laboratory

Serial femtosecond crystallography (SFX) at an X-ray free election laser is an emerging technique that allows studies using femtosecond x-ray pulses of high brilliance to investigate protein structure and dynamics without radiation damage. A pump-probe time-resolved SFX experiment has recently been performed at the Linac Coherent Light Source, California, on a photosynthetic reaction center from *Blastochloris viridis.* Photosynthetic reaction center absorbs a photon at a special pair of bacterial chlorophylls beginning an electron transfer reaction that results in the eventual generation of the electrochemical gradient required for the production of ATP. This electron transfer chain has a high quantum yield, and time-resolved crystallography allows us to investigate ultrafast structural changes after photon absorbtion that may contribute to this efficiency. Structural changes were investigated both in the femtosecond time range and at two longer time points (5 ps and 300 ps). Processing of the data from the picosecond time-points reveals numerous structural changes arround the special pair of chlorphylls and along the electron transfer chain. Processing of the femtosecond data and structural modelling is still ongoing.

Pathogenic effector secretion in *Yersinia* is coupled to the dissociation of the key protein YscU

Oanh Ho, Tomas Edgren*, Tobias Karlberg**, Herwig Schüler ** and Magnus Wolf-Watz

Chemistry Department, Umeå University, Sweden
* Department of Molecular Biology and The Laboratory for Molecular Infection Medicine Sweden (MIMS), Umeå University, Sweden
** Department of Medical Biochemistry and Biophysics (MBB), Karolinska Institutet, Sweden

The type III secretion system (T3SS) is a macromolecular assembly that enables transport of pathogenic effector proteins (Yop proteins) into eukaryotic host cells. *Yersinia pseudotuberculosis* protein YscU has an important role in regulation of the order of substrates secreted by T3SS apparatus by its proteolysis. YscU composes an N-terminal trans-membrane domain and a cytoplasmic domain YscUc which undergoes auto-proteolysis at the conserved NPTH motif, resulting in a "hetero-dimeric" protein with one 7 kDa N-terminal $YscU_{CN}$ and one 10 kDa C-terminal polypeptide fragment (denoted as $YscU_{CC}$). Dissociation of $YscU_C$ into $YscU_{CC}$ and $YscU_{CN}$ has been shown as an important regulator for effector secretion. Here we have identified organic compounds that are able to regulate both secretion levels of effector proteins and HELA cell cytotoxicity. By application of nuclear magnetic resonance (NMR) spectroscopy and crystallization, we have demonstrated that these compounds inhibit the dissociation of $YscU_C$. Hence the mode of action of these molecules are novel in that they affect dissociation of a hetero-dimeric protein. These results indicated that dissociation of $YscU_C$ is fundamental relevance for function of the T3SS system in Yersinia. The significant similarity between different members of the YscU family suggests that the dissociated inhibition discovered here is also present in other gram negative bacteria with T3SS.

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

Léocadie Henry, Oskar Berntsson, Sebastian Westenhoff

University of Gothenburg, department of Chemistry and Molecular Biology

Phototropins are blue-light photosensors ubiquitous in plants. These proteins control various cellular processes, such as photropism or chloroplast movement, to optimize the photosynthetic efficiency. Phototropins are light-activated serine/threonine protein kinases structurally composed of two blue-light absorbing light-oxygen-voltage (LOV) domains at the N-terminus. Photoexcitation of the LOV domain results in receptor autophosphorylation and an initiation of phototropin signaling. Structural similarities have been found in different phototropins by small angle X-ray scattering (SAXS). However it appears that the two LOV domains in phototropins have different tasks in different species raising the possibility that they could undergo different structural changes. Furthermore, the mechanism of phototropins still remains largely discussed. Thus, we aim to determine, by time-resolved X-ray solution scattering, the molecular mechanism and conformational changes of the different domains of phototropin form *Chlamydomonas reinhardtii* upon blue-light illumination.

Human spermatozoa with a twist

John Heumann*, Azusa Suzuki**, Garry Morgan**, Per Widlund***, Johanna Höög****

* Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, CO, USA **Krefting Research Centre, University of Gothenburg, Gothenburg, Sweden ***Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden **** Department of Chemistry and Molecular Biology, University of Gothenburg, Gothenburg, Sweden

Microtubules are an important part of the cellular cytoskeleton, as they, for example, separate the chromosomes during cell division and aide cellular motility in multiple ways. They are 25 nm wide hollow tubules built of tubulin heterodimers. A large number of microtubule-associated proteins (MAPs) are used by the cell to regulate their dynamics, for example by promoting growth or triggering depolymerisation (catastrophe). While the vast majority of MAPs associate with the outer surface of microtubules, relatively little is known about the microtubule lumen and the proteins inside of it. Microtubule inner proteins (MIPs) are found inside the microtubule lumen of cilia and flagella, and bind at specific locations on the doublet microtubule lattice[1]. Another protein, often called "the carrot" extends from the flagellar tip into the A-tubule of the flagellar axoneme[2]. However, so far, no larger protein complex, or complex structure, has been described inside a microtubule.

We performed cryo-electron tomography on healthy human spermatozoa, frozen intact in their ejaculate. A several micrometer long repetitive structure was apparent inside the microtubule lumen in sperm tails, which was revealed by sub-tomogram averaging to be a helix. The helical structure is positioned off-centre inside of the microtubule lumen, only touching a part of the microtubule lattice.

This is, to our knowledge, the first time a complex structure is seen inside the microtubule lumen, and because of its positioning, we speculate that it might be a microtubule stabilizing structure.

1. Nicastro, D. *et al.* The molecular architecture of axonemes revealed by cryoelectron tomography. *Science* **313,** 944–948 (2006).
2. Dentler, W. L. Structures linking the tips of ciliary and flagellar microtubules to the membrane. *J. Cell. Sci.* **42,** 207–220 (1980).

Tetraspanins in control of epithelial-to-mesenchymal transition

Henryk J. Korza[1], Karin Skaar[1], Petra Sekyrova[2], Michael Andäng[2†], Martin Högbom[1†]

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

2) Department of Pharmacology and Physiology, Karolinska Institute, Stockholm, Sweden

Tetraspanins are cell surface membrane proteins found in all higher eukaryotes. They are thought to act as a scaffold for assembly of multimolecular complexes of other membrane-bound proteins. Tetraspanins show a wide range of functions and take part in several fundamental processes such as signaling, cell interaction, motility, adhesion and proliferation as well as viral entry. Some members of the family have been reported as important players in cancer development and progression. The expression level of some tetraspanins is linked to metastasis and disease outcome.

It is known that eukaryotic cells with a deregulated cell cycle may eventually give rise to malignancies. Moreover, these uncontrollably dividing cells may develop the ability to spread and migrate throughout the body, which leads to metastasis. The active migration ability of tumor cells is linked with a process known as epithelial to mesenchymal transition (EMT).

Our goal is to test if and how tetraspanins are involved in the EMT and migration. To address this question, we have used qPCR to determine the mRNA expression levels in NMuMG epithelial cells from mouse mammary gland. We have detected substantial changes in the expression levels of several tetraspanins upon the EMT transition. The most interesting candidates were subjected to functional analysis by gene silencing. We have found that some tetraspanins have strong influence on cell motility and adhesion in our model. We have undertaken the transcriptional activity analysis and metabolic profiling to pinpoint the influenced pathways.

Control of catalytic activity in the EGF receptor

John Kuriyan, University of California, Berkeley

The epidermal growth factor receptor (EGFR) and its relatives, Her2, Her3 and Her4 play important roles in cell growth and differentiation and are misregulated in numerous cancers. The binding of growth factors to these receptors induces the dimerization and activation of their cytoplasmic kinase domains, resulting in the phosphorylation of tyrosine residues in their C-terminal tails and recruitment of downstream effectors. Ligand binding causes a conformational change that enables dimerization, with the dimer interface mediated entirely by the receptor. These findings suggested that EGFR might be activated by a mechanism shared by many other receptor tyrosine kinases, in which ligand-induced dimerization brings the kinase domains close enough together to activate each other through trans-phosphorylation. But, unexpectedly, some years ago we discovered that the catalytic domain is activated by the formation of an asymmetric dimer in which one kinase domain, the 'activator' acts as an allosteric activator for a second 'receiver' kinase domain. This activation mechanism resembles the activation of cyclin-dependent kinases by cyclins, with the EGFR kinase domain acting as its own 'cyclin'. More recently we have focused on the nature of the coupling of the extracellular domain through the TM-JM segment to the kinase domain and the mechanism of tail phosphorylation, the most important outcome of receptor activation. These questions are especially intriguing in light of the fact that distinct tyrosine phosphorylation sites recruit signaling proteins with entirely different roles in the signaling pathway, with some being downstream effectors, while others exerting feedback inhibition on the receptor. I will describe our current studies aimed at uncovering answers to these questions.

Hypoxic Signalling and the Cellular Redox Tumor Environment Determine Sensitivity to MTH1 inhibition

Lars Bräutigam*, Linda Pudelko*, Ann-Sofie Jemth*, Helge Gad*, Mohit Narwal, Robert Gustafsson, Stella Karsten*, Jordi Carreras-Puigvert*, Evert Homan*, Carsten Berndt**, Ulrika Warpman Berglund*, Pål Stenmark, and Thomas Helleday*

Department of Biochemistry and Biophysics, Stockholm University, Stockholm, Sweden
*Science for Life Laboratory, Division of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden
**Department of Neurology, Medical faculty, Heinrich-Heine-University Düsseldorf, Life Science Center, Düsseldorf, Germany.

Redox signalling contributes to the regulation of cancer cell proliferation and survival. To compensate for oxidative stress, tumor cells overexpress MTH1 and need this enzyme to survive, whereas normal cells do not require MTH1 for regular function. Therefore, requirement of MTH1 enzymatic activity, only in cancer cells with redox imbalance, have made this protein an interesting drug target.

We used zebrafish as model organism to study the role of MTH1 protein in cancer cells. Here we show that increasing oxidative stress in non-cancerous cells sensitized them to the effects of MTH1 inhibition, whereas decreasing oxidative stress protected against inhibition. Introduction of 8-oxo-dGTP and 2-OH-dATP to zebrafish embryos was highly toxic in the absence of MTH1. Furthermore, chemically or genetically mimicking activated hypoxia signalling in zabrafish embryos triggered MTH1 inhibition in them. Transgenic zebrafish line was used so that cellular redox status could be studies *in vivo*. Analysis of results suggested that unusual redox environment could cause sensitization.

In addition, zebrafish MTH1 protein was purified and its crystal structure was solved in complex with a known potent inhibitor. In summary, this study suggested that MTH1 inhibition could be a common approach to cure cancer cells characterized by redox imbalance.

Reference: Bräutigam *et al*. *Cancer Research* **2016**, pii: canres.2380.2015.

The supramolecular packing of the gel-forming MUC5B and MUC2 mucins and its importance for cystic fibrosis

Harriet E. Nilsson[1,2], Sergio Trillo Muyo[2], Anna Ermund[2], Malin Bäckström[2,3], Elisabeth Thomsson[3], Daniel Ambort[2], Philip J. B. Koeck[4], David J. Thornton[5], Gunnar C. Hansson[2]

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Introduction: The genetically related gel forming mucins, MUC2 (intestine), MUC5B (airways), MUC5AC (airways, stomach) and MUC6 (stomach) have large sizes with heavily glycosylated mucin domains in the central part. The C-termini form intermolecular dimers. The N-terminal regions are evolutionarily similar with identical domain organization important for the oligomerization. MUC5B is vital for normal mucociliary clearance of the lungs whereas MUC2 in colon forms an inner dense and attached stratified layer impermeable to bacteria, and an outer loose and unattached layer habituating commensal bacteria. The MUC2 N-terminus (D1-D2-D′D3 domains) was shown to form concatenated polygon-structures under low pH- and high calcium conditions (1).

Aim: To understand the cellular packing of the MUC5B and MUC2 mucins and how this influences their secretion.

Method: The N-terminal and D´D3 domains of MUC2 and MUC5B were expressed, purified and analyzed by subsequent gel filtration, transmission electron microscopy and single particle image processing.

Results: MUC5B multimerizes by disulfide bonds between the D3-domains giving rise to a linear structure (2). Analysis of the MUC5B N-terminus at lower pH and higher calcium concentration revealed a tight dimer+dimer packing (D2 symmetry). This way of packing the MUC5B in the granulae will allow a slow unwinding of a linear molecule.

Conclusion: The MUC5B and MUC2 mucins are packed in the mucin granulae in a way allowing the formation of linear strands or net-like structures, respectively.

References:

1. Ambort, D., Johansson, M. E. V., Gustafsson, J. K., Nilsson, H., Ermund, A., Johansson, B. R., Koeck, P. J. B., Hebert, H., and Hansson, G. C. (2012) Proc. Natl. Acad. Sci. U. S. A. 109, 5645-5650

2. Ridley, C., Kouvatsos, N., Raynal, B. D., Howard, M., Collins, R. F., Desseyn, J. L., Jowitt, T. A., Baldock, C., Davis, C. W., Hardingham, T. E., and Thornton, D. J. (2014) J. Biol. Chem. 289, 16409-16420

Protein Crystallization: Simply *grab* the needle from the haystack

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Initial screening for conditions yielding protein crystals is often compared to *poking for a needle in a haystack* since it is a multi-parameter approach that may become very cumbersome. The process is additionally aggravated by current screening methods that tend to judge results (i.e. observation of crystalline matter) after changing several variables simultaneously. Since this is clearly no good strategy for any multi-parameter-system, it often leads to wrong conclusions and delay of projects.

JBScreen Thermofluor is a pre-crystallization screen for protein stability (and thus: protein crystallizability [1]) that eliminates the undesired overlay effects of traditional screening.

By **focusing separately on each relevant parameter** such as pH, ionic strength, specific ions and buffers [2-4], the haystack is systematically withdrawn and the optimum buffer condition(s) become apparent.

JBScreen Thermofluor is sensitive to assay volumes down to 5 µl and can also be applied in the absence of Thermofluor dye. New options will be discussed to apply JBScreen Thermofluor for hydrophobic targets such as protein-ligand complexes and/or membrane proteins.

Further, we developed **JBScreen LCP** for Crystallization of Integral Membrane Proteins. JBScreen LCP is a 96 condition screen designed for crystallization in the Lipidic Cubic Phase (LCP). It is based on data mining of 192 integral membrane proteins that were successfully crystallized by the *in meso* method and have yielded structures at atomic resolution [5,6]. We present several **LCP lipids/systems** suitable for native membranes that help to speed up membrane protein crystallization.

References:

[1] Price *et al.* (2009) Understanding the physical properties that control protein crystallization by analysis of large-scale experimental data *Nat. Biotech.* **27**:51.
[2] Newman (2004) Novel buffer systems for macromolecular crystallization. *Acta Cryst. D* **60**:610.
[3] http://www.rcsb.org/pdb/home/home.do
[4] www.jenabioscience.com/cms/en/1/catalog/2570
[5] Caffrey (2015) A comprehensive review of the lipid cubic phase or *in meso* method for crystallizing membrane and soluble proteins and complexes. *Acta Cryst F* **71**:3.
[6] www.jenabioscience.com/cms/en/1/catalog/2624

AQP2 interaction with lysosomal sorting protein LIP5

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Selection of membrane proteins for endocytosis into multivesicular bodies (MVBs) and possible degradation in lysosomes, is an important mechanism for controlling protein expression in the plasma membrane. AQP2 undergoes this process during regulation: upon phosphorylation the protein is shuttled to the apical membrane, which results in the concentration of urine. Conversely, down-regulation of the aquaporin commence with ubiquitination with subsequent internalization and lysosomal degradation.
The details of this regulation for AQP2 are still yet to be elucidated. The lysosomal trafficking regulator–interacting protein 5 (LIP5) is presumably important for the sorting and degradation of membrane proteins, and has been shown to interact with the C-terminal tail of AQP[1]. The aim with this project is to investigate the complex between these proteins by microscale thermophoresis and X-ray crystallography to obtain an enhanced understanding of the interaction.

1. LIP5 interacts with aquaporin 2 and facilitates its lysosomal degradation, van Balkom BW, Boone M, Hendriks G, Kamsteeg EJ, Robben JH, Stronks HC, van der Voorde A, van Herp F, van der Sluijs P, Deen PM, *J Am Soc Nephrol.* 2009 May;20(5):990-1001.

Monomer structure of a human somatostatin-14 amyloid

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The monomeric structure of a peptide hormone molecule of human somatostatin (SST)-14 in a fibril was determined to atomic resolution with magic angle spinning solid-state NMR spectroscopy.

In recent years the idea that a number of peptide hormones and neuropeptides are transiently stored in aggregated form has accumulated support[1]. These 'functional amyloids' are believed to be packed into dense-core vesicles[2], which function as temporary depots of messenger peptides in secretory cells. SST-14 is one of these molecules that occurs physiologically both aggregated and as a soluble monomer[3]. The amyloid with only 14 amino acids per molecule forms a classic instance of a biological sample that can be examined with solid-state NMR methods.

Using a 1:3 (uniformly labelled:natural abundance) diluted sample and correlating 2D and 3D experiments, the chemical shifts of the backbone of SST-14 could be fully assigned. Long-range experiments subsequently provided distance restraints that are spectrally unambiguous at 0.2 ppm tolerance. These were combined with a prediction of secondary structure elements by secondary chemical shift calculations and dihedral angle restraints predicted by TALOS+[4]. The collected data led to the structure presented here.

[1] Nespovitaya, N., Gath, J., Barylyuk, K., Seuring, C., Meier, B. H., & Riek, R. (2016). *Journal of the American Chemical Society, 138*(3), 846-856.

[2] Van den Pol, A. N. (2012). *Neuron, 76*(1), 98-115.

[3] Maji, S. K., Perrin, M. H., Sawaya, M. R., Jessberger, S., Vadodaria, K., Rissman, R. A., ... & Riek, R. (2009). *Science, 325*(5938), 328-332.

[4] Shen, Y., Delaglio, F., Cornilescu, G., & Bax, A. (2009). *Journal of biomolecular NMR*, *44*(4), 213-223.

Changes in dynamics and conformational entropy of the Galectin-3 carbohydrate recognition domain upon binding two designed ligands

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In rational drug design, the development of new drugs is facilitated by knowledge of the three-dimensional structure of the protein-ligand complex and the thermodynamics of binding. Using NMR spin relaxation derived order parameters we have probed the backbone, conformational entropy changes of the carbohydrate-binding domain of galectin-3, when binding two design ligands (Ligand A and B). Galectin-3 plays a crucial role in cancer cell activity and fibrosis which makes it a potential drug candidate. The two complexes show similar changes on a structural (chemical shifts) and dynamic level (order parameters) compared to the apo form. However subtle changes in backbone dynamics between the ligands results in a decrease of 8kJ/mol in conformational entropy when going from A to B. This is reflected by ITC where the overall entropic contribution to binding is less unfavorable for A compared to B. Nevertheless, the binding free energy for B is slightly more favorable compared to A meaning that the enthalpy of binding is overcompensating the loss in entropy. This nicely shows that high binding affinity can as well be achieved by entropic as enthalpic factors.

Structural mechanism of substrate recognition by the antibiotic resistance enzyme AadA

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Aminoglycosides are antibiotics that bind to the bacterial ribosome and disturb protein synthesis. However, their efficacy is challenged by resistance in the environment. Clinically, the most important resistance mechanism is inactivation of the aminoglycoside by aminoglycoside modifying enzymes. ANT(3'')(9) or AadA enzymes confer resistance against the aminoglycosides streptomycin and spectinomycin by adenylation of the 3''- and 9-hydroxyl position respectively.

The apo structure of AadA from *Salmonella enterica* was previously determined in our laboratory [1]. Analysis of the structure together with ITC data suggested that the active site was in the cleft between the two domains and that ATP would bind before the aminoglycoside substrate.

A striking remaining question was how AadA could bind and modify two distinctly different drug molecules.

To answer this question, we determined complex structures of wild type AadA with ATP at 1.9 Å resolution and with a mixture of substrate and product of ATP and streptomycin at 2.7 Å. In addition, we solved structures of an active-site mutant in complex with ATP and streptomycin or dihydrostreptomycin at resolutions of 1.7 Å and 1.3 Å respectively. We also compared enzymatic activity of the wild type AadA and active-site mutants towards both antibiotics.

Our structures show how ATP induces a conformational change in AadA to position the two domains for aminoglycoside binding and allows detailed characterization of the streptomycin binding site. The structures and the kinetic data obtained allow prediction of how the chemically very different spectinomycin molecule would bind.

References:

1. Chen Y., Näsvall J., Wu S, Andersson D.I. & Selmer M. (2015), "Structure of AadA from *Salmonella enterica*: a monomeric aminoglycoside (3'')(9) adenyltransferase", ***Acta Cryst. D*** 71:2267-77

Emergence and evolution of an interaction between intrinsically disordered proteins

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Protein-protein interactions involving intrinsically disordered proteins are important for numerous cellular functions and common in all organisms[1]. However, it is not clear how such interactions emerge and evolve on a molecular level. We have performed phylogenetic reconstruction and biophysical characterization of two interacting disordered protein domains, CID and NCBD, from the transcriptional co-regulators NCOA and CREB-binding protein, respectively. The CID domain appeared in NCOA after the divergence of protostomes and deuterostomes and before the first whole genome duplication in the vertebrate lineage 450-600 million years (myr) ago. The affinity for the most ancient CID/NCBD complex we could resurrect was relatively low (K_d~5 μM). At the time of the first whole genome duplication the affinity had significantly increased (K_d~200 nM). This affinity was then maintained in further speciation in the deuterostome lineage as shown by the affinity of the CID/NCBD complex from the fish/tetrapod ancestor (K_d~400 nM) and the similar cross-species modern affinities for different combinations of human CID domains and NCBD domains from human, sea lamprey and zebra fish (K_d values in the range 40-600 nM). Molecular dynamics simulations show that the ancestral low affinity CID/NCBD complex displayed higher conformational heterogeneity than the younger high affinity variants. Our findings suggest a general mechanism for the evolution of new interactions involving intrinsically disordered proteins whereby a low affinity complex evolve by optimizing affinity through direct interactions as well as dynamics, while tolerating several potentially disruptive mutations.

[1]Dunker AK, Bondos SE, Huang F, Oldfield CJ. 2015. Intrinsically disordered proteins and multicellular organisms. *Semin. Cell Dev. Biol.* 37:44–55.

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Structure of the antenna baseplate from a green sulphur bacterium

Dynamic catalysis and signalling in microsomal prostaglandin E2 synthase

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Microsomal prostaglandin E2 synthase type 1 (mPGES-1) is responsible for the formation of the potent lipid mediator prostaglandin E2 under proinflammatory conditions, and this enzyme has received considerable attention as a drug target. Recently, a high-resolution crystal structure of human mPGES-1 was presented, with Ser-127 being proposed as the hydrogen-bond donor stabilizing thiolate anion formation within the cofactor, glutathione (GSH). We have combined site-directed mutagenesis and activity assays with a structural dynamics analysis to probe the functional roles of such putative catalytic residues. We found that Ser-127 is not required for activity, whereas an interaction between Arg-126 and Asp-49 is essential for catalysis. We postulate that both residues, in addition to a crystallographic water, serve critical roles within the enzymatic mechanism. After characterizing the size or charge conservative mutations Arg-126–Gln, Asp-49–Asn, and Arg- 126–Lys, we inferred that a crystallographic water acts as a general base during GSH thiolate formation, stabilized by interaction with Arg-126, which is itself modulated by its respective interaction with Asp-49. We subsequently found hidden conformational ensembles within the crystal structure that correlate well with our biochemical data. The resulting contact signaling network connects Asp-49 to distal residues involved in GSH binding and is ligand dependent. Our work has broad implications for development of efficient mPGES-1 inhibitors, potential anti-inflammatory and anticancer agents.

Brock, J. S., Hamberg, M., Balagunaseelan, N., Goodman, M., Morgenstern, R., Strandback, E., et al. (2016). A dynamic Asp-Arg interaction is essential for catalysis in microsomal prostaglandin E2 synthase. *Proceedings of the National Academy of Sciences of the United States of America, 113*(4), 201522891–977. http://doi.org/10.1073/pnas.1522891113

MX software setup at NSC Triolith

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National Supercomputer Centre (NSC) in Linköping and Protein Science Facility (PSF) from Karolinska Institutet in Stockholm runs a pilot project to evaluate macromolecular crystallography (MX) software applications running at NSC Triolith. The pilot homepage (http://ki.se/en/mbb/get-started-at-national-supercomputer-centre-nsc) aim to guide new members, share software settings and support an efficient supercomputer workflow.

The Swedish light source MAX IV decided to fund a pilot extension called PReSTO, to support integrated structural biology calculations including macromolecular crystallography (MX), Nuclear Magnetic Resonance (NMR) and cryo-electron microscopy (cryo-EM). The integrated structural biology workflow is supported by NSC thinlinc software enabling remote graphic applications such as COOT/VASCo/PyMol for model building, calculation and visualization of protein surface properties and structures. Karolinska Institutet researchers, recruited to NSC by the pilot, recently shared the first acknowledgement for running molecular dynamics software at NSC Triolith(*1*).

We first installed the parallel data processing software XDS and its (GUI) scripting derivatives XDSGUI/XDSAPP. We also installed hkl2map, the SHELX C/D/E GUI, where SHELXD is parallel and the two major software packages CCP4 and PHENIX. We installed Rosetta to be used with "*phenix mr rosetta*" and "*phenix rosetta refinement*" modules and discovered MRage, a parallel implementation of the leading molecular replacement software PHASER inside the phenix-GUI. The phenix-GUI is ideal for making module parameter files that can be used in sbatch scripts for convenient submission from the Triolith login node to the Triolith compute nodes. We found a way to make project specific software installations enabling frequent updates of PHENIX and DIALS. We installed parallel MX software developed for supercomputer usage such as Arcimboldo_lite and Shake and Bake. We recently installed the parallel and leading software packages for phasing (SHARP) and refinement (BUSTER) from GlobalPhasing.

MX software today are typically using a single compute node with maximum 32 cores (16 at Triolith), however when rosetta is used for structure rebuilding/refinement, for tricky molecular replacements, and to make X-ray data processing as fast as todays X-ray data collection, we would benefit from software that can use multiples of 16 cores across several nodes. Now we will install ccp4i2, more PyMol plugins, and parallel electrostatic and pKa prediction software, Crystallography and NMR System, and potentially the Cambridge Structural Database System (CSDS) for more accurate ligand protein structure complexes. We also need to establish close collaboration with Max IV/Lunarc and develop NSC user training sessions to collect MX community feedback.

1. J. S. Brock *et al.*, A dynamic Asp-Arg interaction is essential for catalysis in microsomal prostaglandin E2 synthase. *Proceedings of the National Academy of Sciences of the United States of America* **113**, 972-977 (2016).

Structural and functional analysis of calcium ion mediated binding of 5-lipoxygenase to nanodiscs

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An important step in the production of inflammatory mediators of the leukotriene family is the Ca^{2+} mediated recruitment of 5 Lipoxygenase (5LO) to nuclear membranes. To study this reaction *in vitro*, the natural membrane mimicking environment of nanodiscs was used. Nanodiscs with 10.5 nm inner diameter were made with the lipid POPC and membrane scaffolding protein MSP1E3D1. Monomeric and dimeric 5LO were investigated. Monomeric 5LO mixed with Ca^{2+} and nanodiscs are shown to form stable complexes that 1) produce the expected leukotriene products from arachidonic acid and 2) can be, for the first time, visualised by native gel electrophoresis and negative stain transmission electron microscopy and 3) show a highest ratio of two 5LO per nanodisc. We interpret this as one 5LO on each side of the disc. The dimer of 5LO is visualised by negative stain transmission electron microscopy and is shown to not bind to nanodiscs. This study shows the advantages of nanodiscs to obtain basic structural information as well as functional information of a complex between a monotopic membrane protein and the membrane.

References

Kumar RB, Zhu L, Idborg H, Rådmark O, Jakobsson PJ, Rinaldo-Matthis A, Hebert H, Jegerschöld C. (2016) Structural and Functional Analysis of Calcium Ion Mediated Binding of 5-Lipoxygenase to Nanodiscs. PLoS ONE 11(3): e0152116. doi: 10.1371/journal.pone.0152116

Cryo-EM study of *S. pombe* nucleosome

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Eukaryotic genome is packaged into compact chromatin structure that has to be made accessible for vital cellular processes such as replication and transcription. The basic unit of DNA organization are nucleosomes, where ~147 base pairs DNA are wrapped around a histone protein octamer. Although the histone proteins are highly conserved across species, there are some species-specific differences. The fission yeast *Schizosaccharomyces pombe* is an important model organism for higher organisms in chromatin biology but its nucleosome structure has not been solved. We have used cryo-electron microscopy to study the nucleosome core particle structure of *S.pombe*. For this, recombinant *S.pombe* histones were purified, octamers refolded and nucleosomes reconstituted. Images of vitrified specimens were collected at a 200 kV acceleration voltage and used for single particle analysis. The results will be compared to previously determined nucleosome structures of *Xenopus laevis*[1] and *Saccharomyces cerevisiae*[2].

1. Davey, C.A., Sargent, D.F., Luger, K., Maeder, A.W. & Richmond, T.J. (2002): Solvent mediated interactions in the structure of the nucleosome core particle at 1.9Å resolution. J. Mol. Biol. 319:1097-1113.

2. White, C.L., Suto, R.K. & Luger, K. (2001): Structure of the yeast nucleosome core particle reveals fundamental changes in internucleosomal interactions. EMBO J. 20: 5207-5218.

Performance of alignment algorithms and ligand fitting in computational structural biology

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A newly implemented alignment routine for ligand fitting in real space density will be presented. The core routine utilizes a highly efficient shape recognition algorithm that can be coupled with a random torsion sampling technique. These improvements facilitates the tools to sample a larger set of ligand conformations during a fixed runtime. The main novelty of the new algorithm is the runtime speed decrease of the core alignment routine.

In order to optimally utilize the available shape characteristics of the electron density a small set of pruning and analysis routines have also been developed. These include an optimal sharpening routine that utilizes a golden ratio search of the map kurtosis as well as a modified real space filtering technique. The effect of these map filters on ligand fitting will be presented.

Results from the new fitting procedure have been implemented and tested on a large set of crystal structures and found to yield quantitatively similar results as those achievable with commonly available routines. The comparative fitting results for the unfiltered routines also show to be roughly equally successful at reproducing deposited ligand binding modes in known structures.

Use of small fragments of the Amyloid-beta peptide to prevent amyloid aggregation

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Alzheimer´s disease is an incurable neurodegenerative disorder linked to misfolding and aggregation of the amyloid β-peptide (Aβ) [1]. What causes Alzheimer´s disease is not fully understood, but it is believed that the transition of unstructured monomeric Aβ into β-sheet rich oligomers and fibers is a key element [2]. A recent study reports the first familiar autosomal recessive APP mutation (APPA673V) that causes AD only in the homozygous state whereas heterozygous carriers may be unaffected [3], and another study reports the protective variant APPA673T [4]. The mutation A2V in hAβ already destabilizes aggregates in vitro when present as hexapeptide only ($A\beta_{1-6}A2V$) through binding to full-length hAβwt and thus delays amyloid fibril formation [3]. In preliminary studies we also found plaque formation in vivo to be inhibited by this peptide. To exploit this mechanism as potential treatment, we are investigating $hA\beta_{42}$ incubated under different conditions to determine protocols that to produce oligomers or fibrils, respectively. Samples containing $hA\beta_{42}$ and $hA\beta_{40}$ together with $A\beta_{1-6}A2V$, $A\beta_{1-6}A2T$ has been investigated by Thioflavin T assay to explore conditions to prevent Aβ aggregation. ^{15}N-labeled $A\beta_{40}$ together with $A\beta_{1-6}A2V$, $A\beta_{1-6}A2T$ have been further analyzed by ^{15}N-HSQC where we examined structural induction in the intrinsic disordered $A\beta_{40}$ peptide by co-incubation with $A\beta_{1-6}A2V$ and $A\beta_{1-6}A2T$. ^{15}N-HSQC will also be used, together with Thioflavin T to investigate $A\beta_{1-6}A2V$ or $A\beta_{1-6}A2T$ can dissolve amyloid aggregates of the Aβ42 and $A\beta_{40}$ peptide

References

[1] J. Hardy, D.J. Selkoe, The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics, Science 297 (2002) 353-356.

[2] M. Stefani, C.M. Dobson, Protein aggregation and aggregate toxicity: new insights into protein folding, misfolding diseases and biological evolution, J Mol Med (Berl) 81 (2003) 678-699.

[3] G. Di Fede, M. Catania, M. Morbin, G. Rossi, S. Suardi, G. Mazzoleni, M. Merlin, A.R. Giovagnoli, S. Prioni, A. Erbetta, C. Falcone, M. Gobbi, L. Colombo, A. Bastone, M. Beeg, C. Manzoni, B. Francescucci, A. Spagnoli, L. Cantu, E. Del Favero, E. Levy, M. Salmona, F. Tagliavini, A recessive mutation in the APP gene with dominant-negative effect on amyloidogenesis, Science 323 (2009) 1473-1477.

[4] T. Jonsson, J.K. Atwal, S. Steinberg, J. Snaedal, P.V. Jonsson, S. Bjornsson, H. Stefansson, P. Sulem, D. Gudbjartsson, J. Maloney, K. Hoyte, A. Gustafson, Y. Liu, Y. Lu, T. Bhangale, R.R. Graham, J. Huttenlocher, G. Bjornsdottir, O.A. Andreassen, E.G. Jonsson, A. Palotie, T.W. Behrens, O.T. Magnusson, A. Kong, U. Thorsteinsdottir, R.J. Watts, K. Stefansson, A mutation in APP protects against Alzheimer's disease and age-related cognitive decline, Nature 488 (2012) 96-99.

Overexpression, purification and functional characterization of *Nicotiana benthamiana* XIP1;1 aquaporin

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Aquaporins, also referred to as Major Intrinsic Proteins (MIPs), facilitate the permeation of water and/or other small uncharged molecules across membranes in all kingdoms of life. The X Intrinsic Proteins (XIPs), forming a new MIP subfamily, were recently discovered in the genomes of plants, fungi and protozoa. Since there is a dearth of information regarding the structure and biological function of XIPs, a further molecular characterization is needed. Here, we describe the heterologous overexpression of functional *Nicotiana benthamiana* XIP (*Nb*XIP) protein in the methylotrophic yeast *Pichia pastoris*. We also present data from the subsequent purification trials and initial substrate specific studies in artificial lipid vesicles. We also present a homology model based on the recently published high resolutoin X-ray structure of the ammonia and water transporting aquaporin TIP2;1 from *A. thaliana*.

Novel interaction between Aquaporin7 and Perilipin1 suggests a PKA-dependent mechanism for regulation of glycerol in adipocytes

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Aquaglyceroporins are integral membrane proteins known to facilitate transport of glycerol and water. The aquaglyceroporin AQP7 is expressed in adipose tissue where it regulates glycerol efflux as it translocates to the plasma membrane during lipolysis as a result of catecholamine stimulation. Depletion of AQP7 in mice leads to development of obesity and adipocyte hypertrophy, suggesting an important role in the metabolism. We have discovered that AQP7 interacts with Perilipin1 (Plin1), an abundant protein in adipocytes that coats the lipid droplet, and that phosphorylation by protein kinase A (PKA) of the cytosolic N-terminus of AQP7 diminishes the interaction, which allows AQP7 to move to the plasma membrane. We aim to determine the three-dimensional structure of AQP7 solely and in complex with Plin1 with X-ray crystallography. As we have shown that the N-terminus of AQP7 is primarily involved in the interplay with Plin1, further crystallization efforts are focused on a variant of human AQP7 with C-terminus truncated. The modified AQP7 has successfully been expressed in *Pichia pastoris*, purified to homogeneity and showed high stability. At the moment there are no structures available for any mammalian aquaglyceroporins and a high-resolution structure of AQP7, alone and in complex with Plin1, will help us understand the specificity of aquaglyceroporins and give us further insights into the regulation of glycerol in adipocytes.

Structural studies of transition metal transport

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Every living cell requires a number of transition metals, mostly as cofactors for enzymes. One of the most abundant is zinc, which binds to about 15% of the human proteome, and redox properties of copper are suitable for catalysing redox reactions. Nevertheless, elevated concentrations of any transition metal are toxic to the cell, for instance copper can generate reactive oxygen species which are detrimental to macromolecules (proteins, lipids and nucleic acids). For that reason the homeostasis of transition metals is under strict control, and both import and export are tightly regulated.

Transition metal transport is catalysed by proteins. The work presented here focuses on structural studies of metal transporting membrane proteins by using X-ray crystallography. The proteins are produced as GFP fusions to promising levels (about 1 mg protein per litre culture), then extracted from plasma membranes and purified by affinity and size-exclusion chromatography. Monodisperse and stable fractions are currently being subjected to crystallization trials employing different detergent and lipid-based approaches.

Insights Into Aquaporin 2 Trafficking and Regulation by X-ray Crystallography

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The concentration of urine is an essential process in mammals to prevent excessive water loss. This occurs in the collecting ducts (CDs) of the kidneys. Part of the regulation is mediated by vasopressin, which is released by the pituitary gland and perceived in CD apical cells by the vasopressin-2 receptor (VS2R). The water channel aquaporin 2 (AQP2) is the target of the regulation. Activation of VS2R results in a signalling cascade and the phosphorylation of AQP2 at S256 at its C-terminal domain. This displaces AQP2 from its storage position in intracellular storage vesicles to the apical membrane, where it acts as a water channel [1]. In order to shed light on the mechanisms behind AQP2 trafficking, we investigate the structure of AQP2 containing the phosphomimicking S256E mutation by X-ray crystallography. AQP2 S256E has been expressed in *Pichia pastoris* and recombinant protein purified. Crystals have been obtained, currently with a 3.5 Å resolution. We are currently trying to solve the structure as well as optimizing crystallization to improve resolution. Moreover the recent crystal structure of hAQP2 solved by our group [2] displayed AQP2 binding to two cadmium ions. This indicates that AQP2 could bind calcium *in vivo.* To investigate this, we perform multi-approach calcium binding assays with AQP2.

References:

1) Sasaki. Aquaporin 2: From its discovery to molecular structure of medical implications. Molecular Aspects of Medicine, 33 536.546 (2012)

2) Frick et al. X-ray structure of human aquaporin 2 and its implications for nephrogenic diabetes insipidus and trafficking. PNAS 111:17 6305-6310 (2014)

Neutron crystallography studies on Galectin-3

Galectins are a family of proteins belonging to the superfamily of lectins, broadly defined by a conserved carbohydrate recognition domain (called Gal3C) of about 130 residues responsible for carbohydrate binding, primarily beta galactosides. Galectin-3 is one of the best-known and most studied galectins due to its involvement in various biological events such as embryogenesis, cell adhesion, proliferation, apoptosis, mRNA splicing and regulation of the immune system (Wang & Guo, 2016).

Due to its involvement in various diseases, galectin-3 is a current pharmaceutical target and a large number of inhibitors have been and are currently being synthesized. This, together with the available X-ray crystallographic and NMR data, provides a unique opportunity to study in detail the protein-ligand binding mechanism. However, the exact position of the hydrogen atoms, and thus the hydrogen bond network between the protein and the ligand, cannot be obtained though these techniques.

In our study we present the first neutron crystallographic structures of galectin-3C in complex with lactose, glycerol and one synthetic inhibitor. Neutron crystallography is the only technique that is able to determine experimentally the position of the hydrogen atoms at normal resolutions. We also present the refinement of these structures through a quantum mechanical potential in nCNS. We believe that this knowledge can lead to a better understanding of the protein-ligand binding mechanism and provide useful knowledge for the development of high affinity inhibitors.

Studies of the interaction between hAQP5 and PIP

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Human aquaporin 5 (AQP5) is a membrane-bound water channel that maintains water balance in lacrimal and salivary glands. A defect in this protein's trafficking from intracellular storage vesicles to the apical membrane is thought to play a role in development of Sjögren's syndrome - a chronic autoimmune disease. Prolactin-inducible protein (PIP) is a cytoplasmic protein, which was found to contribute to the pathogenesis of this disease. An interaction between the AQP5 C-terminus and PIP was suggested but further biochemical or structural data are still unavailable. This project aims to study the proposed interaction. Both proteins were overexpressed in the yeast *Pichia pastoris* and purified using column chromatography.

Microscale thermophoresis (MST) is a method which measures a movement of molecules along a temperature gradient created by an IR laser. This way changes in size, charge and hydration shell of molecules are detected using covalently bound dyes or fluorescent fusion proteins. By titrating a concentration of a ligand against a fixed concentration of a labelled molecule it is possible to construct a binding curve. All experiments are performed in a solution loaded in a capillary.

In this study PIP was labeled with red fluorescent dye which binds to lysine residues. The non-labeled AQP5 was titrated. Each MST experiment was performed in triplicates. PIP bound to the full-length AQP5 with a K_D of 0.6 μM. As a negative control truncated AQP5, which was completely lacking the C-terminus, was used. In this case the K_D shifted to approximately 7 μM which may correspond to non-specific binding.

The K_D values will be validated using ITC. The effect of glycosylation of PIP will be studied by mutating the glycosylation site. The interaction in vivo will be studied using bimolecular fluorescent complementation essay in *Saccharomyces cerevisiae*.

Superantigens bind and activate the gp130 receptor on human fat cells

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Superantigens (SAgs) are bacterial toxins that are able to cause massive immune responses, which may lead to severe disease. SAgs are produced by the bacterium *Staphylococcus aureus,* and also called staphylococcal enterotoxins. Recent findings show that staphylococcal enterotoxin A (SEA) modulates insulin signaling as well as insulin biological responses in fat cells, supporting that a bacterial infection might contribute to the development of insulin resistance and type 2 diabetes. SEA is capable of activating the IL-6 cytokine signaling co-receptor gp130 (abundantly expressed on fat cells) by binding to its extracellular domains. Activation results in phosphorylation of the signal transducer and activator of transcription STAT3 within the fat cell. The phosphorylated STAT3 interferes then with the insulin pathway. We have investigated if other SAgs are capable of binding to gp130 and activating the receptor, by looking at the binding using surface plasmon resonance (SPR) and STAT3 phosphorylation upon SAg stimulation of human fat cells. The results show that several SAgs are capable of binding and activating gp130, which further emphasizes that SAgs might have a role in the development of insulin resistance and type 2 diabetes.

Purification and Characterization of *Anopheles gambiae* TRPA1

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Abstract

TRPA1 is a member of transient receptor potential non-selective cation channels superfamily that have the ability to detect the changes in environment and mediate response. It consists of six trans-membrane helices and perform the function as homo or hetero tetramer. Since it is a membrane protein and show low-level expression, therefore it is not an easy task to purify this protein in large amount for structural studies. Our objective is to overexpress and purify TRPA1 in large amount for crystal structure. We have successfully cloned *Anopheles gambiae* TRPA1 (full length and N-terminal truncated constructs) into the pPICZB vector for overexpression in methylotrophic yeast *Pichia pastoris.* Single channel electro physiological recordings showed that both isolated proteins are functional and respond to allyl isothiocyanate (AITC) and heat. The results conclusively show that TRPA1 from *Anopheles gambiae* is an inherent temperature- and chemoreceptor, and similar to what has been reported for the human TRPA1 ortholog, the N-terminal ankyrin repeat domain may tune the response but is not required for the activation by these stimuli.

Effects of truncations in the C-terminal domain of human TRPA1

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Living organisms have to be able to sense and respond to potential harmful environmental stimuli such as very low/high temperatures and detrimental chemicals, which could lead to tissue damage. In humans, the non-selective cation channel TRPA1 (hTRPA1) is one of those molecular sensors and can be activated by various noxious chemicals as well as by cold.

hTRPA1 belongs to the **T**ransient **R**eceptor **P**otential (**TRP**) superfamily, which consists of seven subfamilies which mediate different sensations. Structural information have been scarce for hTRPA1, only recently a single-particle cryo-EM structure has been published with a resolution of 4 Å [1]. It showed that the ion channel contains six transmembrane helices (S1-S6), a long N-terminal ankyrin repeat domain (ARD) (14-17), as well as parts of the long C-terminus, containing a coiled-coil domain. The functional unit is a homo tetramer, where the S5-S6 of the different subunits form a central pore. A large part of the protein comprises of two highly flexible cytosolic regions (N- and C-terminus), being therefore a challenging target for crystallography.

In a previous study Moparthi et al. showed that the N-terminal ARD is not necessary for cold or chemical activation, and its removal improved the expression in the heterologous *Pichia pastoris* expression system [2]. In this study we focus on truncations of the C-terminus of the human TRPA1 to investigate its role in chemical and cold activation, effects on expression levels and protein stability, as well as on crystallization. We cloned three different C-terminal truncations and expressed them in small batches in *P. pastoris*. One of the truncations showed sufficient expression and purification attempts have been initiated.

[1] C.E. Paulsen et al., Nature, 525 (2015) 552.
[2] L. Moparthi et al., Proc Natl Acad Sci U S A, 111 (2014) 16901-16906.

Solution structure modeling and cryo-EM reconstruction of the Hsp21 dodecamer

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Small heat shock proteins (sHsp) play an important role as paramedics in the cell, with a number of human diseases associated with malfunctioning sHsps. The sHsps rapidly sequester destabilized target proteins and thereby overcome the kinetic competition with aggregation, acting as Nature's sponges to avoid protein aggregation. The sHsps act on early unfolding intermediates and capture unfolded states of proteins even present for only a small fraction of the time. Oligomerization and rapid subunit exchange are crucial for sHsp's chaperone activity, yet the molecular mechanisms of why the oligomers are needed are not fully understood and the structural features that mediate the dynamic nature of the oligomer and the variability in subunit interactions remains to be defined. We used cryo-EM, small angle X-ray scattering, homology modeling and crosslinking mass spectrometry to investigate Hsp21, a chloroplast-localized sHsp from *Arabidopsis thaliana*. On average, six of the twelve N-terminal arms were found to be highly dynamic and accessible for interaction with substrate proteins on the dodecamer outside. There was a 30° rotation of the discs in relation to each other and pairwise interactions between the C-terminal tails between the discs. These interactions were lost by V181A substitution, resulting in a mixture of hexamers and dimers and a compromised chaperone activity, providing further emphasis to the requirement of the oligomeric conformation for sHsp function.

Iron induced oligomerization of human frataxin

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Frataxin is a mitochondrial matrix residing protein. Human frataxin is processed by the mitochondrial matrix peptidase (MMP) generating three different lengths, FXN^{42-210}, FXN^{56-210} and FXN^{81-210} (1). The function of frataxin is tied to its capacity to bind, store and deliver iron to various biochemical processes like iron-sulfur cluster and heme biosynthesis (2,3). The shorter variant of human frataxin, FXN^{81-210}, has previously only been observed as a monomer and is believed to remain monomeric even in the presence of iron.

With this work, is iron-dependent oligomerization of human frataxin FXN^{81-210} shown for the first time using dynamic light scattering and transmission electron microscope. The oligomerization process of FXN^{81-210} requires both iron and oxygen to proceed. Hence, during anaerobic Fe(II) incubation frataxin FXN^{81-210} remained monomeric and only when oxygen was introduced oligomers started to occur. By adding H_2O_2 a natural occurring ROS in the mitochondrial matrix the iron oxidation and oligomerization process were accelerated and larger spherical oligomers were detected. The 6:1 ratio of iron to protein was critical to generate the larger oligomers.

Employing X-ray absorption spectroscopy at the Fe K-edge resolved structural details of the Fe-ions bound in the large FXN^{81-210} oligomers. The subunits of the large oligomers are held together by Fe(III) octahedra of O (N) ligands from coordinating amino acid side groups (six Asp/Glu and one His) and molecular oxygen. The typical intermetal distances as found in the ferrihydrite-like biomineral core of iron-loaded horse spleen ferritin propose presence of edge-sharing and double corner sharing Fe(III) octahedra.

1) Koutnikova, et al. (1998) Hum Mol Genet 7, 1485-1489

2) Yoon et al. (2003) J Am Chem Soc 125, 6078-6084

3) Gakh et al. (2010) J Biol Chem 285, 38486-38501

Structural basis for oxygen activation at a heterodinuclear manganese/iron cofactor

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Two groups of prokaryotic di-metal carboxylate proteins that are found predominantly in pathogens and extremophiles harbor a heterodinuclear Mn/Fe cofactor, the class Ic ribonucleotide reductase R2 proteins and R2-like ligand-binding oxidases (R2lox). We have previously shown that the Mn/Fe cofactor of R2lox self-assembles from Mn^{II} and Fe^{II} *in vitro* and catalyzes a challenging two-electron oxidation of the protein scaffold, yielding a tyrosine-valine ether cross-link (1). R2lox proteins are therefore of interest as biological catalysts for oxidative chemistry. Here we present a detailed structural analysis of R2lox in the non-activated, reduced and oxidized resting Mn/Fe- and Fe/Fe-bound states, as well as the non-activated Mn/Mn-bound state (2). Using X-ray crystallography and X-ray absorption spectroscopy, we show that the active site ligand configuration of R2lox is essentially the same regardless of cofactor composition. Both the Mn/Fe and the diiron cofactor activate oxygen and catalyze formation of the ether cross-link, whereas the dimanganese cluster does not. The structures delineate likely routes for gated oxygen and substrate access to the active site that are controlled by the redox state of the cofactor. These results suggest that oxygen activation proceeds via the same mechanism at the Mn/Fe and Fe/Fe center, and that R2lox proteins might utilize either cofactor *in vivo* based on metal availability.

1. Griese JJ*, et al.* (2013) *Proc. Natl. Acad. Sci. USA* 110(43):17189-17194.
2. Griese JJ*, et al.* (2015) *J. Biol. Chem.* 290(42):25254-25272.

Structural studies of clostridial neurotoxins binding to protein and ganglioside receptors

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The clostridial neurotoxin family is composed of the tetanus and botulinum neurotoxins which cause the diseases tetanus and botulism. These extremely potent toxins recognize motorneurons with high affinity and specificity and result in inhibition of neurotransmission, causing paralysis. Our aim is to determine the mechanism of binding of the clostridial neurotoxins to their receptors using biophysical methods, in particular X-ray crystallography. Understanding these complex interactions should help in the development of vaccines and anti-toxin therapies as well as in the engineering of novel biopharmaceuticals.

Biochemical and cryo-EM studies of the CorA magnesium channel

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Magnesium (Mg^{2+}) is an essential divalent cation, and is transported into cells via Mg^{2+} transport proteins. CorA is the best-characterized family of Mg^{2+} channels to date, and there are several structures determined of the closed- as well as the apo state. However there is no structural information of CorA (or any other Mg^{2+} channel) in a conducting conformation. Thus, a central goal with my PhD studies is to use single particle cryo-EM to obtain a high-resolution structure of CorA in a conducting state, to reveal how CorA handles Mg^{2+} ions in the selection and permeation processes.

CorA is a cone shaped homo-pentamer with a centrally located ion conduction pathway and a large cytoplasmic domain. Gating of the channel is believed to occur by Mg^{2+} (ligand) binding/unbinding to sites situated at the subunit interfaces in cytoplasmic domain through a classical negative feedback loop, thus assuring the intracellular Mg^{2+} levels are sufficiently high. Currently, I am trying to design a version of the CorA protein that is desensitized to Mg^{2+} (by performing mutations at the ligand binding sites). The idea is to decouple the gating of the channel from the Mg^{2+} import process (*i.e.* allow incoming Mg^{2+} to enter the pore, but not close the channel once they have entered). To monitor the process biochemically we employ “the pentameric shift assay” that explores the ability of CorA version to scaffold the pentamer as well as an *in vivo* assay to monitor channel function. Once a suitable candidate has been found we intend to subject this to cryo-EM analysis to visualise the Mg^{2+} coordination within the ion-conduction pore. Taken together, we hope that a detailed understanding of the CorA system can serve as a connectional platform for understanding Mg^{2+} transport in other protein families.

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Despite the advances in functional genomics has revolutionized the approach of researchers to protein characterization, it is clear that this task cannot be achieved solely by the comparison of genome sequences. Traditional enzymology, in contrast, requires purification and dilution of proteins which disrupts interaction possible partners and do not reproduce the reality in the cell milieu. Several -omics approaches have been tried in the last decades to develop high-throughput functional studies with mixed results and metabolomics has been successfully used in plant research to identify protein substrates and pathways by comparing wild-type organisms with silenced mutants. In this work we explore the potential of metabolomics for protein characterization by looking at the change in the metabolome due to protein overexpression. The promising prospective of metabolomics is combined with x-ray crystallography studies of the three membrane proteins used in the pool of metabolomics targets.

Crystal structure of the *B. anthracis* class Ib ribonucleotide reductase R2 subunit

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Ribonucleotide reductases (RNR) utilise radical chemistry to reduce nucleotides to their corresponding deoxyribonucleotides for DNA synthesis and repair in all organisms[1]. Class Ia and Ib enzymes are structurally homologous, yet utilize different dinuclear metal cofactors for the generation of a stable tyrosyl radical (Y•) in the R2 subunit of the RNR, which is required for catalysis in the R1 subunit. In class Ia the active cofactor Fe^{III}_2-Y• self-assembles from Fe^{II}_2-R2, O_2 and a reducing equivalent, whereas class Ib enzymes utilize a Mn^{III}_2-Y• cofactor in their R2 subunit. The oxidizing equivalent for the Mn^{II}_2-R2 is provided by a flavodoxin-like protein in the presence of O_2. However, class Ib enzymes are also active *in vitro* when loaded with Fe, but at a much lower rate[2].

We are investigating the mechanisms behind metal selectivity and specificity in these classes of RNR with X-ray crystallography, using the *Bacillus anthracis* class Ib R2 protein as one of our model systems. In order to characterize any possible differences in protein fold or metal coordination depending on metal identity, apoprotein crystals were soaked with an excess of manganese and/or iron under aerobic or anaerobic conditions. Metal ions were identified using X-ray anomalous dispersion.

Preliminary results suggest minimal differences between the Mn- and Fe-containing protein structures, the metal coordination sites being essentially identical. Also, there is evidence for metal reduction during data collection. For this reason a low X-ray exposure dataset was collected on an aerobically iron-soaked crystal. This data allows us to gain deeper insight into the architecture of the metal site upon oxygen activation.

1. Nordlund P, Reichard P. *Annual Review of Biochemistry*. 2006;75: 681 -706.

2. Cotruvo JA, Stubbe J. *Biochemistry*. 2010;49(6):1297-1309.

Structure based drug design targeting cancer

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Nucleotides that have been oxidized by reactive oxygen species can if inserted into DNA cause mutations. The human protein MutT Homolog 1 (MTH1) effectively hydrolyze oxidized nucleotides, thereby sanitizing the dNTP pool and preventing the molecules to be incorporated into DNA. In many current therapies for cancer, DNA damage is induced using radiotherapy or chemotherapy. Inhibiting MTH1 is an alternative way of to introduce DNA damage in cancer cells. MTH1 is overexpressed in many cancer cells and shRNA depletion of MTH1 prevents tumor growth.

We have formed a large interdisciplinary research collaboration that develops inhibitors targeting MTH1. We use X-ray crystallography to study MTH1 and gain insight into the detailed binding of the inhibitors. Co-crystallizations of the human MTH1 protein with inhibitors are crucial for the development of inhibitors with high affinity, specificity and favorable ADME properties.

We have solved several high resolution crystal structures of MTH1 with different nanomolar inhibitors and gained insight into how to efficiently develop these inhibitors into promising drug candidates, that have also been further evaluated using both cell, mouse and zebrafish studies.

Gad, H. *et al.* MTH1 inhibition eradicates cancer by preventing sanitation of the dNTP pool. *Nature* 508, 215–221 (2014).

Bräutigam, L. *et al.* Hypoxic signaling and the cellular redox tumor environment determine sensitivity to MTH1 inhibition. *Cancer Res*, 2016 Feb 9, DOI: 10.1158/0008-5472.CAN-15-2380

Structural studies of MTH1, an anticancer target

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Reactive oxygen radicals, ROS, are continuously produced as by-products of regular cellular metabolism. ROS cause damage to DNA by oxidizing free nucleotide bases. If the oxidized nucleotides are incorporated into DNA, they can cause mutations and thereby increase the risk of cancer. MTH1 hydrolyzes oxidized nucleotide triphosphates, preventing them from being incorporated into DNA, thereby decreases the risk of cancer. However, MTH1 is also essential for the ability of cancer cells to survive their inherently high ROS levels. Enabling and validating inhibition of MTH1 is of high importance in the search of a novel cancer treatment.

We have crystallized MTH1 alone and together with its products and inhibitors to elucidate the substrate specificity and the structure activity relationship between inhibitors and MTH1. We present the structures of human MTH1 (1.9 Å) and its complex with the product 8-oxo-dGMP (1.8 Å) and we propose an unexpected mechanism for substrate recognition. [1]

We show that cancer cells require MTH1 activity to avoid incorporation of oxidized dNTPs. We validate MTH1 as an anticancer target *in vivo* and describe inhibitors that potently and selectively engage and inhibit MTH1 in cells. The inhibition of MTH1 leads to DNA damage, cytotoxicity and therapeutic responses in patient-derived mouse xenografts. This exemplifies the concept for anticancer treatment and validates MTH1 as being cancer phenotypic lethal.[2]

[1] Svensson, L.M. et al. *Crystal structure of human MTH1 and the 8-oxo-dGMP product complex.* FEBS Letters. 2011 Aug 19; 585(16): 2617-21. doi: 10.1016/j.febslet.2011.07.017.

[2] Gad, H. et al. *MTH1 inhibition eradicates cancer by preventing sanitation of the dNTP pool*. Nature. 2014 April 10; 508(7495): 215-21. doi: 10.1038/nature13181.

Heterogeneity and Turnover of Intermediates during Amyloid‑β (Aβ) Peptide Aggregation Studied by Fluorescence Correlation Spectroscopy

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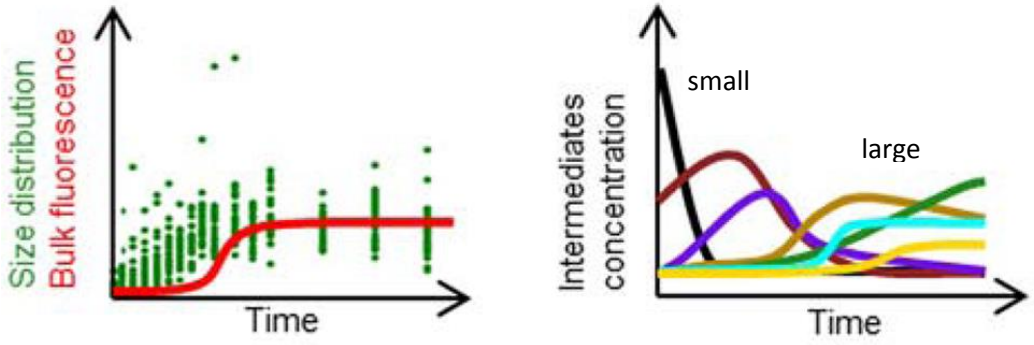
Amyloid-β peptide aggregation has previously been studied by fluorescence correlation spectroscopy (FCS) using peptides with a covalently linked fluorescent label. This approach has yielded significant new information, but it also has some limitations: the covalently labeled Aβ peptides can behave differently than unlabeled peptides, and the concentration range that can be tested by FCS is limited. In contrast to covalently labeled peptides, Thioflavin T (ThT) in micromolar concentrations does not significantly affect the kinetics of Aβ aggregation. By using ThT as a fluorescent marker, we are not monitoring Aβ monomers but rather amyloid aggregates rich in β-structure that give rise to ThT fluorescence.

In this study[1], FCS and ThT flourescense were used to monitor the time dependent growth of structured aggregates and characterize multiple components during the aggregation of Aβ peptides in a heterogeneous aqueous solution. To this aim, we collected data during a relatively large number of observation periods, 30 consecutive measurements lasting 10 s each, at what we consider to be a constant time point in the slow aggregation process. This approach enabled monitoring the formation of nanomolar concentrations of structured amyloid aggregates and demonstrated the changing distribution of amyloid aggregate sizes throughout the aggregation process. We identified aggregates of different sizes with molecular weight from 260 kDa to more than 1×10^6 kDa and revealed the hitherto unobserved kinetic turnover of intermediates during Aβ aggregation. The effect of different Aβ concentrations, Aβ:ThT ratios, differences between the 40 (Aβ40) and 42 (Aβ42) residue long variants of Aβ, and the effect of stirring were also examined.



Reference
1. Ann Tiiman, Jüri Jarvet, Astrid Gräslund, and Vladana Vukojević, *Biochemistry* **2015** *54*, 7203-7211

Structural and functional study of R2-like ligand-binding oxidases

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Ribonucleotide reductase R2 proteins belong to the ferritin-like superfamily and utilize dinuclear metal centers to generate a free radical involved in the synthesis of deoxyribonucleotides [1]. Further analyses of R2 homologues have led to the discovery of a novel group of R2-like proteins, namely R2-like ligand-binding oxidases (R2lox), with a completely different function and predominantly found in pathogens and extremophiles. Crystal structures of these proteins [2,3] show that, although the R2-protein fold is conserved, this latter is remodeled in order to accommodate an unexpected ligand, in interaction with a dinuclear metal cluster, characterized by EPR as an antiferromagnetically coupled Mn(III)/Fe(III) dimer linked by a μ-hydroxo/bis-μ-carboxylato bridging network [4]. This metal center catalyzes, upon oxygen activation, the formation of an ether cross-link in the protein scaffold and, compared to R2 proteins, appear to employ partly different mechanisms to assemble [5]. In addition, it was recently shown that the redox state of the cofactor impacts the protein structure, leading to the formation of likely routes for oxygen and substrate access to the active site [6]. However, until now, the physiological substrate and reaction catalyzed are unknown. Using X-ray crystallography and complementary approaches, the aim of our project is to provide new insights into the function of this novel group of proteins.

References

1. Nordlund P, Reichard P (2006) *Annu Rev Biochem* 75:681-706
2. Andersson CS, Högbom M (2009) *Proc Natl Acad Sci USA* 106(14):5633-5638
3. Griese JJ, *et al.* (2013) *Proc Natl Acad Sci USA* 110(43):17189–17194
4. Shafaat HS, *et al.* (2014) *J Am Chem Soc* 136(38):13399-13409
5. Griese JJ, *et al.* (2014) *J Biol Inorg Chem* 19(6):759-74
6. Griese JJ, *et al.* (2015) *J Biol Chem* 290(42):25254-72

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

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

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

Partnering up: How NADH dehydrogenase II of the bacterial respiratory chain can achieve efficient electron transfer

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In the bacterial respiratory chain there are two types of NADH dehydrogenases, one that is similar to ours producing a proton gradient across the membrane and one that does not. This second type of NADH dehydrogenase, NdhII, is a peripheral membrane protein and the primary one used by *E. coli* under aerobic conditions[1].

NdhII transfers electrons from NADH to ubiquinones (UQ). These electrons are then used by cytochrome bo3 oxidase in the final step of the respiratory chain. NdhII is essential and there are no homologs of this enzyme in mammals, making it a very interesting target for drug development.

It has been proposed that NdhII functions on its own as a dimer and that it has a binding site for both a quinone and NADH[2], but the details of the quinone binding site is still unclear. There are small amounts of UQ with short isoprenoid tails present in *E. coli* but the majority has an 8-unit long tail[3]. The proposed mechanism works well in vitro with UQ-1, producing a reduced quinone pool that can be used to consume oxygen. However, when we test this system with the longer, naturally more abundant, UQ's the system is less efficient.

It has previously been speculated that NdhII could have a membrane bound partner[4], which would aid the transfer of reducing equivalents to the longer UQ's residing within the membrane. We believe that we have found such a partner for this enzyme. This partner would allow NdhII to more efficiently transfer the reduction power of NADH to the more abundant UQ-8 pool. This would better explain how this enzyme can be such an important key player of the *E. coli* respiratory chain.

References:

1. Unden, G. & Bongaerts, J. *Biochim. Biophys. Acta - Bioenerg.* **1320,** 217–234 (1997).
2. Heikal, A. *et al. Mol. Microbiol.* **91,** 950–964 (2014).
3. Soballe, B. & Poole, R. K. *Microbiology* **145,** 1817–1830 (1999).
4. Björklöf, K. *et al. FEBS Lett.* **467,** 105–110 (2000).

Abstract

The botulinum neurotoxin (BoNT), produced by the Gram-positive bacterium *Clostridium botulinum*, is known to be the most toxic protein discovered to date. Its human median lethal dose stands around 10 ng/kg when inhaled and around 2 ng/kg when administrated intravenously or intramuscularly. The high toxicity of the BoNT is achieved by its N-terminal Zn^{2+}-metalloprotease domain, which is able to cleave SNARE proteins (Soluble NSF Attachment protein Receptor) that are responsible for mediating vesicle fusion. This process leaves motor neurons unable to release acetylcholine, ultimately leading to muscle paralysis.

The protein is synthesized as an inactive precursor that is then cleaved resulting in a light chain (LC) and a heavy chain (HC), kept together by a loop of the HC that holds the LC acting as a belt, and also by a disulfide bridge. The mature neurotoxin consists of three domains: the LC N-terminal metalloprotease domain, the HC N-terminal translocation domain and the HC C-terminal receptor-binding domain. These three domains take consecutive parts in the toxicity mechanism of the toxin, as the binding domain delivers the protein to its target, the translocation domain helps the complex crossing the cell membrane and finally the metalloprotease domain cleaves its target proteins, resulting in the toxic activity.

We structurally and biophysically study the BoNTs, their receptors and several proteins that are closely connected to the toxins. Our studies will help us to further understand the intricate mechanisms of the BoNTs.

Purification of Native Cytochrome bcc-aa3 super-complex from *Mycobacterium smegmatis*

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Mycobacterial diseases present substantial risk to human health. This posits to understand the mycobacterial processes in details. Respiratory complexes/super-complexes are important targets in this regard. Thus there is greater need to characterize the respiratory super-complexes biochemically as well as structurally. The purification of these super-complexes is first step in this direction.

Here, we will be presenting data on the purification of *Mycobacterium smegmatis* Cytochrome bcc-aa3 super-complex in native conditions. Existing literature suggests that the super-complex is quite stable in DDM detergent[1,2]. Membranes were purified from the wild type bacteria and the proteins were solubilized in 1% DDM. Since we did not utilize any specific protein tag, thus several purification steps such as DEAE chromatography, Q-sepharose chromatography, sucrose density gradient and gel filtration are required. The purified super-complex (dithionite reduced) shows all the characteristic spectral absorption peaks for Cytochrome a (602 nm), Cytochrome b (562 nm) and Cytochrome c (552 nm), see the fig.1 below. Blue-native gel analysis suggests that the complex migrates as 4 different species ranging from 200-600 kDa (fig. 2). The complex is quite stable for 2-3 weeks when stored at 0°C in ice. The further characterization of the complex is in progress.

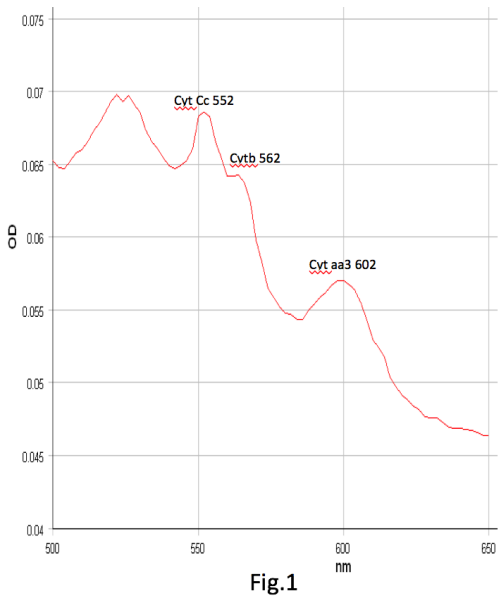

Fig.1

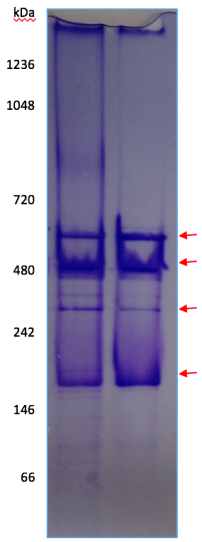

Fig. 2

1. James A. Megehee *et. al.* (2006), *Microbiology*, 152, 823–829.
2. Mi-Sun Kim *et. al.* (2015), *JBC* 14350–14360.

The role of acidic residues in gating of the CorA magnesium channel

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Magnesium (Mg^{2+}) is an essential trace element that plays a pivotal role in numerous physiological and cellular processes. Therefore specific transport proteins regulate the influx and efflux of magnesium. Among eukaryotes and prokaryotes different proteins for magnesium transport have been identified. The Mg^{2+} channel CorA found in prokaryotes is the best characterized to date and serves as a prototype for Mg^{2+} transport and regulation.

There are several CorA structures available. These show that the channel form a homopentamer with a five-fold rotational symmetry. Each monomer contains a large cytoplasmic domain and transmembrane domain with two helices connected by a periplasmic loop. [1] Both structural and biochemical studies suggest it is the large cytoplasmic domain that sense and regulate intracellular levels of Mg^{2+}. The current model is that at low intracellular levels, five M1 sites (composed of acidic residues) at the subunit interfaces of the cytoplasmic domain are in close proximity, leading to electrostatic repulsion and cause the channel to open. When Mg^{2+} is present at sufficient amounts, the M1 site bind Mg^{2+}, neutralizing the acidic interfaces and thus stabilizing interactions between adjacent monomers. This decreases the repulsive force so that the channel can maintain in its closed conformation. [2]

My goal is to better understand the impact of acidic residues at the subunit interfaces in channel gating. I use CorA from *E. coli* as a model protein (see poster by Hena Sandhu). My starting point is a channel version for which all the acidic residues of the M1 site is neutralized because they are replaced with alanine. This causes a significant reduction in gating events and the protein is non-functional (previous work in the group). In my project I test if I can induce gating by introducing acidic residues at novel positions at the interface (outside of the M1 site), and monitor the outcome by testing for a regain of function using an *in vivo* assay. Interestingly, preliminary data indicate that a single acidic residue – at several different interfacial positions – can restore channel function. My hope is that the project will help us understand what evolutionary pressure the CorA channels are under, and to explain why the regulatory M1 site deviate between family members.

References

1. Palombo, I. et al. 2013. *Why Is the GMN Motif Conserved in the CorA/Mrs2/Alr1 Superfamily of Magnesium Transport Proteins?* Biochemistry 52, pp 4842-4847.
2. Palombo, I. et al. 2012. *The Periplasmatic Loop Provides Stability to the Open State of the CorA Magnesium Channel.* J. Biol. Chem 287, pp 27547-55.

Structural basis for magnesium and cobalt hexammine binding in the pore of the Mg^{2+} channel CorA

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The prokaryotic CorA channel is an important model system for understanding Mg^{2+} uptake and regulation in cells. So far, only a closed, non-conductive conformation has been characterized structurally, which limits our understanding of ion-conduction and gating processes. Here, we report the first crystal structure of the cytosolic pentameric sensor domain of CorA from *Escherichia coli* in complex with Mg^{2+}, in what appears to be a semi-open conformation. We identify a single M1 regulatory site in a novel position and conformation in the subunit-interface that contributes insight into gating mechanics. The 23 Å long cytosolic portion of the CorA ion pore exhibit properties suggestive of an open conducting state: it is highly symmetric and two Mg^{2+} ions are trapped at clearly defined ion binding sites. The site closest to the membrane represents a novel high-affinity binding site comprised of two rings of Asn and Gln residues. Soaking experiments reveal that cobalt hexammine, a structural analogue of hydrated Mg^{2+} and known inhibitor of CorA, outcompetes Mg^{2+} at this site. This represents the first structural information of how Mg^{2+} and cobalt hexammine associate to the CorA pore. By combining crystallographic data with molecular dynamic simulations we provide a molecular basis for Mg^{2+} association to the CorA pore during permeation.

Functional and structural studies of proteins involved in long-chain alkane oxidation

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Long-chain alkanes are often found in oil polluted environments, can cause troubles in crude oil processing and show a higher persistency than shorter-chain alkanes in nature. Organisms found in oil contaminated settings live under extreme conditions therefore need metabolic pathways for employing the only carbon sources available. Proteins involved in long-chain alkane utilization are widespread in nature and are also found in pathogenic organisms such as *Pseudomonas aeruginosa* and *Mycobacterium tuberculosis*. Several of these metalloproteins involved in long-chain alkane utilization have been recombinantly produced to high levels in our lab. The reaction mechanisms of terminal oxidation of long-chain alkanes are now, by using isolated membranes, further investigated by spectroscopic methods and functional assays, aswell as with structural studies.

Towards A Better Understanding of Metal Specificity in Di-metal Carboxylate Proteins

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Reduction of ribonucleotides to deoxyribonucleotides required for DNA synthesis is catalyzed by ribonucleotide reductase (RNR), which is the only enzyme known to do so. The *Escherichia coli* RNR is a dimer of two distinct homodimers, namely the R1 and R2 subunits. The R1 subunit holds the redox active cysteine residue, the substrate-binding site and the allosteric effector region, whereas the R2 subunit holds a diiron site, which upon oxygen activation generates a single radical, to be transported to the R1 subunit for catalysis (1). Recently a new class of RNR was found to hold a heterodinuclear Mn-Fe metal center instead of the classical di-iron cofactor (2,3). This discovery shows that the metal affinity, specificity and the redox tuning of the metal centers are carefully regulated in each of the RNR classes. In this study we try point mutational studies on class I *E.coli* RNR to identify the participating elements in its metal coordination sphere affecting its metal affinity and specificity.

References

1.Nordlund P, Reichard P (2006) Ribonucleotide reductases. *Annu. Rev. Biochem*. 75:681-706.

2.Högbom M, *et al.* (2004) The radical site in *Chlamydial ribonucleotide* reductase defines a new R2 subclass. *Science* 305(5681):245-248.

3.Jiang W, *et al.* (2007) A manganese(IV)/iron(III) cofactor in *Chlamydia trachomatis* ribonucleotide reductase. *Science* 316(5828):1188-1191.

Silver ions affect the amyloid-β aggregation in Alzheimer´s disease

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Alzheimer's disease (AD), an irreversible neurodegenerative disease affecting memory and cognition, belongs to the category of ´misfolded protein diseases´ and is characterized by insoluble, amyloid fibrils in the brain. The amyloid fibrils consist of amyloid-beta (Aβ) peptides and also contain various metal ions such as copper, iron, calcium and zinc. The mechanism for the pathology of AD is still not known. Disturbed metal homeostasis is one hallmark of AD, and an additional hallmark is elevated concentration of Aβ in brains of AD patients compared to healthy controls. Metal binding has been proposed to be involved in the pathology. Mainly copper, zinc and manganese ions have previously been described to bind at the N-terminal part of the peptide [1-3]. We found changed biophysical properties of Aβ caused by binding of silver ions, studied with a combination of spectroscopic methods such as nuclear magnetic resonance and circular dichroism, as well as aggregation kinetics. Preliminary results show modulation of the aggregation process in a silver concentration-dependent manner. In future research metal binding properties of various metal ions will be further investigated and studied in relation to possible occurrence in AD patients.

References

[1] J. Danielsson, R. Pierattelli, L. Banci, and A. Gräslund, "High-resolution NMR studies of the zinc-binding site of the Alzheimer's amyloid beta-peptide.," FEBS J., vol. 274, pp. 46–59, 2007.

[2] A. Abelein, A. Gräslund, and J. Danielsson, "Zinc as chaperone-mimicking agent for retardation of amyloid β peptide fibril formation," Proc. Natl. Acad. Sci., vol. 112, no. 17, pp. 5407–5412, 2015.

[3] C. Wallin, Y. S. Kulkarni, A. Abelein, J. Jarvet, Q. Liao, B. Strodel, L. Olsson, J. Luo, J. P. Abrahams, S. B. Sholts, P. M. Roos, S. C. L. Kamerlin, A. Gräslund, and S. K. T. S. Wärmländer, "Characterization of Mn(II) ion binding to the amyloid-β peptide in Alzheimer's disease," J Trace Elem Med Biol, vol. in press, 2016.

Acquisition and processing of high quality 5D spectrum using the sparse fast Fourier transform

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Rapid development of non-uniform sampled (NUS) NMR experiments enables an acquisition of 4-7 dimensional spectra with high resolution. Analysis of such experiments can be challenging because algorithms for spectrum reconstruction from NUS data require heavy computational power and data storage. Sparse fast Fourier transform (SFFT) is a non-iterative method for signal collection and processing, which allows to reconstruct large size and dimensionality spectrum from extremely sparse data (~0.01-3%)[1]. SFFT algorithm combines the Fourier projection-slice theorem and NUS NMR and works in 2 steps: (i) determination of signals' positions in a frequency domain using discrete line projections; (ii) estimation of intensities of identified frequencies. The remaining points are assumed to be zero and omitted from calculation and data storage. Since in a sparse spectrum the number of both measured and reconstructed points is small, SFFT allows to process high quality NMR spectra of any size and dimensionality.

Here we demonstrate an application of SFFT algorithm for 5D HACACONH spectrum of Azurin with 64 complex time points in each indirect dimension. We recorded 29 unique discrete line projections and additional 5% of randomly sampled NUS points. In total, NUS data set included 1896 (0.01%) time domain points. SFFT algorithm identified 57448 out of $1.7*10^{11}$ (0.00003%) non-zero points in the frequency domain. Values of intensities of these points were estimate and stored, final data set requires less then 10Mb. We were able to detect 120 cross-peaks that match to expected amount of signals in Azurin.

Our findings show that SFFT is well suited for the large spectra and can provide high quality reconstruction from less then 1% sparse NUS data set.

1. Hassanieh, Mayzel, Shi, Katabi and Orekhov (2015). Fast multi-dimensional NMR acquisition and processing using the sparse FFT. *J. of Biomolecular NMR*, 63(1), 9–19.

Binding of human proteins to amyloid-β protofibrils

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder. It is believed that the neurodegeneration of AD is linked to the presence of prefibrillar aggregates of the amyloid β-peptide (Aβ) in the brain but the exact role of these aggregates in disease pathology is not fully understood. However, any mechanism of AD pathology involving Aβ must proceed via interactions between the Aβ aggregates and other molecules (Figure 1). We here used an engineered Aβ variant, $A\beta_{42}cc$ [1,2] to map the protein interaction network of Aβ protofibrils in human serum and cerebrospinal fluid. We found that the protofibrils attract a substantial number of protein binding partners. Many of the 101 proteins that were found to interact with Aβ42cc protofibrils are involved in lipid transport and metabolism, the complement system, and in hemostasis. Four selected binding proteins were subjected to kinetic binding analysis. All of these were found to bind strongly to Aβ protofibrils. Taken together, our results suggest that protein ligands might be considered in studies of neurotoxic mechanisms involving Aβ aggregates.

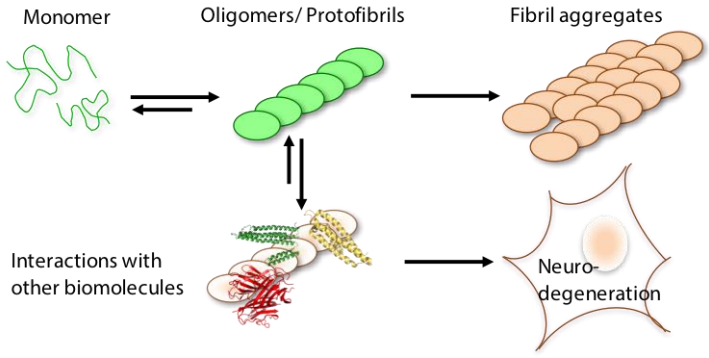

Figure 1. The mechanism by which Aβ becomes neurotoxic and causes neurodegeneration must involve interactions with other molecules.

References

1. Sandberg, A. *et al*. Proc. Natl. Acad. Sci. U. S. A. 2010, 107, 15595–15600.

2. Dubnovitsky, A. *et al*. PLoS ONE, 2013, 8, e66101.

Biotechnological applications of cross-β structured protein nanofibers

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Fiber formation of the yeast proteins Sup35 and Ure2 is phenotypically expressed in S*accharomyces cerevisiae* as the inheritable factors [PSI+] and [URE3], respectively [1-2]. Nanofibers are formed by the N-terminal domains of Sup35 (1-61) and Ure2 (1-80), which self-assemble with β-sheet secondary structure [3]. The overall strength and robustness of these fibers offer many advantages that could be exploited biotechnologically [4]. For instance, functionalization of the fibers is accomplished by designing fusion variants of the protein with a functional domain. Protein-nanofibers with novel properties are then obtained from co-assembly of the functionalized and un-functionalized protein, which can serve a wide variety of purposes.

References: [1] Glover, J. R., A. S. Kowal, et al. (1997). "Self-seeded fibers formed by Sup35, the protein determinant of [PSI+], a heritable prion-like factor of S. cerevisiae." Cell 89(5): 811-819; [2] Maddelein, M. L. and R. B. Wickner (1999). "Two prion-inducing regions of Ure2p are nonoverlapping." Molecular and cellular biology 19(6): 4516-4524; [3] Ngo, S., L. Gu, et al. (2011). "Hierarchical organization in the amyloid core of yeast prion protein Ure2." The Journal of biological chemistry 286(34): 29691-29699. [4] Smith, J. F., T. P. Knowles, et al. (2006). "Characterization of the nanoscale properties of individual amyloid fibrils." Proceedings of the National Academy of Sciences of the United States of America 103(43): 15806-15811.

A Journey to explore the Function and structure of TRP

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Transient receptor potential (TRP) transmembrane proteins are (typically) nonselective cation channels which become permeable in response to a wide variety of physical, chemical and thermal signals, thereby allowing cellular sensation [Latorre 2009]. TRP-channels have been linked to a variety of diseases including inflammatory and neuropathic pain, urinary incontinence and cancer [Eid 2011]. In addition, mutations in TRP genes have been implicated in e.g. neurodegenerative disorders and kidney diseases [Nilius 2011]. A long-standing question is how TRP-channels sense a wide variety of signals which is ultimately linked to understanding how TRP-linked diseases occur and how they can be treated. To answer such questions, high resolution structural information is indispensable, but so far only one structure exists of two full length TRP-channels, those of TRPV1 and TRPA1 determined recently using cryo-electron microscopy (cryo-EM) [Liao 2013, Paulsen 2015].

With this proposal I aim to shed more light on the structure, function, regulation, gating and established complexes of TRP-channels using a combinatorial and interdisciplinary approach, where X-ray crystallography, cryo-EM and biochemical analysis is combined.

Identifying suitable TRP targets for structural studies and determining the structure of human TRP-channels are our specific aims. Of course, to explore further biochemical characterization is our final aim.

Now I have had pretty higher expression level of TRP-channes in yeast cells. Besides that, C-family and M-family have been done FSEC and the peaks seemed good. We have enough reasons to believe that we will get the high-resolution structure of TRP-channels. Journey is blocked of obstacles, but the future is bright.

Reference:

Latorre et al. (2009) Quarterly reviews of biophysics 42: 201-246.

Eid (2011) Current topics in medicinal chemistry 11: 2118-2130.

Nilius and Owsianik (2011) Genome Biol 12:218.

Liao et al. (2013) Nature 504:107-112.

Paulsen et al. (2015) Nature. 520:511-7. Verkman et al. (2014) Nature Reviews 13: 259-277

Regulation of Mitochondrial Apoptosis by Bcl-2 Proteins

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Programmed cell death (apoptosis) is an essential mechanism in life. Key regulators of the intrinsic mitochondrial apoptotic pathway are pro- and anti-apoptotic members of the Bcl-2 family who meet at the mitochondrion's surface - as defined by its outer membrane system - where they arbitrate a life or death decision. Our main aim is to address this molecular regulation mechanism occurring at this mitochondrial outer membrane (MOM) level. For this purpose we use the anti-apoptotic Bcl-2 protein itself which is an integral membrane protein, and its counterpart, the pro-apoptotic Bax protein, to address specifically the importance of the MOM system in the recruitment of Bax.

We could show that oxidatively damaged mitochondrial outer membranes – generated upon oxidative stress - increase the affinity and therefore the translocation of Bax towards these membranes dramatically, and even promote partial penetration of full-length Bax [1]. This process was characterized by a combination of solid state NMR spectroscopy, differential scanning calorimetry and CD spectroscopy [1]. More recently, we found by fluorescence based leakage assays that Bax under these conditions is already able to induce MOM pore formation without additional mediator proteins (e.g. tBid); albeit at a pore size not sufficient for cytochrome c release [2]. Surprisingly, the Bax-induced leakage increased with the amount of oxidized lipid present without any detectable threshold neither for oxidized phospholipids (OxPl) nor Bax. And the leakage rate correlated with i) Bax to lipid ratio and ii) the oxidized lipid content.

This observation of an OxPls induced significant increase in Bax membrane affinity, challenged the traditional view of Bax activation. We therefore proposed a model (s. Figure 1), where Bax in a dynamic equilibrium shuttles between cytosol and the membrane. And upon apoptotic stress this equilibrium shifts towards the membrane-bound state; a prerequisite for Bax induced permeabilization of the MOM and the final execution of cell death.

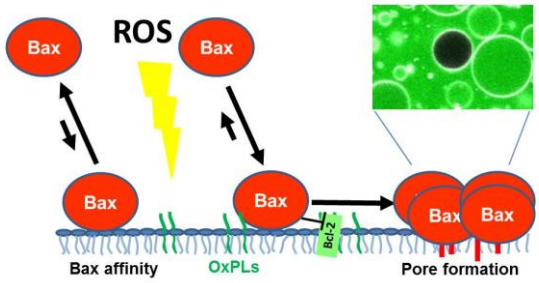

Figure 1: Our concept of the mitochondrial outer membrane as an active partner in apoptotic regulation: (left) in a normal cell, Bcl-2 inhibits the few Bax molecules upon membrane binding; (middle) Generation of OxPls (green) increases population of membrane-bound Bax which can block Bcl-2 and forms pre-apototic pores if tBid is absent (right).

[1] M. Wallgren, M. Lidman, Q. Dat Pham, .. G. Gröbner. The oxidized phospholipid PazePC modulates interactions between Bax and mitochondrial membranes. BBA Biomembranes 1818 (2012) 2718 - 2724.
[2] M. Lidman, Š. Pokorná, A.P.G. Dingeldein, T. Sparrman, .. G. Gröbner. The oxidized phospholipid PazePC promotes the formation of Bax pores in mitochondrial membranes. BBA Biomembranes (2016) in press.

Dynamically driven substrate specificity

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Using NMR we have shown that the specificity of substrate binding of adenylate kinase is determined by the dynamic behavior of the enzyme. Binding of substrate induces the necessary conformational change from the open to the closed state following the induced fit pathway. A substrate that is less effective in causing this conformational change binds less tightly to the protein. Hence, the magnitude of the equilibrium constant for the induced fit rearrangement, K_{conf}, is a major driver for substrate selectivity of adenylate kinase. Our results also shows that the effectivity of the binding and subsequent conformational change have a large effect on the over-all turnover rate of adenylate kinase, the opening of this domain have previously been shown to be the rate limiting step in the adenylate kinase reaction[1].

Adenylate kinase is an essential kinase that catalyzes the reversible transfer of a phosphate group from adenosine triphosphate to adenosine mono phosphate. This enzyme is necessary to keep the energy balance in the cell and is found in organisms from bacteria and fungi to humans.

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

Structural studies of active site mutants in Pol epsilon

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DNA polymerase epsilon (ε) is a multisubunit B-family DNA Polymerase that is involved in leading strand synthesis during DNA replication in eukaryotes. DNA polymerase ε in yeast consists of four subunits Pol2, Dpb2, Dpb3 and Dpb4. Pol2 contains an N-terminal catalytic core ($Pol2_{CORE}$) with both the polymerase and the exonuclease active sites, and a separate C-terminal domain. We have recently solved the X-ray structure of the ternary complex of $Pol2_{CORE}$ bound to DNA and an incoming nucleotide[1]. Like other B-family DNA polymerases, $Pol2_{CORE}$ consists of finger, palm, thumb, exonuclease and N-terminal domain. In addition to this, $Pol2_{CORE}$ has a novel P-domain, which contributes to its high processivity. At the polymerase active site (Fig.1), the incoming nucleotide and two catalytic Aspartates (D877 and D640) coordinate the Mg^{2+}ion. Another active site residue (see below), methionine 644, which is conserved as leucine in Pol δ and RB69, has been shown to be critical for selection of correct dNTP during replication. Our biochemical analysis of Pol ε mutants M644G and M644L suggested contrasting properties: M644G is a mutator polymerase, while M644L is an antimutator polymerase[2]. In addition, K967 and R988 were shown to be important for the switch between the polymerase and the exonuclease active sites[2] (Fig.1). To study the mechanisms further, our aim is to solve the structures of these mutants, which may provide a structural explanation why the switch between the exonuclease and the polymerase active sites is altered.

References

1. Hogg,M., Osterman,P., Bylund,G.O., Ganai,R.A., Lundstrom,E.B., Sauer-Eriksson,A.E. and Johansson,E. (2014) Structural basis for processive DNA synthesis by yeast DNA polymerase varepsilon. *Nat. Struct. Mol. Biol.*, **21**, 49–55.
2. Ganai,R.A., Bylund,G.O., and Johansson,E. (2015) Switching between polymerase and exonuclease sites in DNA polymerase ε. *Nucleic Acids Res.*, **43 (2)**, 932-42

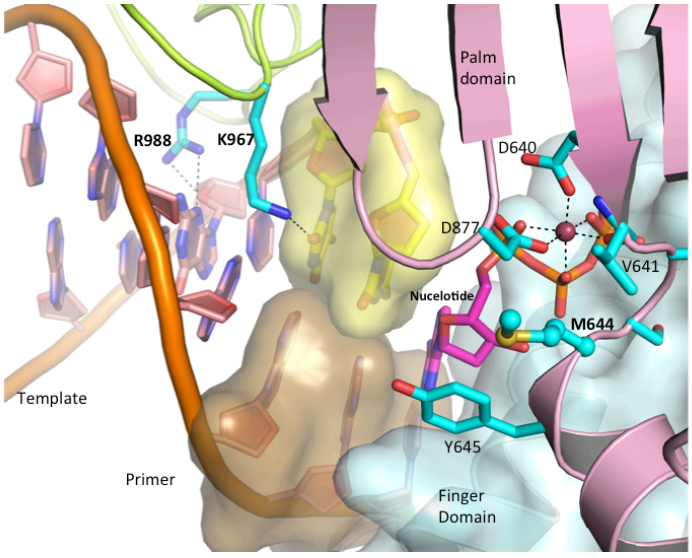

Fig.1. The catalytic active site of $Pol2_{CORE}$.

Dimeric cyanobacterial 1-Cys Prx6 is a moonlighting protein

Yogesh Mishra*, Michael Hall*, Patrik Storm*, Kwangho Nam*, Stefan Jansson**, Wolfgang P. Schröder* and Uwe H. Sauer*

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Peroxiredoxins (Prxs) are vital regulators of intracellular reactive oxygen species (ROS) levels in almost all organisms and their activity depends on one or two catalytic cysteine residues, but no metal, as for classical Prxs[1].

Here, we report results for the 1-Cys Prx6 protein from Anabaena sp. (AnPrx6)[2]. By combining activity assays, X-ray crystallography, NMR, SAXS, mass-spectroscopy and MD simulations we have gained new insights into 1-Cys Prx action. In particular, active site asymmetry and consorted movements of key active site residues affect the activity. Furthermore, we show that the dimeric AnPrx6 is a moonlighting protein with peroxidase and molecular chaperone activity, without change of oligomeric state. The dual function might have contributed to the survival of cyanobacteria[3] in the harsh environments on earth over billions of years.

References:
1. Wood ZA, et al. (2003) Trends Biochem Sci 28(1):32-40.
2. Mishra Y, et al. (2011) Acta Crystallogr F 67(10):1203-1206.
3. Dietz KJ (2011) Antioxid Redox Signal 15(4):1129-1159.

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. This transferable antibiotic resistance is mostly associated with mobile genetic elements such as plasmids. With conjugation, bacteria have evolved a highly effective system for the transfer of DNA from a donor to a recipient cell, mediated by proteins of the Type IV Secretion System (T4SS) [1]. T4SSs are therefore a major contributor to the spread of antibiotic resistance in many clinically relevant pathogens [2].

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

We were able to purify and crystallize different truncations of B and obtained several datasets up to 1.8 Å resolution. Structure solution with heavy atom and SeMet derivative crystals is currently ongoing. Moreover, ITC and SPR based interaction studies provide insight in the molecular function of B in cell adhesion and biofilm formation.

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

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

Structural Insight into the Function of the mitochondrial Bcl-2 Membrane Protein

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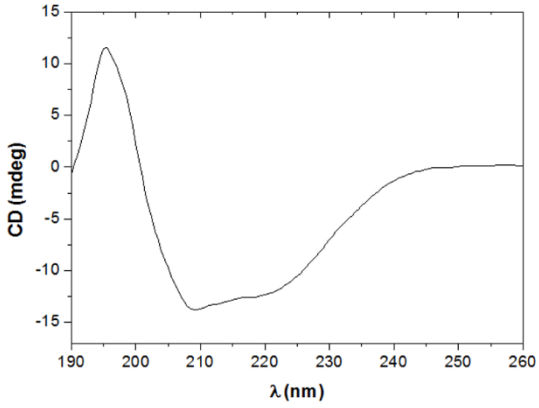
Mitochondria are not only the powerhouse of the cell, but also involved in cellular suicide via apoptosis. However, it is still a mystery how tumour cells escape their fate in cancer therapies. Normally, these treatments interfere with the mitochondrial apoptotic pathway; a major regulator in mammalian cells death, where pro- and anti-apoptotic Bcl-2 proteins meet at the mitochondrial membrane and tightly regulate the fate of a cell. Treatment-resistant tumour cells often possess mitochondria which are enriched in the pro-survival Bcl-2 membrane protein which blocks any apoptotic signals (e.g. as induced by drugs or the Bax protein) and therefore prevents cell death. Our main aim is to elucidate this function of Bcl-2, by providing structural insight of the protein when residing in its natural environment, the mitochondrial outer membrane system. In particular we will also understand how it functions – as a conformational switch presumably – when it inhibits the apoptotic Bax protein at the membrane to exert its pore-forming and cell death inducing activities.

So far no structure of the unmodified, full-length Bcl-2 protein exists. We have established an expression and purification protocol in *E. coli* for the full-length Bcl-2 protein yielding mg amounts, where it is reconstituted in Brij-35 micelles. Solution NMR and CD data of Bcl-2 in Brij-35 confirms that the obtained Bcl-2 protein is comparable with Bcl-2 using a cell-free expression approach previously developed by us and co-workers [1]. We are now focusing on screening for detergents compatible with Bcl-2 for NMR spectroscopy to study the mechanism regulating the opposing activities of Bcl-2 and Bax in an membrane environment, but also to obtain the structure of full-length Bcl-2. Subsequently, conformational changes occuring in both proteins upon Bax association to Bcl-2-containing membranes will then be monitored. Based on the model obtained for Bcl-2 we will delop a mechanistic model which combine the conformational flexibility of Bcl-2 with its molecular recognition of the apoptotic Bax protein; information useful to understand their concerted action in life/death decisions.



CD spectrum of 5 µM full-length ^{15}N-labelled Bcl-2 in 0.05% Brij-35 obtained from recombinant expression.

[1] Pedersen A, Wallgren M, Karlsson G, Gröbner G. Expression and purification of full-length anti-apoptotic Bcl-2 using cell-free protein synthesis. Protein Expr Purif. 2011 Jun;77(2):220-3

Preparation of microcrystals for structural serial femtosecond crystallography of photoactive phytochrome.

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Gothenburg University

Conventional protein crystallography is often based on the idea of making the one perfect protein crystal for structural determination. A larger crystal is often easier to handle, more resistant to radiation damage during data collection and can indicate good crystal quality. Hence, there is often a strive for getting fewer and larger crystals. However, Serial femtosecond crystallography, SFX, is based upon the diffraction before destruction principle and requires large quantities of small microcrystals since every radiation exposed crystal only provides one diffraction image. The crystals are injected into the x-ray radiation with a normal hit rate around 10% indicating also that a lot of sample only goes to waste. This demands accessible ways to produce large quantities of well diffracting microcrystals. Small crystals also eliminate the problem of non homogenous laser penetration of the crystals in light activated structural studies.

Based upon the conditions for normal crystallization of the chromphore binding domain CBD from bacteriophytochrome from *Deinococcus radiodurans*, protocols for both crushing macrocrystals and batch crystallization of microcrystals in large quantities were formulated. The key aspects laid in variation of the protein/reservoir ratio and in lowering the crystallization temperature. The resulting microcrystals yielded in good quality protein structures from SFX experiments at both SACLA and LCLS paving the way for further time resolved studies of the structural dynamics in photoactive phytochromes. The method for microcrystallisation can also be useful for producing microcrystals of other proteins that do or does not form large well diffracting crystals.

The last light

Greger Hammarin , Rajiv Harimoorthy, Guo Chen

Gothenburg University

A story about the very last users at old MAXLAB

In the days following the 20th Swedish Conference on Protein Structure and Function conference the new synchrotron facility MAX IV will be inaugurated on the brightest day of the year and it will surely be the setting for many stories in the future. This, however, is the story about the very last experiment conducted at the old facility before it closed down at sunset on Lucia-day December 13th last year.

When the overhead lights in the machine hall were dimmed and a procession of singing Lucias with burning candles hair walked around the larger of the three rings (MAX II) and machine director Mikael Eriksson made ready cut the power a small group of researches were busy collecting the final images at beamline 911-2 (Cassiopeia-Schedar). This is the story about that experiment, what they were taking images of and why they continued to take images while bystanders were drinking Glögg.

Effect of electric fields on the kinetics of microtubule polymerization

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**Center for Advanced Molecular Imaging, La Trobe Institute for Molecular Science, La Trobe University, Melbourne, Australia.

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

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

Structural characterization of *ba_3* type cytochrome *c* oxidase at room temperature

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Ba_3 type Cytochrome *c* oxidase (ba_3 oxidase) is an integral membrane protein of the respiratory chain that catalyzes the reduction of dioxygen to water in some prokaryotes. *Ba_3* oxidase belong to the heme/copper oxidases (HCO) and despite the numerous structures available of all different types of HCO, the details of the proton transfer mechanism not fully understood.

Ba_3 oxidase from *T. thermophiles* was crystallized in lipid-cubic phase, X-ray free-electron laser (XFEL) diffraction data collected on microcrystals at SACLA, Japan, and the room temperature structure solved to a resolution of 2.4Å. This is one step closer to our goal to do time-resolved serial femtosecond crystallography using XFEL radiation to investigate the structural changes in cytochrome *c* oxidases upon flash photolysis of carbon monoxide and during the reaction with oxygen.

Purification and crystallization of a cytochrome P450 3A5

Carolina Berg, Cecilia Safari, Gisela Brändén

University of Gothenburg

Cytochrome P450 enzymes (CYPs) constitute a superfamily of enzymes involved in diverse biosynthetic and metabolic processes. They are involved in the degradation of xenobiotics, such as pharmaceutical drugs, in the human body. There is a severe risk of toxicity if the CYPs are inhibited by a pharmaceutical compound. Therefor it's of great importance to better understand the CYP proteins and how to avoid inhibition.

CYP3A5 is the dominant CYP isoform in the lung, but its structure is not yet known. Detailed structural and functional understanding of CYP3A5 could be very useful in the development of inhaled drugs, such as asthma medications.

The primary aim is to extract pure CYP3A5 using protein chromatography in order to use it for further analysis. This involves structural and functional methods, such as X- ray crystallography, in order to characterize how drug-like compounds interact with cytochrome P450s.

Planned Time-resolved Studies for Sensory Rhodopsin II.

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*. Univeristy of Gothenburg

Understanding protein dynamics is key to unlocking the protein structure function relationship. Time-resolved methodologies provide a powerful tool to study protein dynamics[1]. This project focuses on membrane protein crystallography, specifically sensory rhodopsin II (SRII) and the transducer protein HtrII. SRII is a light driven proton pump that is also responsible for photo-taxis[2]. Current studies focus on helix F and G as the major location of signal propagation from SRII to its HtrII dimer[3]. However the extent of this movement and the importance of other structural motifs remains elusive.

These experiments provide a unique opportunity to study a membrane protein complex imparting a structural change between proteins at high resolution. Experiments will be aimed at time-resolved crystallography (TRX) and Serial time-resolved crystallography with proteins crystalised in Lipidic cubic phase which reduces the amount of protein required[4]. The overall objective is to describe intermediate states currently missing from our picture of SRII reaction cycle and provide a complete structural understanding of its function.

1. Vukica Srajer. Et al, Photolysis of the Carbon Monoxide Complex of Myoglobin: Nanosecond Time-resolved Crystallography, Science **274**, 5293, (1996)
2. Valentin I Gordeliy, Jörg Labahn Et al, Molecular basis of transmembrane signalling by sensory rhodopsin II–transducer complex, Nature **419**, 484-487, (2002)
3. Ivan Gushchin et al, Active state of sensory rhodopsin II: structural determinants for signal transfer and proton pumping, Journal of Molecular Biology **412**, 591-600
4. Martin Caffrey, A comprehensive review of the lipid cubic phase or *in meso* method for crystallizing membrane and soluble proteins and complexes, Acta Crystallogr F Struct Biol Commun.1;71(Pt 1):3-18, (2015)

Bimolecular Fluorescence Complementation Used to Purify a Human Aquaporin0 and Calmodulin Protein Complex

Petra Båth, Jennie Sjöhamn*, Richard Neutze and Kristina Hedfalk

University of Gothenburg, *La Trobe University

Bimolecular Fluorescent Complementation is a method wherein a fluorescent protein is split and the two non-fluorescent halves are attached to two different proteins. When these proteins interact the fluorescent protein fuses and the chromophore matures which gives rise to a fluorescent signal. We have used this method to show the interaction between human Aquaporin0 (hAQP0) and Calmodulin (CaM) *in vivo* in *Saccharomyces Cerevisiae* using Yellow fluorescent protein (YFP). The fluorescent signal is significantly lowered upon removal of the C-terminus, which is the expected site of the interaction, or by mutating key residues.

Furthermore, we have shown that we can purify this protein-protein interaction as a complex by using the YFP both as an anchor that holds the two proteins together and as a marker for fluorescent detection throughout the purification. The complex was purified using IMAC and FSEC and confirmed by in-gel fluorescence, blue native PAGE and Western blots using anti-bodies against hAQP0 and CaM.

Elucidating the structure of a dark form phytochrome at biologically relevant temperatures.

E.Claesson[1], P.Edlund, H.Takala[2], R.Dods[1], L.Henry[1], M.Panman[1], H.Lehtivuori[2], O.Berntsson[1], P.Båth[1], E.Gustavsson[1], P.Berntsen[3], S.Boutet[4], D.DePonte[5], J.Ihalainen[2], G.Groenhof[2] and S.Westenhoff[1].

Supported by members of BioXFEL Sience and Technology Center.

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Phytochromes are an important family of light-sensing kinases which control diverse cellular processes in plants, bacteria, and fungi. The photoreceptors are sensitive to visible light in the red and far-red region. Upon light absorption, several structural changes appear in the protein which lead to the activation/deactivation of an output domain. These structural changes are known to originate in the chromophore. However, the earliest steps in the structural evolution during signal transduction are still unclear. Serial femtosecond crystallography (SFX) provides subatomic resolution on sub-picosecond time scales and is therefore an ideal method with which to study the structural changes in the photosensory core of phytochromes.

In this investigation, the room temperature crystal structure of the resting state of the chromophore binding domain (CBD) from the bacterial phytochrome *Deinococcus Radiodurans* is presented. Data were collected from protein microcrystals at the free electron laser at Linac Coherent Light Source (LCLS). The crystal structure is solved to a resolution of 2.1 Å and the structure agrees well with the existing steady-state structures recorded at cryogenic temperatures. The most significant differences between our results and the existing structures are found within the chromophore and in residues close to the chromophore-protein linkage. The results presented here are important steps in unravelling the sub-picosecond structural dynamics of the phytochrome photocycle.

Structural and Interaction studies of pro-survival proteins

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Survivin is a human pro-survival protein of 16kDa which belongs to the Inhibitor Apoptosis Protein (IAP) family and plays an important role in apoptosis and cell cycle [3, 4]. Survivin is overexpressed in many cancers and it is related to chemotherapy resistance, recurrence and bad outcome[1]. In addition, high levels of this anti-apoptotic protein have been found extracellular in plasma and synovial fluid of patients with arthritis rheumatoid[2]. At least five survivin isoforms exist in human, sometimes localize differently in cell[3].

Our project is focused on the search of new interactional partners of survivin and isoforms that helps to understand better its mechanism and drug development. MicroScale Thermophoresis (MST) and Isothermal Titration Calorimetry (ITC) are or will be used to test these interactions. Preliminary MST results show interactions between survivin and other peptides/proteins with dissociation constants in the range of nM-μM. In addition, structural studies by X-ray crystallography has been developed, obtaining data around 4Å.

Hereafter, other techniques will be included to obtain more information about the interactions. ITC will provide independent information about binding enthalpy and entropy of unlabelled proteins. Identified protein complexes will also be studied by X-ray crystallography and NMR.

1. Altieri D. C. (2003) Survivin, versatile modulation of cell division and apoptosis in cancer. Oncogene, 22:8581–8589.
2. Baran M. *et al* (2009) Survivin is an essential mediator of arthritits interacting with urokinase signalling. Journal of Cellular and Molecular Medicine, 13:3797-3808.
3. Mita A. C. *et al* (2008) Survivin: key regulator of mitosis and apoptosis and novel target for cancer therapeutics. Clinical Cancer Research 15:5000-5005.
4. Verdecia M. A. *et al* (2000) Structure of the human anti-apoptotic protein survivin reveals a dimeric arrangement. Nature Structural Biology, 7:602-608.

Applying bimolecular fluorescence complementation to screen and purify membrane protein:protein complexes

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

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

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Phototropins are blue-light photosensors ubiquitous in plants. These proteins control various cellular processes, such as photropism or chloroplast movement, to optimize the photosynthetic efficiency. Phototropins are light-activated serine/threonine protein kinases structurally composed of two blue-light absorbing light-oxygen-voltage (LOV) domains at the N-terminus. Photoexcitation of the LOV domain results in receptor autophosphorylation and an initiation of phototropin signaling. Structural similarities have been found in different phototropins by small angle X-ray scattering (SAXS). However it appears that the two LOV domains in phototropins have different tasks in different species raising the possibility that they could undergo different structural changes. Furthermore, the mechanism of phototropins still remains largely discussed. Thus, we aim to determine, by time-resolved X-ray solution scattering, the molecular mechanism and conformational changes of the different domains of phototropin form *Chlamydomonas reinhardtii* upon blue-light illumination.

Light-induced Changes in a Monomeric Bacteriophytochrome

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Phytochromes are red-light sensing proteins in plants, bacteria and fungi. Upon illumination with red light they undergo structural changes that are relayed to a histidine kinase output domain. In previous work, the light-induced structural dynamics of the photosensory subunit of a bacterial phytochrome were elucidated by X-ray crystal diffraction and time-resolved solution X-ray scattering [1]. Bacterial phytochromes are dimeric proteins. Therefore it is of interest whether the dimeric arrangement is necessary for the structural response upon light illumination.

Here we have used time-resolved X-ray scattering to study a bacterial phytochrome in which the dimerization interfaces were modified [2]. This protein does not dimerize in solution which allowed us to investigate the monomer structural dynamics after light illumination. The time-resolved X-ray scattering was fitted against MD simulations of the dark (Pr) and light state (Pfr). The data reveals two dominant motions: a bend and a twist of the PHY domain. This is in accordance to the structural response of the dimer found in previous studies. We conclude, that the dimeric arrangement is not necessary for the structural activation but may instead be required for activating the output domain of bacterial phytochromes.

[1] Takala, H., Björling, A., Berntsson, O., Lehtivuori, H., Niebling, S., Hoernke, M., Kosheleva, I., Henning, R., Menzel, A., Ihalainen, J.A., and Westenhoff, S. (2014) Signal amplification and transduction in phytochrome photosensors. *Nature.* **509,** 245-248.

[2] Takala, H., Bjorling, A., Linna, M., Westenhoff, S., and Ihalainen, J.A. (2015) Light-induced Changes in the Dimerization Interface of Bacteriophytochromes. *J.Biol.Chem.* **290**, 16383-16392

Structural and dynamical studies of two oxidase enzymes

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The cytochrome c oxidases are membrane-bound enzymes that use the energy derived from reducing molecular oxygen to water, to translocate protons across a biological membrane. The free energy that is stored in this electrochemical gradient can then be used e.g. to synthesize ATP. The proton pathway of ba_3 oxidase, belonging to the B family of oxidases, is yet not well understood in detail. The aim of this project is to produce well diffracting crystals of wild-type and mutant ba3-type oxidase from Thermus thermophilus to trap intermediate states of the enzyme during the oxygen reaction and solve the structures using X-ray crystallography.

The cytochrome P450 protein family (CYPs) is the main metabolizing system for xenobiotics in the body, responsible for the breakdown of about 75 % of all marketed pharmaceuticals. There are more than 30 CYP450 enzymes in humans, the most abundant being CYP3A4. Homology between CYP3A4 and the less studied CYP3A5 has been verified considering catalytic specificity and also amino acid sequence match (85% sequence identity). Despite this, no structural data has been published of CYP 3A5. The aim of this project is to over express a recombinant form of CYP3A5 in *E.Coli* in order to characterize the structure and dynamics of the protein, using crystal-based X-ray methods in combination with spectroscopy.

Save the file as .pdf-format and upload to http://sweprot.se/abstracts/ before 31 March 2016.

Production optimization of human AQP10 for structural determination

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Human aquaporin 10 (hAQP10), an integral membrane protein which belongs to the aquaglyceroporin family, is located in the enterocytes of intestinal cells in the small intestine. hAQP10 is expected to accelerate the transport of water and glycerol through the apical membrane of enterocytes by passive transport. There is a lack of structural information on hAQP10 mainly because glycosylation leads to a diminished probability for protein crystals. For this reason, a non-glycosylated hAQP10 mutant was created, N133Q, giving a fully functional channel protein but with a decreased thermostability of 3-6 °C (REF). To prepare a more stable non-glycosylated protein, amendable for crystallization, a random mutagenesis approach was applied. From a pool of 66 mutants, a triple mutant (M161I in TMD3, G184E in Loop D and T225A in Loop E) was identified showing an increased thermal stability of 2-5 °C. By modeling, we find that one of these mutations, G184E, has the potential to form three new hydrogen bonds (one to N182 in the same monomer and two to R180 in the neighboring monomer), which is a possible explanation to the increased stability observed for the hAqp10 tetramer. To obtain protein crystals, two different approaches are pursued: (1) preparation of microcrystals in lipid cubic phase (LCP) for analysis by small-angle X-ray scattering, and (2) standardized crystallography setup via hanging drop method. In addition, mutants with additional thermostability will be screened for using fluorescence activated cell sorting (FACS). With these varied approaches we aim for the first high-resolution structure of a human aquaglyceroporin.

Structural and functional characterization of fish aquaporin

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Water transport in essential for all kingdoms of life. In order to maintain body fluid homeostasis, fishes have to cope with the osmotic challenge caused by the environment. Transcellular water transport pathways formed by aquaporins are the predominant way for fishes to maintain their water balance. In addition to transporting water, some aquaporins are also able to transport small molecules, such as ammonia, carbon dioxide and glycerol in many physiological functions. Although several aquaporin structures have been determined, the structural basis of substrate permeability and selectivity is still not very clear. We have initiated the structural and functional study of an aquaporin from the freshwater teleost Climbing Perch, *Anabas testudineus* (cpAQP1). This fish is able to survive in fresh water, sea water, land and high ammonia environment. It has been reported that terrestrial and ammonia exposure leads to a significant change of the cpAQP1 expression level in gills and skin, which implies that cpAQP1 may be one of the genes involved in water balance and ammonia excretion [1]. The aim of this project is to determine the crystal structure of cpAQP1, presumably first from a fish and study its permeability and selectivity of small molecules by in vitro liposome experiments. So far, we have successfully heterologous expressed cpAQP1 in *Pichia pastoris*, purified and crystallized it. We will optimize the crystal and determine its structure soon.

Reference:

1. Ip YK, Soh MML, Chen XL, Ong JLY, Chng YR, et al. 2013

Advanced NMR signal processing algorithms.

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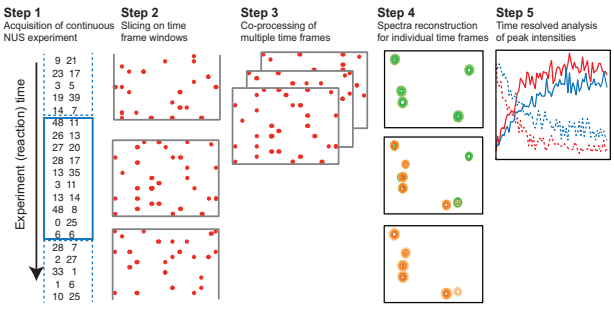
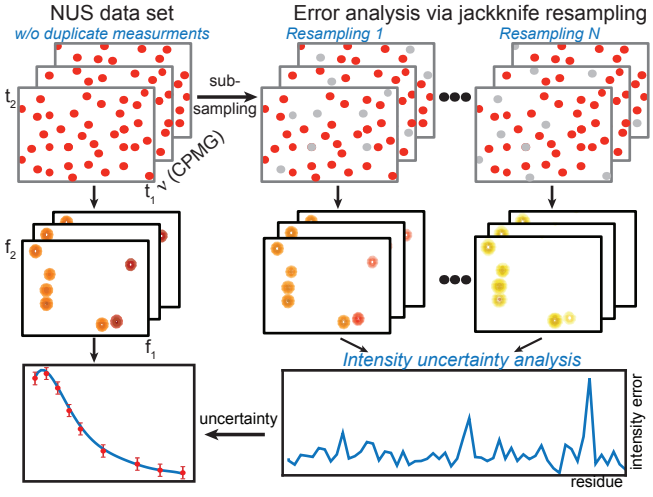
During the last decade non-uniform sampling (NUS) methodology has proven its viability in the field of high-resolution biomolecular NMR spectroscopy where it was primarily used to achieve improvement in spectral resolution. However, applications of NUS for quantitative analysis such as studies of molecular dynamics is only emerging. The method requires caution to avoid biases in the results due to the non-linearity of many techniques developed for NUS spectra reconstruction. In this work we present methods of co-processing and rigorous statistical analysis for improving resolution in the time-resolved (TR) NMR spectroscopy and for quantification of two- and three-dimensional NUS relaxation-dispersion (RD) spectra.



TR NMR spectroscopy allows monitoring and quantification of a kinetic process occurring at the time scale of hundreds of milliseconds and longer. TR studies of Intrinsically Discorded Proteins (IDPs) specifically demand high resolution both in spectral dimensions and in time of the studied kinetic process. The latter requirement traditionally prohibits applications of the multidimensional experiments, which although capable of providing invaluable information about structure and dynamics and almost unlimited spectral resolution, require too lengthy data collection. Our work shows that the problem has a solution in using modern methods of NMR data collection and signal processing [1].

Besides boosting of the resolution, NUS offers new possibilities for improving spectra analysis. We demonstrate for the first time a method of incremental data accumulation with concurrent signal processing for monitoring progress of achieving targets on precision of the peak intensities. Furthermore, we show that jackknife analysis of down sampled spectra yields robust estimates of peak intensities errors, eliminating the need for recording duplicate data points. The methodology should be useful for characterization of millisecond dynamics in small to medium-sized proteins with poorly dispersed spectra [2].



1. Mayzel M., Rosenlow J., Isaksson L., and Orekhov V. Y., *J Biomol NMR*, 58, 129-139 (2014)
2. Mayzel M., Ahlner A., Lundstöm P., and Orekhov V. Y. (in preparation)

o-nitrophenyl Cellobioside as an Active Site Probe for Family 7 Cellobiohydrolases

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Interactions between *o*-nitrophenyl-*β*-D-cellobioside (*o*NPC) and two cellobiohydrolases, Cel7A from *Hypocrea jecorina (Trichoderma reesei)* and Cel7D from *Phanerochaete chrysosporium* were studied by fluorescence spectroscopy and kinetic tests. The hydrolysis rate is slow enough that the substance may be treated as nonreactive in equilibrium binding studies. *o*NPC quenches the natural fluorescence of Cel7A and Cel7D by radiationless energy transfer. Addition of cellobiose recovers the fluorescence of Cel7A, indicating that *o*NPC binds preferentially to the +1,+2 subsites of the enzyme, to which cellobiose is known to have highest affinity. *o*NPC was used as indicator ligand to determine the association constants of cellobiose for catalytically inactive mutants of Cel7A by displacement binding experiment. The experimental data are supplemented by structural modelling of *o*NPC into the active site of the enzymes studied. Further, molecular dynamics simulations were performed to identify active site contributions to affinity, and free energy calculations (free energy perturbation with Hamilteonian replica exchange molecular dynamics) were conducted to understand preferential subsite binding.

Conformational Change of 2-(2’ Hydroxyphenyl)benzenesulfinate Desulfinase During Catalysis

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Increasingly stringent regulation of sulfur oxide emissions and environmental stewardship necessitates more effective fossil fuel desulfurization technologies. While traditional catalytic means of desulfurization effectively remove simple sulfur compounds, more complex thiophenic molecules remain intact; these thiophenic molecules now account for the majority of sulfur emissions from liquid transportation fuels. Biodesulfurization via enzyme catalysis has the potential for highly specific, rapid thiophenic desulfurization occurring at ambient temperature and pressure. The 4-step catabolic pathway converts dibenzothiophene (DBT), a common crude oil contaminant, into the sulfur-free molecule 2-hydroxybiphenyl (2-HBP) without the disruption of carbon-carbon bonds. 2-hydroxybiphenyl desulfinase (DszB), the rate-limiting enzyme in this biocatalytic process, is capable of selectively cleaving carbon-sulfur bonds. Accordingly, fundamental understanding of the molecular mechanisms of DszB must be developed. Based on crystallographic evidence, we hypothesize that DszB undergoes an active site conformational change associated with the catalytic mechanism. Moreover, we anticipate this conformational change is responsible, in part, for enhancing product inhibition. Rhodococcus erythropolis IGTS8 DszB was recombinantly produced and purified via Escherichia coli BW25113 to test these hypotheses. Activity and the resulting conformational change of DszB in the presence of 2-HBP were tested. The activity of recombinant DszB appears comparable to the natively expressed enzyme and is inhibited via competitive binding of the product, 2-HBP. Using circular dichroism, we observed conformational changes in DszB upon introduction of product, 2-HBP.

The role of polar residues in processive chitinase function

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Processive glycoside hydrolases are responsible for the efficient deconstruction of polysaccharides in nature. To accomplish this task, these enzymes attach to the polymer chain via deep binding clefts or tunnel-shaped active site architectures. Hydrolysis then occurs repeatedly without dissociating from the crystalline substrate surface. Our understanding of the relationship of active site geometry and chemical composition with processive glycoside hydrolase function has yet to be generalized in fashion that makes predictable protein engineering feasible. Toward developing a universal, molecular-level description of processive glycoside hydrolase mechanisms, we have initiated studies of model glycoside hydrolases from the *Serratia marcescens* suite of chitinases. Previously, we examined aromatic-mediated interactions with the chitin substrate, demonstrating residues near the cleft entrances had the largest effect on processive function. Here, we extend our characterization of the *S. marcescens* chitinase B (ChiB) active site by focusing on the role of polar residues in chitin binding, hydrolysis, and processive ability. Using kinetic assays, molecular simulation, and free energy calculations, we evaluated the roles of D316, R294, E221, and Y145, which occupy binding subsites along the length of the cleft. Using umbrella sampling calculations and kinetic analysis of the D316A variant, we revealed D316 does not form a "roof" across the substrate with W97 opposite the cleft, contrary to a prior hypothesis based on the static crystallographic structure. The Y145A variant was catalytically inactive; molecular simulation suggested interaction between the catalytic acid and the glycosidic bond was interrupted by formation of a new hydrogen bond with a neighboring C6 hydroxyl. Thus, Y145 is critical to hydrolytic function by encouraging the catalytic residue to engage in productive interactions with the substrate. Evaluation of the R294A and E221A variants established that, while these residues play little to no role in catalysis, they cooperatively bind the substrate with neighboring aromatic residues. Ultimately, we aim to incorporate these findings into a broad, fundamental model capable of describing processive glycoside hydrolase function.

Towards an understanding of the GLUT1 inhibition

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GLUT1 is one out of 14 proteins of the facilitative glucose transporter family. These membrane proteins regulate cell glucose uptake and thus are crucial for cell metabolism[1]. It is known that cancer cells have an elevated glucose uptake due to increased GLUT expression, therefore glucose transporters have emerged as potential drug targets for cancer therapy[2]. GLUT1 is overexpressed in various cancers and thus research on possible inhibitors, which would block GLUT1 activity, has been undertaken in recent years and several classes of inhibitors have been described[3].

Recently, a serie of novel salicylketoxime based compounds have been synthesized and shown to inhibit glucose uptake and cell proliferation in lung cancer cells, as well as possible binding mode at GLUT1 has been suggested based on computational studies[4]. However, in order to fully understand the protein-inhibitor interactions on a molecular level, an X-ray structure of GLUT1 in complex with these compounds is needed, therefore crystallization of such complex is the main focus of this study.

Currently, the expression and purification of both rat and human GLUT1 protein is being optimised to achieve a suitable protein sample for crystallization. Besides the structural biology approach, a serie of inhibitors has been tested in a giant vesicle assay[5] using recombinant GLUT1 protein and two lead compounds with high inhibition have been identified. Moreover, preliminary cell growth assays of selected inhibitors in leukemic cell lines have been carried out.

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

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

[3]Granchi, C. et al. (2014), Bioorg. Med. Chem. Lett., 24: 4915-4925.

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

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

Force field parameterization for 2'-hydroxybiphenyl-2-sulfinate, 2-hydroxybiphenyl, and related analogs

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2'-hydroxybiphenyl-2-sulfinate (HBPS) desulfinase (DszB) catalyzes the cleavage of the carbon-sulfur bond in the final step of dibenzothiophene (DBT) 4S pathway reactions. DszB is notable for its substrate specificity and exhibits product inhibition, responsible for hindering the overall reaction rate. As a result, we seek to understand the differences between substrate and inhibitor binding with DszB by performing molecular dynamic (MD) simulation, which will illustrate the molecular-level binding mechanism. However, we must first develop accurate molecular mechanics (MM) force fields parameters for the molecules of interest. Here, we develop and validate CHARMM-compatible force field parameters for use in classical MD simulations. For HBPS substrate, 2-hydroxybiphenyl (HBP) product, and a set of functional group representatives, such as 2,2'-biphenol (BIPH), 2-biphenyl carboxylic acid (BCA) and a set of highly constrained planar molecules, 1,8-naphthosultam (NTAM) and 1,8-naphthosultone (NAPO), the force field tool kit (ffTK) [1] in VMD was used to optimize molecules charges, bond distances, angles, and dihedral parameters. Optimized geometries were determined from quantum mechanical (QM) calculations. MD simulations of the molecules in explicit and implicit water solutions were conducted to assess the abilities of optimized parameters to recapitulate optimized geometries. Calculated infrared spectroscopies (IR) were obtained and compared with experimental IR spectroscopies for validation of the optimized MM parameters.

1. Mayne, C.G., et al., *Rapid parameterization of small molecules using the Force Field Toolkit.* J Comput Chem, 2013. **34**(32): p. 2757-70.

Multi-functional, primordial-like enzymes from bacteria with streamlined genomes

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Primordial metabolism is thought to have begun with a small number of weakly active but multi-functional enzymes. In contrast, contemporary enzymes typically have single and specific physiological functions. We are interested in understanding the structural, functional and mechanistic differences between primordial and contemporary enzymes, to shed light on processes of enzyme evolution.

Previously, we showed that the *Escherichia coli* cystathionine β-lyase (CBL) has promiscuous (non-physiological) alanine racemase (ALR) activity. CBL catalyzes the penultimate step of methionine biosynthesis, while ALR is essential for peptidoglycan biosynthesis. The two enzymes utilize the same cofactor, pyridoxal 5′-phosphate; however, they do not share any significant sequence or structural similarities. This suggests that modern CBL enzymes may be descended from a bi-functional CBL/ALR ancestor, but that alanine racemase function was replaced in most lineages by an alternate, non-homologous, ALR specialist.

We asked whether any extant bacteria retain multi-functional, primordial-like CBL enzymes. A search of >1,000 sequenced genomes revealed a handful of species in which CBL was present, but ALR was absent. The species lacking ALR all had unusually small genomes. We have characterized the CBL enzymes from three of them: *Pelagibacter ubique*, *Wolbachia pipientis*, and *Thermotoga maritima*. As predicted, each had ALR activity. More surprisingly, the *W. pipientis* and *T. maritima* enzymes were also glutamate racemases. While *W. pipientis* is a methionine auxotroph (and therefore no longer requires CBL activity), it appears that the *T. maritima* CBL uses a single active site to carry out three essential physiological roles. We determined its structure, which hints that gating access to the active site may be important for modulating these activities. Directed evolution, to search for mutations that affect one or more of the activities, is on-going.

These results emphasize that bacteria with streamlined genomes are our best models for studying primordial enzymology and metabolism.

Crystal Structure of Linoleate 13*R*-Manganese Lipoxygenase in Complex with an Adhesion Protein

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[X]Contributed equally to this work

Lipoxygenases (LOX) belong to a gene family with catalytic iron or manganese. The crystal structure of 13*R*-MnLOX of the fungus *Gaeumannomyces graminis* (Gg) and a predicted adhesion protein of *Pichia pastoris* (zonadhesin) was solved by molecular replacement. A comparison of Gg-MnLOX with the MnLOX of *Magnaporthe oryzae* (Mo) shows that the protein fold, substrate tethering, and the manganese ligands are conserved. The latter are superimposable in spite of three and four residues between two His metal ligands of Gg- and Mo-MnLOX, respectively. Gly-332 is positioned in the substrate channel between the entrance and the metal center. Replacements of Gly-332 with Ala and Val could restrict the access of oxygen and substrate to the active site in agreement with experimental data. C_{18} fatty acids are likely clamped with C-11 between $Mn^{2+}OH_2$ and Leu-336 and the 12*Z* double bond between His-290 and hydrophobic resides (Phe-337, Leu-336, and Phe-539). Phe-337 may control the direction of oxygen insertion at C-13, as previously suggested by site-directed mutagenesis. Phe-347 is positioned at the end of the substrate channel and the Phe347Leu mutation positions C_{18} fatty acids for oxygenation at C-9. These key residues are conserved in the active sites of Gg- and Mo-MnLOX, which differ mainly due to the hydrophobic residues in a pocket near the catalytic base and along the end of the substrate channel. Zonadhesin contains β-strands, has in two subdomains with an IgG-like fold, and appears to be the first crystallized fungal protein with structural similarities to collagen adhesins of Gram-positive bacteria.

Expression, purification and structural studies of mRNA methylation enzymes

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Mammalian nuclear RNA methylation enzymes METTL3 and METTL14 form a stable complex that adds N6-adenosine modifications to mRNA and non-coding RNA[1] . Both proteins are predicted to contain methyltransferase domains of the MT-A70 family and to date there is no crystal structure of this domain. The aim of this project is to characterize the structure of the METTL3-METTL14 complex to understand its mechanism and specificity in RNA modification.

We have successfully expressed the full METTL3-METTL14 complex in *sf21* insect cells using the MultiBAC expression system [2] .

Preliminary SAXS experiments were performed for the purified METTL3-METTL14 complex to determine its overall shape. Similar experiments were carried out for METTL3 and truncated METTL3. Crystallization trials are in progress.

References:

1. Liu, J. *et al.* A METTL3-METTL14 complex mediates mammalian nuclear RNA N6-adenosine methylation. *Nat Chem Biol* **10,** 93–95 (2014).
2. Trowitzsch, S.*et al*. New baculovirus expression tools for recombinant protein complex production. *J Struct Biol* **172,** 4554 (2010).

Structural and functional studies on human β-ureidopropionase

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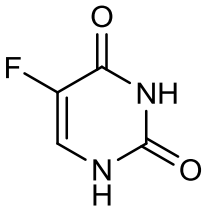
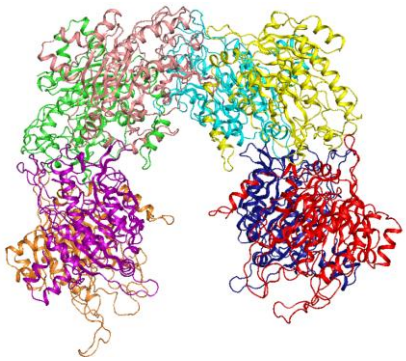
β-Ureidopropionase (EC 3.5.1.6), also known as β-alanine synthase (β-AS), catalyzes the final reaction of the reductive pyrimidine degradation pathway. This pathway converts uracil, thymine and pyrimidine analogues such as 5-fluorouracil (5FU) that are often used as antineoplastic agents to the corresponding β-amino acids, carbon dioxide and ammonia. Cancer patients suffering from β-ureidopropionase deficiency are potentially at risk of developing severe toxicities following intake of 5FU as high doses are administered to account for its usually rapid metabolic inactivation.

In this ongoing work structural and functional studies are performed on human β-ureidopropionase, whose activity is allosterically regulated by reaction substrate and product via an oligomerization-dependent mechanism. The native enzyme concomitantly exists in several different oligomeric states, which has thus far prevented its crystallization. Certain gene mutations found in β-ureidopropionase deficient patients shift their equilibrium towards less or inactive lower molecular species [1].

We are currently targeting putative oligomerization interfaces by site-directed mutagenesis to stabilize specific oligomerization states, and thereby facilitate crystallization and structure determination of the enzyme as well as further investigation of its regulation mechanism.



5-Fluorouracil



β-ureidopropionase

[1] A.B.P. van Kuilenburg, D. Dobritzsch, J. Mejer, M. Krumpel, L.A. Selim, M.S. Rashed, B. Assmann, R. Meinsma, B. Lohkamp, T. Ito, N.G.G.M. Abeling, K. Saito, K. Eto, M. Smitka, M. Engvall, C. Zhang, W. Xu, L. Zoetekouw, R.C.M. Hennekam. **β-ureidopropionase deficiency: Phenotype, genotype and protein structural consequences in 16 patients.** (2012) *Biochimica et Biophysica Acta* **1822**: 1096-1108

Microseed matrix-screening (rMMS): introduction, theory, practice and a new technique for membrane protein crystallization in LCP

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Random Microseed Matrix-Screening (rMMS), where seed crystals are added automatically to random crystallization screens, is a significant recent breakthrough in protein crystallization [1]. During the eight years since the method was published, theoretical understanding of the method has increased [2 - 4], and several important practical variations of the basic method have emerged [5, 6]. We will briefly describe some of these variations, including cross-seeding, and introduce a novel method of making LCP seed stocks by scaling up LCP crystallization conditions. We will also describe a method of generating seed gradients across a plate so that the number of crystals in each LCP bolus can be varied, with a practical example.

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[1] D'Arcy, A., Villard, F, and Marsh, M. "An automated microseed matrix-screening method for protein crystallization." Acta Crystallographica Section D: Biological Crystallography 63.4 (2007): 550-554.

[2] Shaw Stewart, P. D., Kolek, S. A., Briggs, R. A., Chayen, N. E., & Baldock, P. F. (2011). Random microseeding: a theoretical and practical exploration of seed stability and seeding techniques for successful protein crystallization.*Crystal Growth & Design*, *11*(8), 3432-3441.

[3] D'Arcy, A., Bergfors, T., Cowan-Jacob, S. W., & Marsh, M. (2014). Microseed matrix screening for optimization in protein crystallization: what have we learned?. *Acta Crystallographica Section F: Structural Biology Communications*, *70*(9), 1117-1126.

[4] Shaw Stewart, P. D, & Mueller-Dieckmann, J. (2014). Automation in biological crystallization. *Acta Crystallographica Section F: Structural Biology Communications*, *70*(6), 686-696.

[5] Obmolova, G., Malia, T. J., Teplyakov, A., Sweet, R. W., & Gilliland, G. L. (2014). Protein crystallization with microseed matrix screening: application to human germline antibody Fabs. *Structural Biology and Crystallization Communications*, *70*(8).

[6] Abuhammad, A., Lowe, E. D., McDonough, M. A., Shaw Stewart, P. D., Kolek, S. A., Sim, E., & Garman, E. F. (2013). Structure of arylamine N-acetyltransferase from *Mycobacterium tuberculosis* determined by cross-seeding with the homologous protein from *M. marinum*: triumph over adversity. *Acta Crystallographica Section D: Biological Crystallography*, 69(8), 1433-1446.

Structure of the antenna baseplate from a green sulphur bacterium.

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Photosynthetic antenna systems enable organisms to harvest light and transfer the energy to the photosynthetic reaction centre, where the conversion to chemical energy takes place. One of the most complex antenna systems, the chlorosome, found in the photosynthetic green sulphur bacterium *Chlorobaculum tepidum* contains a baseplate, which is a scaffolding super-structure, formed by the protein CsmA and bacteriochlorophyll *a*. Here we present the first atomic-resolution structure of the CsmA baseplate using preparations of intact fully functional, light-harvesting organelles from *Cba. tepidum*, by combining five complementary methods: solid-state NMR spectroscopy, cryo-electron microscopy, isotropic and anisotropic circular dichroism and linear dichroism. We show that the baseplate is composed of rods of repeated dimers of the strongly amphipathic CsmA with pigments sandwiched within the dimer at the hydrophobic side of the helix.