The complete 2011 Experiment Report of ACDC

11/Feb/2011 (ID14-4)

* Clp/Hsp100 (Clausen Group):

Bacterial AAA (ATPases associated with a variety of cellular functions) unfoldases play a crucial role in regulatory proteolysis and protein quality control. To better understand the molecular details of these unfolding machines we aim for structural data of a specific AAA unfoldase from *M. tuberculosis*. We have previously identified two different crystal types, both grown in 15% PEG 4k/0.1 M NaCl/Tris-HCl, pH 8.5 at 19 °C and 25 °C, respectively. The first crystal species belongs to space group P1 (unit cell parameters 122 x 122 x 244 Å), can only be obtained in the presence of ATP analogues and shows diffraction to approximately 8 Å. Only very few crystals diffracted up to 5 Å, *i.e.* into the range required for Molecular Replacement. The second type of crystals, *i.e.* the one obtained at elevated temperature, belongs to space group $P2_12_12_1$ with unit cell parameters of 120 x 132 x 172 Å, is derived from apo enzyme, and shows slightly better diffraction to up to 4.5 Å. In addition, we performed the first heavy atom soaking experiments using both crystal forms, the results of which are the following (about 50 crystals tested): Hg, Pt and Er compounds destroyed the crystal lattice after 15 min of incubation, whereas corresponding short soaks (5 minutes or shorter) did not result in efficient heavy atom binding. In the future, we will enforce our attempts in growing SeMet crystals and co-crystals with stochiometric amounts of the most reactive Hg and Pt derivatives as well as.

* Antigen processing proteases (Brandstetter Group)

Localized mainly to endo/lysosomes, legumain plays an important role inexogenous antigen processing and presentation. The cysteine protease legumain, also known as asparaginyl endopepetidase AEP, is synthesized as a zymogen and is known to undergo pH-dependent autoproteolytic activation whereby N-terminal and C-terminal propeptides are released. However, important mechanistic details of this pH-dependent activation as well as the characteristic pH activity profile remain unclear. We could crystallise human legumain in the tetragonal space group $P4_2$ with unit cell dimensions of 64 x 64 x 79 $Å^3$. Crystals diffracted to better than 2.5 Å resolution. We further suceeded to collect isomorphous mercury derivative data which were used to collect SIRAS data. Structure determination is underway.

* Clostridial collagenases (Brandstetter Group)

We measured specific collagenase-inhibitor complex crystals that were critical to delineate the catalytic mechanism of this enzyme. Furthermore, several mutant crystals were measured to confirm and refine expected mechanisms.

Apr/2011

* PDZ protease (Clausen Group):

We optimized crystals of a bacterial PDZ protease involved in sporulation regulation. CtpB plays a major role in signaling the state of the genetic program from the mother cell to the forespore. The protein was cloned from *B. subtilis* excluding the N-terminal signal sequence and autocleavage sites and harboring a mutation of the active site serine to alanine. Crystals grew in MPD, PEG4000 and Imidazol pH 8 and diffracted up to ~ 2.6 Å. Symmetry was determined to be P1, with a dimer in the unit cell. The structure was solved by molecular replacement using our previous lower resolution CtpB structure as a search model and shows CtpB in a resting state.

* Unc45 (Clausen Group)

The evolutionary conserved myosin chaperone UNC-45 plays a crucial role during muscle development. To better understand the molecular details of this specialized chaperone we initiated a structural analysis of the UNC-45 protein from *C. elegans*. We were able to solve the structure of the full length wild type protein that represented - due to the crystals relatively large unit cell (a=b=85 Å, c=810 Å), a challenging crystallographic project. In this measurement, we aimed for the following structural data:

(1) Complexes with substrate: To address the myosin directed chaperone function of UNC-45 we studied the interaction of the chaperone with its substrate protein. To this end we synthesized peptides corresponding to amino acid stretches of the substrate protein myosin. Wild type SeMet-protein crystals were soaked with potential peptide ligands. Unfortunately the crystals seem to have suffered from the soaking process and their datasets are difficult to index and process. Co-crystallization trials with the peptides are currently ongoing.

(2) Complexes with co-chaperones: In the process of myosin folding UNC-45 additionally recruits the general chaperones Hsp70 and Hsp90. Short characteristic amino acid motifs at the C-termini of these two proteins allow them to interact with the TPR domain of UNC-45. Uncovering the binding mode of these motifs is of particular interested since it could outline the overall orientation of the full length proteins. We tried to co-crystallize native UNC-45 with peptides corresponding to the last 10 – 20 amino acids of Hsp70 or Hsp90. Crystals appeared in the presence of peptides in the same crystallization condition as apo UNC-45 crystals. We aimed to solve the structure by molecular replacement. Since this was not successful so far we are planning to set up new co-crystallization trials with SeMet-derivatized protein.

24/Jul/2011 (ID29)

* Clp/Hsp100 (Clausen Group):

Growing SeMet crystals has been unsuccessful so far for both crystal types. Hence we performed heavy atom soaks using Ta_6Br_{12} clusters in different concentrations and for a range of incubation times. Additionally, we tested slightly different conditions for the cryo buffer. At this beamtime, we have tested 10 crystals of type 2 that had been soaked with Ta_6Br_{12}. However, the incorporation of Ta_6Br_{12} into the crystal lattice turned out to destroy the crystal lattice (as judged from a comparison with native crystals).

*PDZ protease (Clausen Group)

Further crystal-trials with the bacterial PDZ protease CtpB from *B. subtilis* were performed using active forms of the protein, leading to the discovery of a new crystallization condition in sodium malonate pH 7.8. Crystals diffracted to 2.7 Å and had a $P2_1$ space group. Two dimmers of CtpB (four protomers) are contained in the asymmetric unit. The phase problem was again overcome by molecular replacement using our previous CtpB structure as a search model. This new crystal structure of CtpB shows the protease in an active state, with clear differences in the flexibility of the peptide binding PDZ-domains between the two dimmers. Moreover, electron density corresponding to PDZ bound peptide could be identified. Presumably these peptides were co-purified from the *E. coli* expression and co-crystallized.

10/Sept/2011 (ID23.2)

* SAS-6 (Dong Group)

Centrioles are self-replicating cylindrical organelles with an unusual nine-fold symmetry. One of the centriolar proteins, SAS-6, plays an essential role in centriole assembly. We have crystallized the coiled-coil domain of *C. elegans* SAS-6. During this visit we screened 20 SeMet-substituted crystals. Two of them diffracted to 3.3-Å resolution (Space group P61, a=b=141Å, c=73Å). The structure has been determined and functional studies based on the structure have been carried out. The manuscript is now finished and will be submitted in late February 2012.

* PLK-4 (Dong Group)

PLK-4 is a SAK kinase essential for cell division. It regulates centriole duplication and its dysfunction has recently been linked to tumor development. PLK-4 has three domains, the N-terminal catalytic domain, the central substrate binding domain (CPB), and the C-terminal regulatory domain (CTD). While crystal structures of both the NTD and the CTD are already known, structure of the central domain (CPB) has never been reported before. This has greatly limited functional dissection of PLK-4, as the CPB is one of the most important parts of PLK-4, directing its specific targeting of substrates in the cell. We screened about 50 crystals of SeMet-substituted crystals of the CPB during this visit. The best crystals diffracted to ~2.4 Å and belong to the space group P2 (a = 53.4 Å, b = 60.1 Å, c = 87.5 Å, β = 93.31). We collected four fine-slicing data sets, which turned out to be critical for successfully determining the structure. Now we have built the model and are carrying out biochemical and in vivo studies to test our hypothesis how the CPB specifically recognizes its substrates. We are expecting to publish a very interesting story based on the structure in the near future.

* Blood coagulation (Brandstetter Group)

Blood coagulation is a first line of response triggered by vessel injury to stop bleeding. To better understand the tight regulation of this system, we aim to obtain structural data that explain the secondary activation processes as found in coagulation factors VIIIa and IXa. We have measured crystals of superactivated factor IXa mutants with different agonistic and antagonistic compounds. Crystal belonging to space group $P2_12_12_1$ with unit cell constants of 44 x 47 x 98 $Å^3$ diffracted to beyond 2 Å resolution, partially to beyond 1.5 Å resolution. Allosteric cross-talking from cofactor binding sites to the active site spanning more than 30 Å could be experimentally traced by difference Fourier techniques.

27/Oct/2011 (ID23-2)

* Clp/Hsp100 (Clausen Group):

In an alternative approach to crystallize bacterial AAA unfoldase, we used limited proteolysis to identify stable sub-structures of the protein. We were able to identify 2 N-terminally truncated versions (Δ16N, Δ47N) which we cloned, expressed and purified. Crystallization trials led to large crystals that grew in 10% PEG 8k/6% glycerol/100 mM KCl and diffracted poorly in-house. At this beamtime, we have tested approximately 100 of those crystals that differed with regards to incubation temperature, addition of additive(s), heavy atoml soaks and dehydration protocols. However, the diffraction pattern turned out to be very smeary and diffraction was limited to approx. 8 Å regardless of the protocol applied. Secondly, we identified a third class of crystals containing apo full-length unfoldase which grow in a similar condition as the other 2 types, however at reduced temperature. The crystals take much longer to grow and belong to space group P1 (though close to $P2_1$) with unit cell parameters of 97 x 250 x 123 Å and a diffraction limit of approximately 4 Å. At this beamtime we were able to collect 2 native datasets of this new class of crystals. Heavy-atom search for all three crystal types is still ongoing.

* Monoglyceride lipase –bMGL (Gruber Group)

This project focuses on determining the structure of bMGL in complex with various substrate analogues. Monoglyceride lipases catalyse the hydrolysis of monoacylglycerol into free fatty acid and glycerol. So far, we have determined the structure of bMGL in free form. The data collected at ESRF were of bMGL co-crystallised with a substrate analogue containing 18-carbon alkyl chain. The crystals diffracted to 1.8 Å and 1.6 Å respectively. The electron density for the substrate analogue was not observed at the active site. Currently, we are optimizing the crystallization of this complex and will continue to pursue the study in the forthcoming years as well.

* Legume lectins I (Gruber Group)

The legume lectins from the subtribe *Diocleinae*, often referred to as concanavalin A-like lectins, are a typical example of highly similar proteins that show distinct biological activities. The relative position of amino acids within the carbohydrate-binding site are factors that have been reported to contribute to these differences in the activities of *Diocleinae* lectins. The unbound structure of *Dioclea rostrata* lectin (DRL) was previously solved. Our current project aims at solving the structure of DRL with the dimannosides Man(alpha1-3)Man(alpha1-O)Me and Man(alpha1-4)Man(alpha1-O)Me. We collected data sets for both complexes at the ESRF and they are currently being processed. Analysis of DRL in complex with the dimannosides will shed some light in the structure-activity relationship of lectins belonging to the *Diocleinae* subtribe.

* Legume lectins II (Gruber Group)

Plant lectins comprise the best studied group of carbohydrate-binding proteins; and within this group, those purified from species of the *Leguminosae* family are the most studied members. Within this family, lectins isolated from seeds of the *Diocleinae* subtribe exhibit different biological activities, despite their high degree of sequence identity. A lectin purified from the seeds of *C. boliviana* Piper (Cbol), a plant found in the Amazon rainforest, was previously crystallized in the presence of different ligands for the studies of structure-relationship with the

distinct biological activities displayed by this protein. We collected a data at the ESRF for the unbound form of CboI at a resolution of 3.4 Å. We are currently optimizing the crystallization conditions to collect a higher resolution data set, which is necessary for the sort of analysis we are aiming at.

5/Nov/2011 (ID23.1)

* Clostridial collagenases (Brandstetter Group)

Collagen constitutes one-third of body protein in humans, reflecting its extensive role in health and disease. Clostridial collagenases are involved in host colonisation and infiltration by clostridia, including severely pathogenic forms such as C. difficile or C. tetani. Despite their importance, the structural and mechanistic basis of these enzymes has remained elusive. We obtained crystals of several variants of collagenases, albeit mosaic and anisotropically disordered along the long axis. Crystals belong to space group $P2_12_12_1$ with unit cell constants of 58 x 109 x 182 $Å^3$. At this beam time we could collect native diffraction data to better than 3 Å resolution. Additionally, we attempted to collect at the Zn^{2+} edge of this metalloprotease, however the anomalous signal was very small and insufficient for phasing.

1/Dec/2011 (BM14)

* Novel glycosyltransferases (Gruber Group)

Glycosyltransferases are the large group of enzyme interesting for the industrial applications. They are involved in the post-translational modifications of the biotherapeutical proteins. This process can occur both in vivo and in vitro. In our project we focus on the sialyltransferases, a subgroup of glycosyltransferases. These enzymes transfer sialic acid between two molecules. The donor is mostly activated molecule of cytidine-5'-monophospho-N-acetylneuraminic acid (CMP-NANA) or a sialylated carbohydrate. Sialyltransferases are wide spread proteins originating from mammalian as well as bacterial cells. A native data set suitable for structure determination was collected to the resolution of 1.7 Å (C2221 78.44 143.86 83.25 90.0 90.0 90.0). Only apo enzyme crystals are available and we are currently conduction soaking experiments.

*FMN-dependent oxidoreductases (Gruber Group)

Crystal structures of FMN binding proteins from *Pyrococcus horikishii* (Pho) and *Thermus Thermophilus* (Tth) have been determined to 1.8 / 1.5 Å respectively. Several datasets were collected on the native crystals soaked with putative substrates and inhibitors. Complex structures of both proteins with one of the substrates have been elucidated and a mechanistic pathway will be proposed.

* Surfactant protein Lv-ranaspumin (Keller Group)

Lv-ranaspumin is a natural surfactant protein with a molecular mass of 23.5 kDa which was isolated from the foam nest of the frog *Leptodactylus vastus*. Only a partial amino-acid sequence is available for this protein and it shows it to be distinct from any protein sequence reported to date. The protein was purified from the natural source and crystallized. A complete data set was collected to 3.5 Å resolution. The crystal belonged to the orthorhombic space group P212121. Since the complete amino acid sequence remains unknown, a search for a molecular replacement model is not viable. We are currently working on the incorporation of heavy atoms to solve the structure by MIR/MAD. Additionally, lv-ranaspumin possesses eight cysteine residues, which might be sufficient to allow S-SAD phasing. The behaviour of natural surfactant proteins is still poorly understood, and solving lv-ranaspumin will help to elucidate the long-term stability observed for these proteins and their role in formation of the foam nest.

* The allergen Api-Bet-1 (Keller Group)

Api-Bet-1 is an allergen currently under investigation in our group. It crystallize under the space group P4122 and the cell parameters are 52.5 52.5 131.4 90.0 90.0 90.0. The crystal measured at the ESRF diffracted to a resolution of ~3.1 Å. Trials to solve the structure by molecular replacement lead to structures with R-values of ~55%. Further crystallization experiments to optimize the crystallization condition for high quality crystals are in progress.

* SAS-6/SAS-5 complex (Dong Group)

We screened 10 crystals of the SAS-6/SAS-5 complex. Unfortunately, none of them diffracted better than 7-Å resolution. We are now trying the complex using different constructs of both proteins in hope of obtaining well diffracting crystals.

* PLK-4 (Dong Group)

We tested ~20 crystals of a longer construct of PLK-4, containing both the CPB plus the CTD. Most of the crystals diffracted to ~5-7-Å resolution. However, we found a single crystal that diffracted to 3-Å resolution. The structure has been solved by molecular replacement, which indicates a different molecule packing from the structure we determined earlier for the CPB alone. This is very helpful for us to propose an oligermization model that might prove to be essential for its function in vivo.

* Clostridial collagenases (Brandstetter Group)

We again tested collagenase crystals that had seleno methionine incorporated. Many crystals were of insufficient quality with respect to resolution (worse than 4 Å) and mosaicity/anisotropy. We identified two suitable SeMet crystals diffracting to 3.2 Å resolution that eventually allowed the initial phasing of the collagenase crystals.

* Allergen modification (Brandstetter Group)

The major birch pollen allergen Bet v 1 undergoes several posttranslational modifications that are thought to be relevant to its allergenicity. At the same time, closely related Bet v 1 isoforms are hypo-allergenic with low IgE binding and different T-cell polarization. We have crystallised hyper- and hypo-allergenic Bet v 1 isoforms to structurally characterise and map their respective ligand binding behaviour. Crystals belong to monoclinic space group $P2_1$ with unit cell dimensions of 33 x 56 x 38 $Å^3$, β= 94° and diffract to better than 1.5 Å resolution. We have identified a key residue within the hydrophobic binding pocket of Bet v 1 which discriminates hypo- to hyper-allergenic binding properties.