Bag reports, AC-DC first year

10/Sept/2010 (ID23.2)

* 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*. At this beamtime, we have tested 150 crystals (grown in 15 % PEG 4k/0.1 M NaCl/Tris-HCl, pH 8.5) of which the majority diffracted to approximately 8 Å. Only very few diffracted up to 5 Å, *i.e.* into the range required for Molecular Replacement. They belonged to space group $P6_2$ with 3 molecules in the asymmetric unit indicating that the hexameric state had been captured within crystals.

* 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. Several temperature-sensitive mutations of UNC-45 have been identified that show a severe defect in muscle formation in *C. elegans*. One of them, a glycine to glutamate mutation, displays a highly disordered sarcomere structure in a phenotypic analysis. To visualize possible structural alterations in this mutant, we cloned, expressed and purified it and could obtain SeMet-derivatized crystals with a very similar morphology as for the wild-type protein. Nevertheless, growing single crystals was more challenging and for the beamtime in September we tested 30 crystals. Only one of them diffracted to 3 Å and we collected several SAD datasets (180° with inverse beam strategy) on the same crystal at the selenium edge for high redundancy. The crystal showed a shorter unit cell axis (a=b=86 Å, c=718 Å, spacegroup $P6_122$) and we could solve the structure by the use of experimental phases and obtained a nice electron density at 3.4 Å resolution. Model building and refinement is currently ongoing but due to a high flexibility of some parts in the structure, the refinement is not as straightforward as expected. Additionally, we collected several SAD datasets of wild type SeMet-protein soaked with potential peptide ligands. The crystals seem to have suffered from the soaking process and their datasets are difficult to index and process.

05/Nov/2010 (ID23.1)

* *Ce*SAS6 (Gang 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 ~30 SeMet-substituted crystals. Most of them diffracted to 5-7 Å, but a couple diffracted to ~3.7 Å (Space group $P6_1$, a=b=141Å, c=73Å). We have generated experimental E-maps and traced both 150-residue helices. Biochemical studies are now being carried out to test our hypothesis how SAS-6 and SAS-5 cooperate in centriole assembly.

* *Tb*BILBO1 (Gang Dong Group): Sleeping sickness is a disease caused by the parasitic organism *Trypanosoma brucei*. This disease is commonly found in sub-Saharan Africa and leads to significant mortality each year. However, effective treatments of this disease are still unavailable until now. All endo- and exocytosis in *T. brucei* are through a unique cellular compartment, the flagellar pocket (FP), which plays crucial roles in the cell's defense against the host immune system. The 67-kDa BILBO1 is the first characterized FP cytoskeletal protein and is an integral component of the flagellar pocket collar (FPC). This protein is essential for both FP biogenesis and the viability of the parasites. To elucidate the high-resolution structure of the *Tb*BILBO1, we have cloned different domains of this protein (the full-length protein forms large oligomers). During this visit, we screened 20 crystals for the C-terminal domain (42kDa), which typically diffracted to 3-4 Å. The space group is C2 (a=77Å, b=36Å, c=135Å; β=95.6°). We have collected a few SAD/MAD data sets. But due to the high mosaicity, we are now optimizing the crystallization and freezing conditions.

02/Dec/2010 (ID29)

* Clp/Hsp100 (Clausen Group): We again tested 150 AAA unfoldase crystals and additionally identified a second crystal form even diffracting up to 2.0 Å (space group P212121, having again 3 molecules in the asymmetric unit). However, as Molecular Replacement was not successful so far, we have to solve the phase problem by the SAD or SIRAS methods using SeMet substituted protein or heavy atom soaking/co-crystallization.

* OMP assembly (Clausen Group): We performed crystallization experiments with proteins of the β-barrel assembly machinery to get insights how this complex participates in the maturation of outer membrane proteins and interacts with the periplasmic chaperones DegP, SurA and Skp. We managed to grow native crystals of the BamCD sub-complex from *E. coli* (crystallization condition: 0.1 M HEPES pH 7.5, 1.5 M Li_2SO_4) in space group C222 (unit cell

constants a=140, b=110 and c=425A). These crystals diffracted to 3.1 Å. Today, no suitable models for either of the constituent Bam C and BamD proteins have been deposited in the protein data bank. Therefore, we will try to overcome the phase problem by using heavy atom derivatives since SeMet-derivatized proteins did not crystallize.

* Stress response mechanisms (Clausen Group): Protein kinases and their cognate phosphatases represent promising targets to interfere with cellular signaling and/or survival. Detailed structural analyses are required to discern unique features for designing specific inhibitors. To investigate the structural characteristics of YwlE, a low molecular weight protein tyrosine phosphatase that is critical during bacterial stress response and pathogenesis, we crystallized the protein from *B. subtilis* (crystallization condition: 25% PEG400, 100 mM Tris pH 8.3) and solved the structure by molecular replacement using our recently solved structure of YwlE from *B.stearothermophilus* as template. In contrast to YwlE from *B. stearothermophilus*, the *B. subtilis* protein occurred as a monomer. However to our great surprise, one residue of the C-terminal tail of the molecular neighbor protruded into the active site defining the substrate binding pocket. Actually we are testing the current working model regarding to substrate-binding and enzymatic catalysis by mutational and biochemical analyses.

11/Feb/2011 (ID14.4)

* PDZ proteases (Clausen Group): We obtained crystals from a bacterial PDZ-protease involved in sporulation, which signals the state of the genetic program from the spore to the mother cell. The protein was cloned from the thermophile *Geobacillus stearothermophilus*, excluding the N-terminal signal sequence and autocleavage sites. Moreover, the active site serine was mutated to alanin. The crystals, grown in PEG4000, ammonium acetate and sodium citrate pH 5.5, diffracted up to 2.5A resolution, in space group XXX having XXX molecules in the a.u. Unfortunately, the crystals exhibited a slight disorder in direction of the crystallographic c-axis thus weakening the anomalous signal of the SeMet incorporated resides. In upcoming experiments, we plan to screen different additives and ligands to overcome this disorder problem and determine phases by SAD to solve the first crystal structure of this protease class.

* Clp/Hsp100 (Clausen Group): We performed the first heavy atom soaking experiments using both crystal forms, the results of which are the following (about 100 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.

* Kinetochore protein complexes (Stolt-Bergner Group): We are performing structure-function analysis of sub-complexes of the outer kinetochore to determine how essential components are recruited to the kintechore. Crystals of one target protein complex were tested during two different BAG beamtimes, resulting in the collection of a native data set with diffraction to 2.1 angstroms. Structure solution will first be attempted with molecular replacement.

* Secondary transporters (Stolt-Bergner Group): We are focusing on structural characterization of secondary active transporters of the proton oligopeptide transporter family, which drive the transport of di- and tri-peptides across the membrane by coupling this process to the energetically favorable transport of a proton. Crystals of a target integral membrane transporter as well as a complex of the transporter with a Fab fragment were tested during two different BAG beamtimes. The best crystals identified so far showed diffraction to approximately 8-10 angstroms. As these crystals are quite small and diffract poorly, analysis with a micro-focus beam will be attempted next.

* Allergen-IgE-Fab complexes (Keller Group): Alt-a-1 is the major allergen from *Alternaria alternata*. More than 80% of *Alternaria alternata* sensitive individuals recognize the major allergen Alt-a-1. The protein is heat stable dimer of ca. 29 kDa. Development of novel therapeutics requires detailed knowledge of the 3D structure and in particular the epitopes of the dimer, is needed. The crystal of the native protein diffracted up to ~2A. So far no suitable heavy atom derivative required to solving the structure of Alt-a-1 have been found (at this beamtime, we tested about 10 heavy atom soaked crystals). Therefore, further soaking and co-crystallization experiments are in progress.

* Dipeptiylpeptidase III (Gruber Group): The DPP-III family comprises enzymes cleaving off N-terminal dipeptides of small oligopeptides. Their physiological functions are diverse and studies are underway to develop inhibitors for pain medication. We are aiming at determining structures of complexes of human DPP-III with substrates and inhibitors to elucidate the determinants of substrate specificity and to form the basis for structure-based inhibitor design. During the recent BAG-beamtime we collected datasets from crystals of an inactive variant of human DPP-III in complex with tynorphin (VVYPW). The crystals diffracted to about 3 Å resolution. The structure will be solved by molecular replacement.