Report 2010

In the last year we collected data at two ESRF trips (ID23-eh2 09-10/04 and BM14U 11-12/06).

In total we have collected roughly 30 datasets all together, from which 12 structures were determined, of which some still are in the refinement process. We finished structural refinement on tPphA mutants (A/B) a Ser/Thr phosphatase from *T. elongates* at 1.7Å, LysG a transcription regulator from *C. glutamicum*, a mutant of the ATPase CopB from *S. solfataricus*. Several structures on the Ras & Rho inhibition for example RhoA in GppNHp state in complex with a peptidomimetic terphenyl mimicking the RhoA binding site of the Rho effector ROCK at 2.2 Å; phasing by molecular replacement. RhoA in GppNHp in absence of the peptidomimetic terphenyl, as a reference structure for the terphenyl complex at 1.7 Å, as well as the HR1 domain of the Rho kinase ROCK at 3.5 A here however the crystals need to be improved to obtain higher resolution data.

Several datasets were collected on the GAP proteins associated with human diseases project: BAR-PH domain of GRAF1at 4.0 Å. The GAP domain of GRAF1: diffracted to 1.9 Å, and the structure has been build and refined. The complex of the BAR-PH domain of OPHN1 in complex with the GAP domain: yielded a complete native datasets to 3.4 Å. Furthermore the structure of the PEP-Carboxylase from Flaveria was solved at 2.5 Å after collecting a dataset at the BM14U

During the last two trips two structures were solved from the binding protein OpuBC from *S. subtilis* at 1.7 Å and 1.8 Å resolution. These structures contain two different substrates and shed a light on the high substrate affinity and selectivity. Also the structure of a mutant of ProX the glycine betaine binding protein from *A. fulgidus* was solved and reveals how this protein captures its substrates and switches between a high ands low affinity state.

GLOBAL SUMMARY:

We improved native data for the membrane bound light harvesting complex from A. carterae. Due to small crystals and poor reproducibility many crystals were screened. The crystals are relatively stable in the X-ray beam, probably
to the presence of a large number of carotenoids in the complex. Datasets
are now availible for different crystal forms with resolution op to 3.0 Å. Due to increased reproducibility focus shifted to screening for heavy atom derivatives. Pt/Hg soaks yielded two different datasets at 3 Å. Unfortunally up to now the high anisotropy of the crystals did not allow the detection of the heavy atom sites in the datasets.
The structure of ProX an glycine betaine binding protein was solved at 1.6 Å , which revealed a the binding mechanism and explains how this binding proteins bind and release there substrate using a switch from high to low affinity substrate binding (manuscript in preparation)
By solving the octopine dehydrogenase structure in complex with agmatine it is now clarified why this enzyme is highly specific for L-arginine as a substrate. The side chain of arginine induces a conformational change, which is needed to create the binding site for the second substrate pyruvate. This concludes an ordered binding mechanism, which was on debate for a long time (Smits et al 2010).

Besides all the structures solved several datasets were collected on membrane protein crystals which quality improved but are not solved yet. For example the ABC transporters MsbA and NisT. Over 80 crystals were tested on their diffracting quality. The usage of the microfocus beamline improved the
resolution for MsbA from 8 Å to 6 Å using the same crystal.
Since then the crystals we further improved and a low resolution dataset was collected likeky consisting of a MsbA dimer in the asymmetric unit.

We have solved the structure of the c14 ring from spinach chloroplasts last year (Vollmar et al., 2009). This
subcomplex of the membranous Fo domain is the first atomic structure of the rotor ring of a proton translocating FoF1 ATP synthase. Motivate by this success we increased our efforts in crystalising the whole FoF1 ATP synthase. Initial crystals were obtained and tested. Although the diffraction quality is quite poor (15 Å). We are optimistic that the usage of the humidifier, microfocus beamline and improvement of the crystals will increase the resolution.
We solved the structure of PEP-Carboxylase from Flaveria at 2.5 Å resolution and the structure shows some similarities but also significant differences to the known other PEP-Carboxylase structure. The refinment is almost finished and the manuscript prepared.

5 selected publications in the last year that acknowledge the ESRF beamlines:

1) Schulte T., Niedzwiedzki D.M., Birge R.R., Hiller R.G., Polívka T., Hofmann E., Frank H.A. (2009) Identification of a single peridinin sensing Chl-a excitation in reconstituted PCP by crystallography and spectroscopy
Proc. Natl. Acad. Sci., 106, 20764-20769

2) Schulte T., Hiller R.G., Hofmann E. (2010)
X-ray structures of the peridinin–chlorophyll-protein reconstituted with different chlorophylls FEBS Letters., 584, 973-978

3) Wolf S., Freier E., Potschies M., Hofmann E., Gerwert K. (2010)
Directional Proton Transfer in Membrane Proteins Achieved through Protonated Protein-Bound Water Molecules: A Proton Diode
Angewandte Chemie, published online ahead of print, DOI: 10.1002/anie.201001243

4) Christine Oswald, Sander H.J. Smits, Marina Höing, Erhard Bremer, Lutz Schmitt
Structural analysis of the choline-binding protein ChoX in a semi-closed and ligand-free conformation Biological Chemistry 2009 390:11 , 1163-1170

5) Smits SHJ, Meyer T, Mueller A, van Os N, Stoldt M, et al. 2010 Insights into the Mechanism of Ligand Binding to Octopine Dehydrogenase from Pecten maximus by NMR and Crystallography. PLoS ONE 5(8): e12312. doi:10.1371/journal.pone.0012312

Generall comment to beamline performance

In general the performance of the beamlines is very satisfactory. The sample changer performance could be improved. During a standard remote data collection at least twice in 5h manual handling is necessary due to inability
to unmounted samples or due to loss of vial in the sample changer, leading to blocks. Also the overall reliability of bar code reading could be improved. The sample changers seem to be sensitive to misalignment, which means that
the current interval for realignment/inspection is too short.
Remote data collection has proven to be very useful and reliable. We use it now during every trip at least for several hours. We do not use the ISPYB database productively yet. Usage of DNA is not useful for crystals, for which several spots have to be tested. This might be an interesting option to include.
Unfortunally, the second trip was not satisfactory. The BM 14-U beamline was broken and although we could obtain measuring time at other beamlines we missed the multiple wavelength beamline and could not measure the seleno-methionine and heavy atom soaked crystals. However the beamline scientis was very helpful in organizing the other beamlines.
Besides this, the operation on the beamline appeared to be satisfactory. On all trip we were able to measure crystals and had the possibility to spend one hour or so on the testing of new project and or new crystal forms.