EMBL-Heidelberg bag - LS1931 – February 2001 – July2001

Elena Conti

• TAP

The structure of the C-terminal nucleoporin-binding domain of TAP in complex with its partner p15 has been solved and refined.

The structure was solved with a MAD experiment on a mercury derivative that was carried out at the end of January at Elettra. The urgent proposal submitted to Elettra was a result of competition on the project combined to ESRF MAD beamtime available only in May (and that was also required for other BAG projects, see Scheffzek). The structure reveals that 5 of the 8 methionines are disordered, providing an explanation for the failure of the Semet MAD experiments carried out in the previous proposal to give usable phases (see LS1801 report).

We collected 2.8 Å resolution data from crystals of the TAP - p15 heterodimer in complex with a nucleoporin peptide (ID14-2, 1-4-01). The structure of the ternary complex is solved and refined, and the paper is in press.

We also collected data of the TAP-p15 heterodimer in complex with other nucleoporin peptides with different amino acid sequences (ID14-2, 28-5-01). The structures are solved and the electron density shows that both peptides are bound, but the resolution is too low (3.2 Å and 3.4 Å, respectively) to elucidate the details of substrate binding. We plan to extend the resolution of these complexes.

• Exportin

We are continuing to screen for suitable crystal forms of these 150 to 180 kDa ternary complexes. The resolution of the two crystal forms of the 180 kDa complex could not be extended beyond 8 Å, suggesting that we should screen for another crystal form (ID14-2, 1-4-01). The diffraction limit of a fourth crystal from (plate-like crystals) that we obtained recently could not be reasonably assessed due to lack of suitable harvesting / freezing conditions.

In case of the 150kDa complex, the previous crystal forms could be improved only in terms of size, but not in terms of diffraction properties (~8Å), even after establishment of suitable freezing conditions. We since obtained a third crystal form, which diffracts to 6Å and which seems a promising avenue to pursue.

• Importin

We obtained crystals of importin α grown in the presence of a non-canonical NLS peptide and collected data to 3.2 Å resolution (ID14-2, 1-4-01). The structure has been solved by molecular replacement (different crystal form than the native importin α) and partially refined to 3.2 Å resolution. The additional electron density is not clearly attributable to the peptide. The resolution has to be extended.

Dietrich Suck

• **Sm proteins from archaea (PA-Sm1; SS-Sm1)**

So far we have solved the structures of three Sm proteins (AF-Sm1 and AF-Sm2 from *Archaeoglobus fulgidus* and PA-Sm1 from *Pyrococcus abissi*), as well as an AF-Sm1/RNA complex. We have continued our studies of archaeal Sm proteins by testing recently obtained crystals of a PA-Sm1/RNA complex and the *Sulfolobus solfataricus* (a member of the Crenarchaea) SS-Sm1 protein.

Crystals of the PA-Sm1/RNA crystals (space group C2) diffracted to ~3Å (ID14-1), but suffered during data collection. They also showed signs of damage occurring during the freezing procedure. The cell constants are compatible with two heptamers per asymmetric unit corresponding to a solvent content of ~ 61.5%. Crystals and freezing conditions need to be improved.

Preliminary tests were done with the SS-Sm1 crystals. They diffract to ~2.5Å (ID14-1), but were not useful for data collection due to severe disorder and splitting, which apparently had occurred during freezing. The freezing procedure needs to be improved.

• **T4 endonuclease VII**

EndoVII is a junction resolvase with a broad substrate specificity ranging from Holliday junctions to simple mismatched DNA. To further study the structural basis of this unique selectivity we tested several EndoVII/DNA cocrystals containing either 4-way DNA junctions or mismatched DNA duplexes. These crystals were all rather thin (~10-20µ) and did not diffract better than 10-11Å. Their diffraction patterns suggest that the crystals suffered during flash-freezing. We have meanwhile refined the freezing procedure, which resulted in an improvement of the diffraction limit from 18 to ~9Å using a rotating anode source.

• ***E. coli* Hfq**

The Hfq (or host factor I, HF-I) protein is an RNA-binding protein acting as a general post-transcriptional regulator. A 3 Å data set has been collected from tetragonal crystals (space group I4) on ID14-1. We are presently trying to further improve the crystal quality. To obtain phases, we plan MAD experiments using either SeMet-labelled protein or crystals grown in the presence of NaBr or NaI.

Matti Saraste, Klaus Scheffzek

Matti Saraste died in May 2001 and we all miss him, his scientific support and his encouragement. Members of his group are currently associated with Klaus Scheffzek. The projects that depended primarily on Matti's supervision are being partially continued depending on review and scientific support within EMBL or by collaborations with other institutes.

• **α-actinin**

We have collected data of a number of crystals soaked with peptides that have been reported to interact with α-actinin (01.04.01, ID14-1). While we

were able to improve the resolution of our native data to 2.8 Å, we could not detect clearly bound peptide in electron density maps. We will have to improve the soaking conditions, accompanied by biochemical studies. Our expanded set of crystallization conditions will certainly be helpful for our *in situ* binding studies.

• Cytochrome *cbb*$_3$ oxidase

Progress in this project was long hampered by the poor diffraction quality of crystals obtained so far, along with a long c-axis and multiple lattices. A new crystal form is more promising since it usually forms single crystals that diffract to 5 Å. An albeit not highly complete data set of 5.3 Å resolution was collected (23.6.01, ID14-1). We are focusing now on the new crystal form to improve their diffraction quality.

• SAND domain

The structure of the SAND domain has been solved and refined to 1.55 Å. A manuscript describing the work is in preparation. Atomic resolution data have been collected and structure refinement at 1.08 Å resolution is in progress. Since structure determination of the SAND-DNA complex (2.95 Å) using MR based on the refined unligated SAND was unsuccessful so far, we collected MAD/SAD data of crystals containing brominated/iodinated DNA, at somewhat less than 3 Å resolution (ID29, 08.05.01). Data analysis is in progress.

• HPr kinase

After several attempts to obtain a single data set of well diffracting single lattice crystals, we managed to collect two complete native data sets of 1.95 Å and 2.15 Å resolution, respectively, collected from two cryo-frozen crystals of high quality (05.02.01, ID14-4). This enabled us to determine the structure by molecular replacement using the C-terminal domain (50% of asymm. unit) of a homologous protein. The refinement is completed and the manuscript describing the work close to submission. Presumably as a consequence of crystal growth from phosphate buffer we found a number of bound phosphate ions in expected and unexpected but striking positions. To find out more about their significance, we plan to collect data of crystals grown under conditions with no phosphate added. Furthermore, we aim at the structures of enzyme substrate complexes.

• Brutons Tyrosine Kinase (Btk)

We have obtained crystals of the kinase domain of Btk diffracting to ~2.5 Å on ID13 (tc991, 03.11.00), where we could collect a data set of 2.8 Å resolution and of limited completeness (87%). Unfortunately different partial data sets were not compatible, although collected from the same crystal. Similar observations have been made with previous data collections and were ascribed to instabilities in monochromator cooling (C. Riekel). We are now improving the crystals to obtain data of higher quality. Apart from a native structure we aim at inhibitor bound crystals, which we are trying to obtain by either soaking or co-crystallization protocols.

• Neurofibromin (NF1) fragment

Crystals of an NF1 fragment of 33 kDa have been obtained, few of which diffracted to approximately 4 Å at ID14-1 (23.06.01). We were able to

collect a partial data set for preliminary characterization of the crystals that can be frozen using standard protocols. A number of crystals were tested under different cryo conditions. Clearly we need more crystals of larger size and better quality to enhance progress in this project. In the meantime we have observed a morphologically different crystal form that is being included in improvement procedures. We expect the structure to be solved by MIR or MAD and include such experiments in our next proposal.

• **SopE**

The structure of the Cdc42-SopE complex is solved and a manuscript describing the work submitted. The structural model shows SopE in complexed form. To investigate conformational changes occurring on complex formation we need the structure of unligated SopE. Tetragonal crystals show poor diffraction quality (3.5 Å, BM14, 10.05.00). A triclinic crystal form (2.5 Å, ID13, tc83, 07.07.00) could so far not be solved by MR, presumably as a consequence of multiple copies in the asymmetric unit or of conformational differences. We have grown crystals from Se-Met labeled SopE that we want to use for a MAD/SAD approach, to obtain a model of unligated SopE.

• **Protein BX1** (Crossed BAG with MPI Dortmund, Schlichting group)

Partial data sets were collected at ID14-1 (01.04.01) that could be merged into a high quality native data set of 2.4 Å resolution (see report from MPI Dortmund).

Irmi Sinning

The Sinning group has left EMBL in July 2001 and is now part of another BAG.

• **Signal recognition particle (SRP)**

The structure of a binary complex between SRP19 and its cognate RNA has been solved and the paper is in press. This is part of a collaboration between Sinning and Cusack (EMBL-Grenoble BAG). After screening for crystal forms in the previous beamtime allocation (see LS1801 report) and the identification of a suitable one, we solved the structure by MAD on a Bromine K edge. The experiment was performed on ID29 in May using beamtime form the Grenoble-BAG due to heavy load on our MAD beamtime in the spring.

Crystals of SRP54 in a complex with a 47-mer long SRP RNA gave 2.5 Å diffraction at ID13. A MAD experiment on SeMet derivatised crystals was attempted (ID29, 14-7-01) but was not successful. Although the fluorescence scan revealed the presence of selenium in the crystals (probably somewhat oxidized), no phases could be extracted from the experiment. Analysis to trouble shoot is still in progress (the data sets for example do not have good statistics).