EMBL-Heidelberg bag - LS1801 – August 2000 – January 2001

Elena Conti

• TAP

After determining the structure of the RNA-binding domain of TAP, we are now working on the C-terminal nucleoporin-binding domain of TAP, which heterodimerizes with a small protein to function in nuclear export. We obtained well-diffracting crystals of the heterodimer and are in the process of solving the structure by either MIR or MAD.

We have collected a 1.9 Å resolution native data set (ID14-1, 29-11-00) and another native data set (2.4 Å resolution) to confirm isomorphism of the native crystals. We collected data of 4 heavy atom soaks (ID14-1, 29-11-00), which we had identified as promising derivatives from extensive screening at low resolution. One of these, a methylmercury derivative, gave initial SIR phases. Since we were not able to identify additional derivatives, we proceeded by crystallizing the SeMet heterodimer for MAD.

We did two SeMet MAD experiments (ID14-4, 7-12-00), neither of which was successful despite the fact that we could detect a strong fluorescence signal (there are 8 methionines in the 35kDa heterodimer). Possible reasons are poor quality of the data (the Rmerges are around 10%), non isomorphism upon crystal decay (the diffraction pattern was deteriorating at the second data set, with 1 sec exposure per 0.5° rotation to separate spots on the 170 Å axis), beam instability or SeMet disorder. Besides repeating the SeMet MAD experiment (to address the first 3 possibilities), we are considering doing a MAD on the mercury derivative (to address the 4th possibility).

• Exportin

Several crystal forms of a ternary complex (of about 180 kDa) that is involved in nuclear export have been obtained and tested. We decided not to pursue in the future the original needle-like crystal form that gave no diffraction on either ID14 or ID13. We tested bipyramid-like crystals, which diffracted to 8 Å resolution. The diffraction is strong to 8 Å, but drops off rapidly beyond that. A third crystal form obtained after submission of the LS1801 proposal was tested, and gave diffraction to 8Å with some anisotropic spots at 4 to 5Å upon annealing. We are planning to concentrate on improving these two crystal forms.

We also tested two crystal forms of a different exportin complex (of about 150 kDa), which we obtained after submission of the LS1801 proposal. One crystal form diffracts to 8Å. The diffraction limit of the second crystal from could not be determined, due to crystal damage upon freezing.

Dietrich Suck

• Sm proteins

We have continued our studies of archaeal Sm proteins aimed at the structural and functional characterization of these proteins by collecting data of crystals containing a complex of the AF-Sm1 protein from *Archaeoglobus fulgidus* with an oligo-U RNA and of crystals of the AP-Sm1 protein from

Aeropyrum pernix. A 2.75 Å data set of the AF-Sm1/RNA complex was collected (ID13, 3-10-00) and the structure solved using molecular replacement and refined to an R-factor of 22.5%. This structure provides the first high resolution view of an RNP core domain, and the paper describing it is in press.

The data collected of the *A.pernix* AP-Sm1 protein (ID-13, 3-10-00; 3.2 Å resolution) were apparently not of sufficient quality to solve the structure by molecular replacement techniques. We are therefore trying to improve these crystals. The solution of the structure is complicated by the fact that the asymmetric unit of the monoclinic crystals presumably contains 28 protein subunits (arranged in 4 heptameric rings). An asymmetric unit also consisting of four heptamers was present in the (native) *A.fulgidus* AF-Sm1 protein, which clearly has a closely related monomer fold. However, the arrangement of the rings is different. Higher resolution and better quality data are required.

Matti Saraste

• α-actinin

The structure of the rod domain is solved and a manuscript describing the work submitted. Further efforts concentrated on various fragments of α-actinin preferentially those including regulatory modules. Although a number of crystals could be obtained, diffraction quality in most cases was not promising, requiring further efforts in crystal improvement. Basic peptides derived from C-terminal portions of trans membrane receptors have been reported to bind the α-actinin rod. We are currently undertaking efforts to obtain crystals of the rod bound to some of these peptides.

• Cytochrome oxidase cbb3

A limited amount of beam time was used for testing of crystals grown under various conditions. A new crystal form with less tendency to include multiple lattices seems to emerge but needs further characterization in future experiments.

Klaus Scheffzek

• SopE

Initial data sets at 2.6 Å of tiny needle crystals were collected on ID13 previously (tc83, 07.07.00). After heavy improvement in size we could collect data sets of native and in house tested heavy atom soaked crystals of typically 2.3 Å resolution (ID14-1, 07.09.01). This enabled us to solve the structure using a combination of MIRAS and MR. Refinement and analysis are in progress by the end of reporting period.

• HPr kinase

HPr kinase from *S. xylosus* crystallizes in various crystals forms. Based on test experiments on ID13 (tc991, 03.11.00) we found that a crystal form grown from phosphate had exceptional diffraction potential (3 Å) compared

to others. We were able to improve these crystals and discovered that approximately 2 out of 5 crystals tested diffracted to better than 2 Å resolution while the average resolution was considerably less than 3.5 Å. However, crystals often showed multiple lattices and partial data sets did not seem to be compatible, enforcing collection of complete data sets from individual single crystals. At this stage we only obtained a data set at 3 Å. We have refined the crystallization conditions enabling us to supply a large number of crystals of excellent optical quality for future experiments. A partial search model derived from the recently solved structure of a homologous protein has been made available in a collaborative effort, probably facilitating structure determination by using MR.

• **SAND domain**

Due to limited success of MIR or MR strategies we followed a MAD approach of Se-Met labeled SAND crystals and collected a 2.5 Å Se-Met MAD data (BM14, 05.11.00) and a 2.0 Å SAD on a gold derivative. The Se-Met MAD data set, despite some problems with the mosaicity, provided us with the phase information needed to solve the structure. Structure determination and refinement to 1.55 Å based on native data collected earlier (BM14, 10.05.00) is being completed by the end of reporting period. We also collected an atomic resolution data set of crystals diffracting beyond 1 Å on ID14-1 (08.07.00).

Initial crystals (typically 7 Å at ID14-2, 08.07.00) of a SAND-DNA complex were improved to presently 3 Å by using DNA oligo nucleotides of different length in co-crystallization experiments. We collected native data sets to max 2.95 Å resolution (ID14-1, 07.08.00; ID14-2, 27.11.00) that are currently being analysed.

• **Protein P450** (Crossed BAG with MPI Dortmund, Schlichting group)

MAD data were collected on a Hg derivative at BM14 (05.11.00). Data analysis was of limited success by the end of reporting period (see report from MPI Dortmund).

Irmi Sinning

• **Signal recognition particle (SRP)**

We screened for suitable crystal forms of a binary complex between SRP19 and its cognate RNA (collaboration with Cusack, EMBL-Grenoble BAG). Two crystal forms of a 29-mer RNA complex have been tested. A complete data set to 8 Å resolution was collected from an orthorhombic crystal form (ID14-1, 29-11-00). The crystals are promising in terms of diffraction properties (weak diffraction extending to 3 Å) but contain 30 molecules in the asymmetric unit. A tetragonal crystal form with 2 or 4 molecules per asymmetric unit diffracts so far to 8 Å resolution (ID14-3, 26-1-01). Freezing conditions appear to hamper the diffraction (smeary spots) and need to be optimized.

We tested crystals of SRP54 in a complex with a 47-mer SRP RNA that we obtained recently. The crystals give anisotropic diffraction to 3.5 Å resolution (ID14-3, 26-1-01). They decay rapidly even when frozen.

Optimization is under way, as well as preparation of SeMet crystals for MAD experiments.