Matti Saraste, Klaus Scheffzek

• Cytochrome oxidase cbb3
We tested cryo protocols and screened crystals of this membrane protein. The crystals show anisotropic diffraction patterns with a nominal diffraction limit of 4.0-6.0 Å resolution depending on the crystal orientation. The crystals are in a hexagonal space group, with cell dimensions $a = b = 100$ Å and $c = 717$ Å. The quality of the data collected with a MARCCD detector is limited due to the long c axis, which results in problematic indexing and processing. We collected and processed a data set to 8.8 Å resolution that is 85% complete. Additional synchrotron time has been devoted to the set up and data collection with the long image plate scanner (L.I.P.S.) facility available at ESRF beamline ID14-3 via the local contact Steffi Arzt. A first trial experiment in december 1999 was made in order to evaluate the possibility of recording "high resolution" (3.0-3.5 A) reflections. A data set was collected and is under analysis. Further experiments need to be run in order to optimize the offline set up and strategy for data collection of this project.

• Nitric oxid reductase (NOR)
Test beamtime for crystals of the membrane protein NOR has been devoted to the screening of needle-like crystals (~5 x 5 x 500 µm) and of rombohedral-like crystals (20 x 20 x 50 µm). The diffraction limit is about 10 Å resolution, but the high mosaicity suggests inadequate cryo protocols. Improvement of cryo conditions is necessary.

Klaus Scheffzek

• SopE
Crystals of the guanine nucleotide exchange factor SopE diffracted to better than 2.7 Å resolution at BM30 (Feb. 17-19, 2000). We collected a complete data set to 3.0 Å resolution with completeness of 98%, *R*sym of 10% and redundancy of 6 up to 3.2 Å resolution and of 2 from 3.2 Å to 3.0 Å resolution. The highest resolution shell (3.1 Å – 3.0 Å) is 97% complete with *R*sym of 34% and I/sigma of 2. The resolution limit in this data collection might have been decreased by anisotropic diffraction of the crystals.

Irmi Sinning

• Signal recognition particle (SRP) - full-length *A. amb.* FtsY

A 99% complete data set to 3 Å resolution was collected (ID14-2, September 99) from crystals of full-length FtsY *A.amb* with Rsym of 8.9% and I/sigma of 8. These crystals are 20 μ thick needles that in-house do not diffract beyond 8 Å. We are trying to phase the full-length FtsY by molecular replacement (MR) using the coordinates of the NG domain of *E.coli* FtsY and we are also trying with the NG domain of Ffh from *A.amb* as search model. We suspect this is a borderline case for MR since the conservation is about 30% and the previous structures revealed flexibility in the relative orientation of the three subdomains.

Se-Met crystals were tested (ID14-4, February 2000). Despite they are morphologically similar to the crystals of the native protein, the SeMet crystals did not diffract.

• Signal recognition particle (SRP) - NG core domain *R.sulf.* Ffh
We collected (ID14-2, September 99) a 2.2 Å resolution native data set with Rsym of 7%, I/sigma of 11 and completeness of 99%. We had previously determined the structure by SeMet MAD (experiment LS1308), but refinement had been problematic due to the limited resolution (3.5 Å). We are now refining the structure with the current 2.2 Å native data set.

• Signal recognition particle (SRP) - soaks NG core domain *A.amb.* FtsY
We measured data to 2.8 Å resolution of a GMP-PNP soak (ID14-2, September 99). The data are 88% complete, with Rsym of 8.5% and I/sigma of 7. However, no electron density for GMP-PNP could be found in the active site and we are refining conditions to try and get the complex.

• Signal recognition particle (SRP) – test cases
The complex of FtsY *A.amb* with GDP/AlF showed diffraction as the apo form.
Full length Ffh *A.amb.* small needle-like crystals showed no diffraction.
Very small crystals of SRβ showed no diffraction.

• P17

A 1.5 Å resolution native data set was collected (ID14-2 November 99) with Rsym of 6.6% and completeness of 98.1%. Together with a derivative data set collected in-house, these data were sufficient to solve the structure (current Rfree 23%, and Rfactor 20%). This protein is found in the microdomains of the Golgi membranes and shows low sequence homology to PR-proteins in plants and to GlIR. Only one NMR structure with a related fold is available in the PDB data base, but this model did now allow structure solution by molecular replacement. A manuscript is in preparation.

Dietrich Suck

• T4 endonuclease VII

We previously had solved the structures of the wild-type DNA-binding protein T4 endonuclease VII (Endo VII) and of its inactive N62D mutant that indicated substantial structural flexibility in its oligomeric state (Raaijmakers et al., 1999, EMBO J. 18, 1447-1458). This resolvase has broad substrate specificity and the N62D mutant is being used for co-crystallization experiments with various DNA substrates. So far, crystals containing a mismatched DNA oligo have been tested at ESRF. The cubic crystals diffracted to ~7Å (ID14-3 September, 1999).

• Sm-like protein AF-Sm2 (*Archaeglobus fulgidus*)

A native 1.95 Å resolution data set was collected from a hexagonal AF-Sm2 crystal, space group P6, $a = b = 58.4$ Å, $c = 32.1$ Å. We measured 11984 total reflections (4522 unique), with overall *R*sym of 5.1% (outer shell 17.3%), I/sigma of 10.5 (outer shell 4.2) and completeness 97.6% (90.9%outer shell). The structure has been refined using this data set to a *R*free of 23.6% and *R*factor of 20.9%.

• Sm-like protein PA-Sm1 (*Pyrococcus abyssi*)

We collected a data set to 1.9 Å resolution at ID14-2 beamline (ID14-2, November 99). The crystals are triclinic with cell parameters $a = 69.3$ Å, $b = 70.2$ Å, $c = 116.0$ Å, $\alpha = 90.21°$, $\beta = 97.70°$ and $\gamma = 107.48°$. The data set (154678 unique reflections) has overall Rsym of 4.9% (outer shell 14.2%), I/sigma of 14.0 (outer shell 4.0) and completeness of 95.1% (outer shell 92.0%). The asymmetric unit consists of 28 molecules, organized in 4 heptamers. The structure has been solved by molecular replacement with the program AMoRe, using a heptamer derived from the *A. fulgidus* AF-Sm2 structure as a search model. The structure is currently being refined. At present the *R*-free and *R*-factor are 34% and 30%, respectively.

• Cre recombinase.

We collected three data sets of the DNA-binding protein Cre recombinase in complexes with iodinated DNA in order to determine unambiguously the orientation of DNA in the crystals. The data sets have been measured at ID14-2 (September 99 and November 99) and are between 3.1Å and 3.3 Å resolution. In all cases the completeness is higher than 95% (outer shell 96%) and the *R*sym are between 9.5% and 10.2%. The structures have been refined to *R*-free and *R*-factors lower than 26.4% and 20.6% and show insight in the conformational flexibility of the synaptic complex and in the molecular mechanics of the multi-step recombination process.

Elena Conti

• TAP

We determined the structure of the 30kDa RNA-binding domain of TAP.

We first collected two native data sets from the needle-like tetragonal crystals of TAP to check the degree of isomorphism prior to heavy-atom substitutions and obtained Riso of about 8% when comparing natives at 3.0 Å resolution with overall *R*sym of about 5%. We proceeded collecting several partial and complete data sets of potential heavy-atom derivatives between 3.0 and 3.8 Å resolution (ID14-2 September 99, ID14-4 December 99). Despite the fact that mercury and platinum substitutions gave promising *R*iso (between 13% and 28%) with the native data sets, they resulted in uninterpretable difference Patterson maps.

A SeMet MAD experiment was attempted (ID14-4, December 99), but was hampered by the poor diffraction of the SeMet needle-like crystals (particularly after removal of the arsenate-containing harvesting buffer).

Finally, a successful SeMet MAD experiment was carried out with the help of Raimond Ravelli (ID14-4, February 00) on bipiramid-like tetragonal crystals. We collected three data sets at 3.5 Å, 3.6 Å and 3.15 Å resolution respectively at the peak wavelength (12666 ev), at the inflection (12661 ev) and at the high-energy remote (13200 ev) wavelengths. The data sets are 99.8% complete with a redundancy of 6.4. Despite the low resolution, the poor accuracy of the data (overall *R*sym between 9% and 10%) and the small signal (*R*anom of 5.9% at the peak and of 5.1% at the inflection), we could find 12 of the 16 sites present in the 120 kDa tetramer in the asymmetric unit. The resulting phases (FOM of 0.45 with the program sharp) were of good enough quality to allow model building after density modification procedures. We are refining the structure to 3.15 Å resolution (current *R*-free of 33.8% and *R*-factor of 32.5%).