ESRF

| | | |
|---|---|---|
| ESRF | **Experiment title:**<br>BAG-Frankfurt, Sodium-Proton-Antiporter NhaA from *E.coli* , QCR from *S. cerevisiae* | **Experiment number**:<br>MX-135 |
| **Beamline**:<br>ID14EH2 | **Date of experiment**:<br>from: 16.05.03 to: 18.05.03 | **Date of report**:<br>21.01.04 |
| **Shifts:**<br>6 | **Local contact(s)**: : Dr. Cecile JAMIN | *Received at ESRF:* |

**Names and affiliations of applicants** (* indicates experimentalists): C. Hunte*, E. Screpanti*, H. Palsdottir*, S. Solmaz*

Max-Planck-Institute of Biophysics, Dept. Molecular Membrane Biology
Marie-Curie-Str. 15, 60439 Frankfurt, Germany

**e-mail: Carola.Hunte@mpibp-frankfurt.mpg.de**

**Report:**

NhaA is the main $Na^+/H^+$ antiporter in *Escherichia coli*. The protein plays an important role in adaptation of the cells at high sodium concentrations and pH homeostasis. Its activity has a pronounced pH dependence. High resolution structural information for NhaA is not available. The crystals are generally smaller than 0.2 x 0.1 x 0.1 $mm^3$ and diffraction cannot be tested at home radiation sources. Crystal quality has been continuously improved to a diffraction limit of ~4 Å resolution. SeMet-NhaA crystals with similar properties have been measured during the last beamtime, but quality of the data was not sufficient to solve the structure by SAD. Further improvement of crystal quality and derivatives as an alternative strategy for phase determination is tested. Several data sets of NhaA with different additives were collected during this beamtime. The unit cell has been determined to be P21 with a=109 Å, b=126, c=Å 122 Å and ß=90.1°. Again, variations of up to 5 Å of unit cell axes have been

noted. Six data sets were collected: 1. 4.0 Å, $R_{sym}$ 5 %, 75 % complete; 2. 4.0 Å, $R_{sym}$ 4 %, 94 % complete; 3. 4.2 Å, $R_{sym}$ 8 %, 92 % complete; 4. 4.0 Å, $R_{sym}$ 4 %, 91 % complete; 5. 4.0 Å, $R_{sym}$ 6 %, 98 % complete; 6. 4.5 Å, $R_{sym}$ 6 %, 91 % complete. Crystal quality is more consistent and quality of the data is slightly better as before, as judged by $R_{sym}$ and quality of the final frames. However, a data set with higher resolution could not be obtained.

The cytochrome $bc_1$ complex (QCR) is an oligomeric membrane protein which catalyzes electron transfer from ubiquinol to cytochrome c, while the process is coupled to electrogenic translocation of protons across the membrane. Despite recent advances in structural studies including our work, central parts of the molecular mechanism are still not known. Furthermore, the structural basis for transient electron transfer complexes is not well understood. We recently determined the first structure of a respiratory membrane protein complex (yeast QCR) with the mobile electron carrier cytochrome c (Lange & Hunte 2002, 3 Å resolution). Data were collected from a single crystal at 4 °C. To obtain higher resolution, cryo-conditions were established, but initially high mosaicity was the limiting factor. Now, crystallization conditions have been improved and, in addition, soaking of the crystals prior to freezing is possible. A data set of pre-soaked and frozen crystal of the ternary complex of yeast QCR/cytochrome c/antibody fragment was collected: 2.95 Å, $R_{sym}$ 7.2 %, 86 % complete. The structure was refined and analyzed. Specific binding of cytochrome *c* has been confirmed for these conditions, thus paving the way for analysis of the redox partner interaction at different ionic strength, redox-state, pH as well as for obtaining a high resolution structure of this interesting complex.

7 hours of beamtime were lost in the first night, as the software for beamline control hung up and could not be restarted by routine procedures.