ESRF

ESRF	**Experiment title:** BAG-Frankfurt, Sodium-Proton-Antiporter NhaA from *E.coli* , QCR from *S. cerevisiae*	**Experiment number**: MX-135
Beamline: ID14EH4	**Date of experiment**: from: 27.09.03 to: 28.09.03	**Date of report**: 04.03.04
Shifts: 3	**Local contact(s)**: Dr. Gordon Leonard	*Received at ESRF:*

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

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:

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. The binding of cytochrome *c* to QCR is transient and dynamic. The presence of multiple conformations have been suggested. We 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). Our aim is to analyze the structure of the electron transfer complex in respect to redox state, ionic strength and occupancy of substrate in the Qi-site. Cryo-conditions have been established and the data collection strategy was optimized to separate spots and reduce radiation damage. A dataset was collected from a single cryo-cooled, oxidized crystal: Resolution 2.52Å, R_{sym} 5.6%, completeness 96% (space group P2$_1$, unit cell dimensions a=145.56 Å, b=165.10 Å, c=194.61 Å, β 104.26°). The structure has been refined to R_{free} 26.41%, R_{cryst} 23.64%, r.m.s.d bonds/angles 0.0075/1.28. Due to the higher resolution it was possible to include and refine water and lipid molecules. Redox-dependent modifications of the cytochrome *c*/QCR interaction are in progress. To analyze the impact of ionic strength on this binding interaction a dataset of a single crystal, soaked at 180 mM ionic strength, was collected: Resolution 2.6 Å, R_{sym} 8.1%, completeness 96.2%. The structure has been refined to R_{free} 30%, R_{cryst} 27%, r.m.s.d bonds/angles 0.0083/1.6. Analysis of ionic strength dependent changes of the binding interaction is in progress. The structure is compared to that of a complex at 100 mM ionic strength, which was obtained during the previous beamtime. Future plans include improvement of the current resolution as well as completion of the cytochrome *c* binding analysis at different redox states, several ionic strength conditions and varied Qi-site occupancies.

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. More than 20 crystals were analyzed for diffraction quality. Two data sets could be obtained: 1. native, 3.9 Å resolution, R_{sym} 7.9 %, 99 % complete; 2. derivative 4.3 Å, R_{sym} 8.5 %, 94 % complete. Data are used in combination with previously obtained data sets for phase determination.