ESRF

ESRF	**Experiment title:** BAG-Frankfurt	**Experiment number**: MX-135
Beamline: ID14EH2	**Date of experiment**: from: 12.11.03 to: 13.11.03	**Date of report**: 4.03.04
Shifts: 3	**Local contact(s)**: : Dr. Domenique Bourgeois	*Received at ESRF:*

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Report:

ABC transporters constitute a large protein family in both prokaryotic and eukaryotic cells. They translocate a variety of substrates across the membrane, ranging from single ions to large molecules such as polypeptide chains. Some three-dimensional structures of soluble domains have been determined by x-ray crystallography at high resolutions. However, it is still unclear how the substrates are recognized and translocated across the membrane. In this project, we aim to crystallize ABC transporter from *Aquifex aeolicus* and to determine its three-dimensional structure. 10 small crystals were frozen and analysed for diffraction quality. With exposure times between 10 to 60 sec, crystals diffracted up to 5 Å resolution, only. Therefore, no complete data set was collected.

Crystals of the recombinant protein G alpha q (GAQ; source human, expression in *Pichia pastoris*) were obtained. This GTP binding protein couples to GPCRs (G protein coupled receptors). The crystals are needle shaped and small (50-100 micrometers long, diameter <20 μm). No diffraction pattern was observed at in house radiation sources. With 10 sec exposures the crystals were diffracting to ~ 6.5 Å resolution. Crystallization conditions will be refined to improve resolution.

The cytochrome bc_1 complex (QCR) is an oligomeric membrane protein that catalyzes electron transfer from ubiquinol to cytochrome c, while the process is coupled to electrogenic translocation of protons across the membrane. The structure of the complex of QCR and the mobile electron carrier cytochrome *c* has previously been determined at 3 Å resolution (Lange & Hunte, PNAS 99, 2002). We are currently analyzing, whether different redox states, ionic strength conditions, or Qi-site occupancies affect the binding interaction, which is critical for electron transfer. One data set of an sodium ascorbate-reduced crystal was collected: Resolution 2.95Å, R_{sym} 6.6%, completeness 91% (space group $P2_1$, a=145 Å, b=165 Å, c=195 Å, β 104°). The structure has been refined to R_{free} 27.47%, R_{cryst} 24.20%, r.m.s.d bonds/angles 0.0080/1.34. Analysis of redox-dependent changes of cytochrome *c* binding to QCR by comparison with previously determined structures is in progress. To address the question of regulatory long-range interactions between Qi-site and cytochrome c binding, we collected a dataset of a crystal co-crystallized with the Qi-site-specific inhibitor Antimycin A1: Resolution 2.95Å, R_{sym} 10.8%, completeness 87%. Refinement of the structure is in progress (R_{free} 29%, R_{cryst} 25.5%, r.m.s.d bonds/angles 0.0072/1.25). Additional experiments are required to improve the current resolution of the structure and to complete analysis of cytochrome *c* binding in relation to redox state and ionic strength.