ESRF	**Experiment title:** BAG-Frankfurt, Cytochrome bc_1 complex from *S. cerevisiae*	**Experiment number**: MX-135
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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. Strucural studies provided insights into the molecular mechanism, which is not fully understood. We now published work on the binding of an ubiquinol analogue at the Qo site (Palsdottir, H., Lojero, G.C., Trumpower, B.L., Hunte, C., 2003, Structure of the yeast cytochrome bc_1 complex with a hydroxyquinone anion Qo inhibitor bound. *J. Biol. Chem.* **278**, 31303-31311). Data for this study have been previously collected at the ID14EH3.

Summary - Bifurcated electron transfer during ubiquinol oxidation is the key reaction of cytochrome bc_1 complex catalysis. Binding of the competitive inhibitor 5-*n*-heptyl-6-hydroxy-4,7-dioxobenzothiazol to the Q_o site of the cytochrome bc_1 complex from

Saccharomyces cerevisiae was analysed by X-ray crystallography. This alkyl-hydroxy-dioxobenzothiazol is bound in its ionized form as evident from the crystal structure and confirmed by spectroscopic analysis, consistent with a measured pK_a = 6.1 of the hydroxy group in detergent micelles. Stabilizing forces for the hydroxyquinone anion inhibitor include a polarized hydrogen bond to the iron-sulfur cluster ligand His^{181} and on-edge interactions via weak hydrogen bonds with cytochrome *b* residue Tyr^{279}. The hydroxy group of the latter contributes to stabilization of the Rieske protein in the b-position by donating a hydrogen bond. The reported pH dependence of inhibition with lower efficacy at alkaline pH is attributed to protonation of His^{181} with a pK_a of 7.5. Glu^{272}, a proposed primary ligand and proton acceptor of ubiquinol, is not bound to the carbonyl group of the hydroxy-dioxobenzothiazol ring, but is rotated out of the binding pocket toward the heme b_L propionate A, to which it is hydrogen bonded via a single water molecule. The observed hydrogen bonding pattern provides experimental evidence for the previously proposed proton exit pathway involving the heme propionate and a chain of water molecules. Binding of the alkyl-6-hydroxy-4,7-dioxobenzothiazol is discussed as resembling an intermediate step of ubiquinol oxidation, supporting a single occupancy model at the Q_o site.

Furthermore, we are investigating the interaction between QCR and cytochrome *c*. In general, 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). To address open questions regarding regulation of cytochrome *c* binding as well as multiple conformations related to ionic strength and pH, we established cryo-conditions for these crystals. Using a data set collected during the last beamtime, ordered binding of cytochrome c to QCR could be shown under these conditions. Furthermore, some crystals diffract better than 2.0 Å. A high resolution structure is feasible but cumbersome to obtain due to the combination of small crystals, relatively large unit cell and high mosaicity. Two additional data sets of the ternary complex of yeast QCR/cytochrome c/antibody fragment were collected: 2.95 Å, R_{sym} 4.1 %, 98 % complete and 2.8 Å, R_{sym} 6.6 %, 94 % complete. Crystals were pre-treated with different soaking solutions. Structures are currently under analysis, cytochrome *c* binding is confirmed for both conditions.