ESRF	**Experiment title:** BAG: Frankfurt Cytochrome bc_1-complex of *Saccharomyces cerevisiae* (Fv-fragment mediated crystallization)	**Experiment number:** LS-1514
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Report: The mitochondrial cytochrome bc_1-complex, an oligomeric membrane protein, is one of the fundamental components of the respiratory chain. It catalyzes electron transfer from ubiquinol to cytochrome *c*, while the process is coupled to electrogenic translocation of protons across the inner mitochondrial membrane. The proton motive Q-cycle is a widely accepted model for the functioning of this protein. Aiming at a detailed understanding of its mechanism we study the cytochrome bc_1-complex from the yeast *S. cerevisiae*.

The cytochrome bc_1-complex from *S. cerevisiae* was crystallized with the help of a specifically binding antibody-Fv-fragment. The crystals belong to the space group C2 and diffract x-rays better than 2.2 Å resolution at synchrotron radiation sources. We recently determined the strucure of the complex using multiple isomorphous replacement. The structure was refined to a crystallographic R-factor of 21.1% (R_{free} 25.4 %) [1]. One monomer of the homodimeric complex consist of nine polypeptides of the enzyme plus two chains of the Fv-fragment. 2229 amino acid residues, 4 redox-cofactors, the natural substrate coenzyme Q6 and the inhibitor stigmatellin as well as 371 water molecules and five phospholipid molecules are present per monomer.

The structure of the yeast cytochrome bc_1 complex contains the natural substrate coenzyme Q6 bound at the Qi-site of the complex, the site of quinone reduction. The orientation of the quinone headgroup is not unequivocally defined. This may be due to low occupancy, or mobility of the substrate. The nature of substrate-stabilizing amino acid residues and possible proton uptake pathways are under discussion. It is still not clear how the substrate enters and leaves the binding pocket. Analysis of crystals with Qi-site specific inhibitors bound to the cytochrome bc_1 complex will give information on the Inhibitory molecule itself and may add information about the site of quinone reduction. A data set of crystals soaked with funiculosin was collected [2.6 Å resolution, 6.5 % R-merge (overall), 84 % completeness (overall), 1.2 <I/sigI> (outer shell)]. Furthermore, a data set of antimycin co-complex crystals was collected [2.6 Å resolution, 6.5 % R-merge (overall), 91 % completeness (overall), 1.1 <I/sigI> (outer shell)].
Data collection was performed at 4°C. Data of several crystals were scaled to acchieve high completeness.

Fv-fragment mediated crystallization allows the reproducible production of well diffracting crystals of the yeast cytochrome bc_1 complex. A combined approach of X-ray crystallography, biochemical analysis, site-directed mutagenesis and spectroscopy is used to study mechanism and structure/function relationship of this highly important membrane protein.

[1] C. Hunte, T., J. Koepke, C. Lange, T. Rossmanith and H. Michel. Structure at 2.3 Å resolution of the cytochrome bc_1 complex from the yeast *Saccharomyces cerevisiae* co-crystallized with an antibody Fv-fragment. Structure (accepted for publication 2000)
[2] C. Hunte, C. Lange, J. Koepke, H. Michel. X-ray structure of the bc_1 complex suggests roles for phospholipids. Science, submitted