ESRF	**Experiment title:** BAG: Frankfurt Cytochrome bc_1-complex of *Saccharomyces cerevisiae* (Fv-fragment mediated crystallization)	**Experiment number:** LS-1369
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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*. A detailed structure of this protein complex is not only of interest to understand the mechanism of the enzyme, but will aid the development of fungicides, as this membrane protein is the target of a new class of fungicides, the strobilurines.

We obtained crystals of the cytochrome bc_1-complex from *S. cerevisiae* by cocrystallization of the membrane protein complex with the help of a specifically binding antibody-Fv-fragment. The crystals of the cytochrome bc_1-complex initially had an approximate size of 0.4 x 0.3 x 0.3 mm^3, but the size could be increased about 5-fold. Diffraction of the crystals was noted up to 2.4 Å resolution using a rotating anode x-ray source. The crystals belong to the space group C2. Cell dimensions of a=214, b=162, c=147 and and β=117 were determined [1]. Initially, structure determination was tried by molecular replacement using a previously collected native data set (beamline X11, EMBL outstation, DESY, Hamburg) and coordinates of the known bovine and chicken heart cytochrome bc_1-complexes [2, 3, 4].

However, no specific and independent structural features appeared. Therefore, we collected dat sets of 6 different heavy atom derivatives. Phases were obtained up to a resolution of 3.2 Å. The structure has been solved by the method of isomorphous replacement. To improve the structural model a high resolution data set was required. Data collection was performed at the ID 14/3 using the mar CCD detector. In contrast to the previous measurements the beam was not attenuated. Diffraction of the crystals up to 2.2 Å was noted. Still, measurements had to be conducted at 4°C and the quality of diffraction rapidly decreases upon exposure to radiation. Only few exposures are possible at one crystal position and two to four positions of one crystal could be measured. About fourty crystals were analyzed and seventeen of those were used to scale a high resolution data set. To compensate for the overexposure at low resolution, a 3.5 Å data set was collected from a single crystal.

Table 1. Data collection statistics

data set	**resolution [Å]**	**R-merge overall [%]**	**complet. overall [%]**	**<I/sigI> outershell**
native[1]	3.5	6.3	89.2	9.4
native[1]	2.3	6.5	83.8	1.2
native, ubiquin.[1]	2.8	8.4	97	6.3

[1] crystals were grown in the presence of the inhibitor stigmatellin

The statistics of the collected data sets are given in table 1. The aquired data are used for the completion of the structural model and the description of the binding site of the inhibitor stigmatellin. In addition, a data set was collected with a substrate pretreated sample (ubiquinone, cytochrome bc_1-complex).

More high resolution data sets with and without different inhibitors and substrates as well as at different redox conditions have to be collected. The knowledge of the high resolution structure of the yeast protein now allows a combined approach of X-ray crystallography, biochemical analysis, site-directed mutagenesis and spectroscopy to study mechanism and structure/function relationship of this highly important membrane protein. This is not possible for the cytochrome bc_1-complexes of bovine and chicken heart, whose structures became known in 1997 and 1998.

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