ESRF	**Experiment title:** Cytochrome bc_1-complex of *Saccharomyces cerevisiae* (Fv-fragment mediated crystallization)	**Experiment number:** LS-1156
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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*. This protein is biochemically and genetically well characterized and the organism allows a directed mutagenic approach for further studies.

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. This approach was already successfully applied for the crystallization of the cytochrome *c* oxidase [1] from *P. denitrificans* and led to the structure determination of this membrane protein [2]. 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. Initially, structure determination was tried by molecular replacement using the coordinates of the known bovine and chicken heart cytochrome bc_1-complexes [3,4] and a native data set of the yeast protein collected before. However, probably caused by structural

differences no new and specific structural features appeared, Therefore, independent phase information from heavy atom derivatives was needed to solve the structure.
Data collection was performed at the ID14/3 using a 4°C cooling system. The crystals belong to the space group C2. Cell dimensions of a=214, b=162, c=147 and β=117 were determined. The crystals diffract better than 2.4 Å resolution, but they deteriorate fast upon radiation exposure and could not be adapted to cryo-conditions [5].

Table 1. Data collection statistics

data set	**resolution [Å]**	**R-merge overall [%]**	**complet. overall [%]**	**<I/sigI> outershell**
pCMB	3.5	4.1	85.0	9.9
pCMS	3.2	7.5	89.3	6.5
TAMM	3.2	8.4	86.5	5.7
AuCl	3.2	6.5	62.5	3.8

The strategy of data collection had to take into account the extreme liability of the crystals to radiation damage. The setup of the ID14/3 allowed us to collect data sets of sufficient completeness from a single crystal for each of the above noted derivatives. The beam was attenuated with 0.5 mm carbonfilm and we used the mar CCD detector to minimize the time intervall between exposures. The focussed beam enabled us to shift the crystal position up to 6 times during the collection of a data set. Several crystals had to be tried to acchieve optimal results. The statistics of the final derivative data sets are noted in table 1.
In combination with data sets collected at the beamline X11 (EMBL Outstation, DESY, Hamburg) and the MPG-ASMB-BW6 (DESY, Hamburg) phases were obtained up to a resolution of 3.2 Å. With these data work is in progress to solve the structure using isomorphous replacement. High resolution data are now required for the refinement of the structure. Several data sets (substrates, inhibitors, different redox conditions) will have to be collected to analyze the structure/function relationship of this important membrane protein.

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