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ESRF

Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office using the **Electronic Report Submission Application:**

http://193.49.43.2:8080/smis/servlet/UserUtils?start

Reports supporting requests for additional beam time

Reports can now be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title:** Cytochrome bc_1-complex of *Saccharomyces cerevisiae* (Fv-fragment mediated crystallization)	**Experiment number**:
Beamline: ID14/EH3	**Date of experiment**: from: 2.12.2000 to: 4.12.2000	**Date of report**: 26.02.01
Shifts: 6	**Local contact(s)**: L. Dumon	*Received at ESRF:*

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Report:

We recently solved the structure of the cytochrome bc_1-complex from *S. cerevisiae* bound to an antibody Fv fragment at 2.3 Å resolution [1,2]. This mitochondrial 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. Still, the molecular basis of fundamental processes of the enzyme mechanism is not known, e.g. the oxidation of quinol, the identity of primary proton donors during quinone reduction or the importance buried water molecules shown in the yeast structure for proton transfer pathways.

For the first time we obtained crystals without stigmatellin but in the presence of a different Qo site inhibitor. While the spacegroup (C2) of these crystals remained unchanged, the cell constants were slightly altered. We obtained a data set at 2.5 Å resolution, 92 % completeness and 6.6 % R-merge by scaling data from several crystals. The refined structure shows

conformational changes of side chains in the Qo binding pocket compared to the stigmatellin containing structure. The observed alterations will be discussed in respect to the processes of quinol oxidation, since the binding of stigmatellin is thought to resemble an intermediate step of the oxidation.

Two data sets were measured after soaking co-complex crystals with Qi site specific inhibitors: I. 2.8 Å resolution, 7.2 % R-merge (overall), 77 % completeness; II. 2.9 Å resolution, 6.7 % R-merge (overall), 80 % completeness. These data will be used to characterize the quinone binding site.

The yeast cytochrome bc_1-complex is crystallized together with a bound antibody Fv fragment. The fragment is mediating crystal contacts and is therefore essential for formation of the crystal lattice. We modified the recombinant fragment to facilitate faster protein purification. Co-crystals of the cytochrome bc_1 complex and these fragments were obtained. We determined the space group C2 and cell constants of 620Å, 327Å and 217Å for these crystals. X-ray diffraction was below 5 Å resolution, i.e. these crystals are unsuitable for data collection.

All data collection was performed at 4 °C. The start of data collection was delayed due to a defect in the cooling of the CCD-detector.

[1] C. Hunte, T., J. Koepke, C. Lange, T. Rossmanith and H. Michel (2000) 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 8, 669-684.

[2] J. Nett, C. Hunte, B.L. Trumpower (2000) Changes to the length of the flexible linker region of the Rieske protein impair the interaction of ubiquinol with the cytochrome bc_1 complex. Eur. J. Biochem. 267, 5777-5782.