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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: 17.2.2001 to: 19.2.2001	**Date of report**: 26.02.01
Shifts: 6	**Local contact(s)**: E. Mitchell	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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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.

The structure of the yeast cytochrome bc_1-complex contains five tightly bound phospholipid molecules. It is known from biochemical studies that phospholipid molecules are essential for the function of this enzyme. We are investigating the role of these phospholipid molecules. Are they merely required to ensure structural integrity or are some of them – especially cardiolipin – of functional importance. We obtained crystals of a modified protein preparation, which

retains a higher amount of phospholipid during purification. A data set of these crystals was collected: 2.65 Å resolution, 92 % completeness, 6.7 % R-merge. In addition, protein of standard preparations [1] was crystallized and the crystals were treated with phospholipid containing buffer. Soaking did not effect the diffraction quality of the crystals and data were collected to 2.6 Å resolution, 91 % completeness, 5.1 % R-merge. Refinement of these data is in progress.

The exact molecular interaction of the cytochrome bc_1-complex with its substrate cytochrome *c* is not known. We obtained several type of small crystals of a ternary complex consisting of cytochrome bc_1-complex, Fv fragment and cytochrome *c* (Type I-III). Similar to the original crystals no cryo-conditions are available. We modified crystallization conditions favouring stronger interaction of cytochrome c with the cytochrome bc_1-complex. Although crystals can be grown very reproducibly, crystals larger than 0.2 x 0.2 x 0.2 mm are extremely rare. We collected a data set for one crystal at 3.0 Å resolution, 93 % completeness, 11 % R-merge at 4 °C. The crystal belong to the space group P21 with unit cell parameters a=147Å, b=166Å, c=196Å, β=104°. Molecular replacement confirmed the solution, which was obtained with the previous data set at 3.6 Å resolution. A dimer of the cytochrome bc_1-complex is present in the assymetric unit. It cannot be concluded yet, if cytochrome *c* is bound in an ordered manner.

The only known structure of a co-complex from an enzyme and cytochome *c* is that of cytochrome c peroxidase and cytochrome c. No structures are available yet of co-complexes between components of the respiratory chain and cytochrome c.

Data collection was hampered by very low intensity of the beam (technical problem of ID14eh3). Due to a major power cut at the ESRF the data collection had to be stopped after 4 1/2 shifts.

[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.