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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.

	Experiment title:	**Experiment number**:
	X-ray crystallographic study of the H^+-ATPase from *N. crassa* with and without bound ligands	LS 1150
Beamline: ID14-1,2,3	**Date of experiment**: from: Jan 1999 to: Feb 2000	**Date of report**: 21 Feb 02
Shifts: 18	**Local contact(s)**:	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

*Dean R. Madden, Max Planck Institute for Medical Research, Heidelberg, Germany
*James Féthière, "
*Thomas Keitel, "
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Report:

This represents the final, summary report of our experimental measurements for the long-term project LS-1150. Three sessions were scheduled and used. Due to experimental difficulties that could not be surmounted (see below), we decided not to continue the project after Feb 2000, and did not schedule a fourth session.

The goals of the project were to determine the structure of the plasma membrane H^+-ATPase from *N. crassa* in the presence and absence of vanadate, a transition-state analog. Although a molecular envelope had been determined for this protein using electron crystallography (Auer et al., 1998), the resolution was limited to ~20 Å perpendicular to the membrane and 8 Å in the plane of the membrane. Our goal was to obtain a structure at higher and more isotropic resolution and to pursue structure determination in different conformational states. Indeed, as the project began, our crystals yielded the highest resolution yet reported for a P-type ATPase. Plausible rotation function solutions were obtained using the EM structure as a search model, but the translation function searches had been unsuccessful. Thus, experimental phase information was required.

Based on preliminary measurements (LS-660 and LS-940), it was known that the crystals form in space group *R*3 and that frequently exhibited merohedral twinning, yielding an apparent *R*32 symmetry. Furthermore, only ~10% of the crystals screened yielded data to a resolution of ~6 Å. Finally, the data exhibited some anisotropy. To permit phase determination, these experimental problems needed to be overcome. Given the weak laboratory diffraction, the necessary screening could only be achieved in the context of a long-term project. In the first round of measurements, we identified crystallization conditions that increased the proportion of crystals diffracting to 6-7Å from 10% to 70%. Correction for

the observed anisotropy of diffraction was straightforward. During all three visits, we identified conditions for the preparation of native, cross-linked, and heavy-atom soaked crystals with improved resolution and reduced twinning fractions, as judged by the statistical analysis of the intensity distributions (French & Wilson, 1978). Our choice of heavy atoms was guided by knowledge of compounds that interact biochemically with the H^+-ATPase or that had been used successfully in phase determination of other membrane proteins. Crystals were screened by collection and analysis of partial datasets (10-15°), which permitted identification and elimination of highly twinned specimens. In total, 97 pre-screened crystals were analyzed; twelve full datasets exhibited relatively low twinning, with a maximum resolution of 5.8 Å (using a limit of 30% for R_{merge} in highest resolution shell). Despite reduced twinning fractions in those crystals from which full datasets were collected, in no case was it possible to identify heavy-atom derivatives based on isomorphous differences. A major problem was the variability of the twin fraction, which complicated comparisons with the native datasets. Anomalous signals were analyzed, but in no case could we identify derivatives by this method either. Finally, in an attempt to circumvent the twinning problem, computational untwinning was performed using twin fractions determined from the intensity distributions. Once again, neither isomorphous nor anomalous difference signals could be interpreted. Ultimately, it appeared unlikely that we could circumvent the twinning problems either biochemically or computationally. This made it high unlikely that we would be able to determine phases experimentally.

In summary, although we were able to solve the problems of highly variable diffraction limits and crystal anisotropy, the twinning problem could not be controlled. Following the 3rd measurement period, the structure of the homologous Ca^{++}-ATPase from rabbit sarcoplasmic reticulum was determined at a resolution of 2.6 Å (Toyoshima et al., 2000). Molecular replacement with this model also failed to generate a solution vs. our native datasets. Given the much lower resolution limit of our crystals compared to the published structure and the persistent twinning difficulties, we decided to cease work on this crystal form and therefore to end the long-term project. We continue to pursue alternative crystallization conditions in a variety of ligand-bound states.

References:

Auer, M. et al. (1998) Nature 392:840.
French, S. & Wilson, K. (1978) Acta Cryst. A34:517.
Toyoshima, C. et al. (2000) Nature 405: 647.