EUROPEAN SYNCHROTRON RADIATION FACILITY

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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: Crystal structure of Bovine Rhodopsin	Experiment number: LS1489
Beamline: ID13	**Date of experiment**: from: September 1999 to: October 2000	**Date of report**:
Shifts: 18	**Local contact(s)**:Manfred Burghammer	*Received at ESRF:*

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Report:

Long term report September 99 – October 2001

We had observed the first diffraction spots to 4Å from our rhodopsin crystals when we submitted the long-term proposal. Over the past 18 months we have made six visits to ID13 (18 shifts) and have seen gradual improvements in resolution. We summarise here the work carried out in each visit:

1. 5-6/9/99: We screened for conditions giving better diffraction, and found that using certain detergents as additives improved diffraction in the direction perpendicular to the needle. We collected our first complete native data sets each from a single crystal.
2. 18-19/2/00: We carried out more detergent screens and found one that worked most consistently. We started screening for heavy atom derivatives. Our first trials were with Hg, Pt and Xe. With dedicated help from the beamline scientist Manfred Burghammer, we were able to collect complete data sets from several potential derivative crystals, each at the L(III) edge of the heavy atom.
3. 25-26/2/00: We continued our derivative search using Hg and Au compounds both on crystals of native and enzymatically modified rhodopsin.
4. 17-18/6/00: We started trying stabilising conditions on the crystals in our efforts to reduce variability in the heavy atom soaked crystals. And we found conditions that made the spots sharp perpendicular to the needle axis. Because of the severe non-isomorphism between native and derivative crystals, we attempted double heavy atom soaks with Hg and W, aiming to find isomorphous pairs between the doubly and singly soaked crystals. In another attempt to achieve isomorphism we also collected data of the same derivative at

two wavelengths above and below the L(III) edge. Also tested effects of different temperature growing conditions.

5. 20-21/7/00: By this stage our derivatives were diffracting to higher resolution than our natives, so we went back to the native conditions to try and improve resolution.
6. 30-31/10/00: Further modified growth conditions gave us native crystals that diffracted to 2.6Å, which was the best we had ever seen. We collected complete data sets on these crystals.

Conclusion:

Our rhodopsin crystals belong to the space group P3(1). The cell dimensions for the native crystals are a=b=103.8Å, c=76.6Å with two molecules in the asymmetric unit. Over the course of the long term project we have steadily improved the resolution limit from 4Å, with an Rmerge of 17%, to 2.6Å, with an Rmerge of 11%. We have now solved this structure by molecular replacement and cross-crystal averaging of non-isomorphous native and derivative forms.

It has been a very difficult project for a number of reasons. The rhodopsin crystals grow as needles less than 40µm in cross-section but up to 0.4 mm long. The micro-focused beam on ID13 with the option of either a 10µm or 30µm aperture was essential for adequate signal-to-background ratio. The diffraction limit varied from crystal to crystal and at different positions along the crystal. Furthermore beam damage in such small crystals was severe, so that often no more than 30˚ of data could be collected at a single position on the crystal. On ID13 when we have found a crystal that diffracts well at one position, the micro-diffractometer set up allowed us to translate along the needle axis and collect all the useful data. The optics on the diffractometer is excellent and assured good centring of very small crystals. Without all these advance technical features many of our data sets could not have been obtained.

Anisotropic diffraction limit was a serious problem at the start of this long-term project. However, by using detergent additives and improving the stabilising protocol during the freezing, we have finally observed diffraction to 2.6Å in all directions, even though the diffraction perpendicular to the needle axis was still more radiation sensitive than that along the needle.

Occasionally crystals suffered from merohedral twinning. Because beam damage forced us to merge data collected from multiple crystals, twinning in one crystal could prevent us from obtaining complete data. This was particularly damaging for heavy atom derivative data.

Our crystals soaked in heavy atom solutions showed unusually large non-isomorphism to the native crystals, with unit cell changes of 4-14Å along the *a*-axis. This has prevented us from solving the structure by the isomorphous replacement method. Nevertheless, we have turned the non-isomorphism to advantage by carrying out cross-crystal averaging, which has contributed significantly to removing the model bias in the molecular replacement from a published model for the P4(1) crystal form at a lower resolution.

Long-term access to the ID13 facilities was essential to the success of this project. We would like to thank Manfred Burghammer and Christian Riekel for their support and expertise.