EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

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: X-ray structure of the XPD protein	**Experiment number**: 14-U-913
Beamline:	**Date of experiment**: from: 28/06/2007 to: 29/06/2007	**Date of report**: 18/07/2007
Shifts:	**Local contact(s)**:Hassan Belrhali	*Received at ESRF:*

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Report:

Crystals of the XPD protein have been measured at BM14 and a total amount of 5 datasets have been collected so far. The protein crystals belong to the space group $P6_5$ with unit cell constants as indicated in table 1. The statistics of the best two data sets (used for substructure determination) are given in the table below. Both data sets are from the native protein with the iron sulfur cluster present as confirmed by an edge scan at the iron edge and have been collected at a wavelength of 1.7Å and 1.0Å, respectively. Other datasets include XPD co-crystallized with an iodized ATP (1.7Å), a quick Platinum (PT)-soak (1.0Å), and another native set at a wavelength of 1.7Å.

The substructure of the iron sulfur cluster could be solved using Sharp. The substructure was solved using the best two data sets for a 2-wavelength MAD experiment. Combinations with any of the other data sets did not yield any improvement so far. Working on other combinations of datasets employing MIRAS or SIRAS strategies is still in progress and initial processing of the data indicates that the PT-soak could be a promising strategy to get sufficient phase information, combined with the iron sulfur cluster. This is based on a difference Fourier map revealing additional peaks that could represent PT sites. To further pursue this the soaking time and PT derivative needs to be optimised and more crystals have to be tested. However, due to the limited resolution of a maximum of 2.9Å and the size of the protein (620 amino acids) the building of a sensible model has not succeeded yet.

To improve the diffraction quality of the crystals a new purification and crystallization scheme is currently being employed. The key point is to purify and crystallize the protein under anaerobic conditions. Since in previous purifications heavy precipation was observed after the affinity purification step the protein was purified and crystallized under anaerobic conditions leading to higher yield and much lesser precipitation during purification. Crystallisation experiments are currently running.

Table1 data colection statistics on the currently best XPD data sets

Wavelength	1.7 Å	1.0 Å
Unit cell	a = b = 79.2 Å, c = 174.6 Å α = β = 90° , γ = 120°	a = b = 79.2 Å, c = 174.6 Å α = β = 90° , γ = 120°
Space group	$P6_5$ (170)	$P6_5$ (170)
Independent reflexions	20109	27919
High res. shell	3.30 – 3.20	3.0 - 2.9
redundancy	7.4 (7.3)	1.9 (0.7)
R (sym)	6.9 (53.5)	5.0 (55.3)
I/s (I)	23.8 (3.9)	14.4 (1.0)
completeness	99.4 (99.3)	92.4 (42.6)