

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.

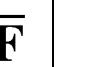
Deadlines for submission of Experimental Reports

- 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:
Beamline:	Date of experiment: from: 22/09/2010 to: 23/09/2010		Date of report: 02/05/2011
Shifts:	Local contact(s):		
Names and affiliations of applicants (* indicates experimentalists): Coquille Sandrine, University of Geneva. Thore Stéphane, University of Geneva.			

Report:

Proposal title: Crystallographic studies of enzymes responsible for the synthesis of vitamin B1.

Proposal number 1177.

Assigned number of Shift: 2.

We came to the synchrotron with numerous untested crystals of two proteins involved in the thiamine biosynthesis pathway.

The first protein, ThiC, had given preliminary hits in our crystallization screens which could not be reproduced. We therefore tried to freeze the pseudo-crystals directly from the drops and brought these samples to the synchrotron. We hoped that we will observe diffraction spots giving us strong encouragement for the ThiC project. Unfortunately, these samples did not show any diffraction properties, possibly reflecting the very limited quality of these crystals or the numerous problems that can be encountered during stabilization and freezing procedures.

The second protein, Thi5, had been crystallized in numerous conditions giving small and beautiful bi-pyramidal shaped crystals. Attempts to grow larger crystal had been unsuccessful

at that time. As for the ThiC project, these crystals could not be tested at home. Nevertheless, due to their strong birefringence and reproducibility, we hoped that good diffraction should be observed. We had verified that these crystal were made of our protein and we had stabilized them using several different techniques (oil, high PEG, high Ethylene Glycol, high sugar, step-wise increment, etc). We brought along approximately 30 to 40 crystals. We spend most of the time (approximately 10-12 hours) working on these crystals. We could test every crystal that we carried along during the two allocated shifts. However, these crystals did not show the expected diffraction quality. Indeed, diffraction spots could be observed to about ~5-6 Å in very few cases. Indexing has been very difficult and numerous space groups can fit the measured spots. In addition, the calculated mosaicity was very large (more than 2-3 degrees) indicating serious problems of crystallogenesis. None of the different freezing conditions had any impact on the diffraction properties from these crystals indicating that the problem is certainly occurring during crystal growth.

Since our last visit, we have tested multiple constructs of the Thi5 protein with the goal to induce another crystal form leading to different X-ray diffraction properties.

In conclusion, these two shifts have allowed us to reduce our effort in the reproduction of several crystallization conditions for the protein ThiC and showed that the crystal obtained with the Thi5 protein had to be significantly optimized in order to determine its three-dimensional structure. We have continued our effort toward the structure determination of these proteins and have obtained new crystals for both projects.