EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

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:** Structural studies of non-classical cytoplasmic polymerase and enzyme involved in thiamine biosynthesis	**Experiment number**: MX-1318
Beamline: ID14-4	**Date of experiment**: from: 04/07/2011 to: 05/07/2011	**Date of report**: 13/07/2011
Shifts: 2	**Local contact(s)**: Stéphanie Monaco	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

***Coquille Sandrine, University of Geneva.**

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Report:

Proposal title: Structural studies of non-classical cytoplasmic polymerase and enzyme involved in thiamine biosynthesis.

Proposal number MX-1318.
Assigned number of Shift: 2.

We came to the synchrotron with numerous crystals (~120) of two proteins involved in the poly(U) polymerization and in the thiamine biosynthesis pathway.

The first protein, Cid1, had given hits in our crystallization screens which could be reproduced and optimized. We had tried to freeze these crystals in various cryo-conditions and tested on our home-source many crystals. We could at that time observe diffraction spots until 3.0 angstroms. We hoped that we will observe diffraction spots until at least 2.5 angstroms using the apo form of the enzyme. We actually were even more successful and could record an excellent dataset to 1.95 angstroms resolution. We could finally determine our exact space group which was previously not possible due to bad low resolution completeness. Additionally, we had brought numerous crystals of the enzyme with various substrates and after heavy atom soakings. We could collect several datasets from soaked crystals and with heavy atoms. Unfortunately, the selected heavy atoms do not seem to be

present in the crystal which lets us without suitable data for de novo phasing. We are now working on different heavy atoms and on obtaining seleno-methionine containing crystals. Regarding the substrate, we cannot yet assess whether they are bound or not.

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 and we decided to start mutating surface residues in an attempt to modify crystal contacts. Since our last visit, we also tested multiple constructs of the Thi5 protein with the goal to induce another crystal form leading to different X-ray diffraction properties. Finally, we could crystallize the thi5 homologue from another fungus. We brought along more than 70 crystals out of which we could tests ~30. We could collect datasets to about 2.75 angstroms from two mutants and from the homologous protein with very reasonable Rfactor, especially for the mutant's one. Each variant has different space group (specifically C2221 and P422) which let us hope that we will be able to solve the structure using the molecular replacement technique (although the sequence identity with the available atomic models is below 30 %...). In addition, we wish to improve the crystal quality for the homologous protein because they showed very weak diffractions to ~3.25 angstroms. Furthermore, several crystals grown in presence of substrate have not been tested due to time limitations.

In conclusion, these two shifts have allowed us to successfully collect multiple datasets for both projects. Regarding the first project, high resolution datasets (more than 2.0 angstroms) for the apo protein have been collected as well as several others with substrate or product-soaked protein. For the second project, high resolution diffracting crystals have been identified indicating that our strategy consisting of mutating surface residues was the right one to follow. These datasets are good enough that we can start the molecular replacement searches for solving our structures. We have also measured diffraction data some substrate-soaked crystals which may or may not contain additional density. Structure determination will first have to be done before we can assess that last point.