EUROPEAN SYNCHROTRON RADIATION FACILITY

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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 via the User Portal:

https://wwws.esrf.fr/misapps/SMISWebClient/protected/welcome.do

Reports supporting requests for additional beam time

Reports can 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:** **Crystallographic studies of proteins involved in vitamin B1 biosynthesis and polyuridylation of mRNA**	**Experiment number**: MX-1392
Beamline: ID14-4	**Date of experiment**: from: 03.06.2012 to: 05.06.2012	**Date of report**: 26.06.2012
Shifts: 2	**Local contact(s)**: **CREPIN Thibaut**	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Prof. Thore Stéphane, University of Geneva

Munoz Paola, University of Geneva

Coquille Sandrine, University of Geneva

Report:

Proposal title: Crystallographic studies of proteins involved in vitamin B1 biosynthesis and polyuridylation of mRNA.

Proposal number MX-1392.
Assigned number of Shifts: 2.

We came to the synchrotron with numerous ligand-soaked crystals (~140) of two proteins involved in poly(U) polymerization and in the thiamine biosynthesis pathway.

The aim of the first project was to determine the structure of the substrate- or product-bound forms of Thi5, an enzyme implicated in the vitamin B1 biosynthesis pathway in yeast.
Therefore, we tested Thi5 protein crystals soaked or co-crystallized with PLP (pyridoxal 5'-phosphate) or HMP (hydroxymethyl-2-methylpyrimidine), respectively the expected subtrate and product of Thi5. Moreover, we tested Thi5 protein crystals soaked with iron atoms, which seem to be required for the enzymatic reaction of Thi5, in order to have a complete overview of this mechanism.
We have collected 8 data sets at a resolution of 2.7 to 3.2 Å with the different combinations of soaking. The analysis of these diffraction data are on going and the preliminary results indicate clearly the presence of PLP (and HMP?) in the active site of Thi5. However, the iron ions seem to be absent in our models.

The aim of second project was to determine the structure of Cid1, a template-independent poly(U) polymerase which we have recently obtained the UTP-bound crystal structure by SAD method (Munoz-Tello *et al.*, 2012), in complex with its product and substrate. Since this protein is able to add either a poly(U) or poly(A) tail, we did several soaks and cocrystallization trials of the native Cid1 and an inactive mutant with ATP, UTP, UMpNpp and ApCpp with a minimal product in other to better understand Cid1's function and selectivity. We hope that this mutant will particularly block the substrate and product in the active site. We collected 13 data sets at 2.5 and up to 1.5 Å resolution with the different combinations of ligand and product. So far, none of the ATP/ApU soaked crystals or UTP/UpU crystals had the two forms in the active site: most of them had either only the UTP, or nothing. Interestingly, one data set of the inactive protein showed an ApU molecule in the active site.

In conclusion, these two shifts have allowed us to successfully collect multiple datasets for both projects. The data collected for the first project allowed us to build a model of the substrate-bound (and product-bound?) forms of Thi5 and gave us some insights into the reaction mechanism of this enzyme. For the second project, the analysis of the data sets collected will give us a hint on Cid1 polymerization mechanism and substrate/product accomodation. Preliminary results show the abscence of a ATP-bound form of the protein which will hep us understand the out-competition of UTP over ATP of this enzyme. Other 3 data sets have to still be analyzed for the presence or absence of the substrate/product molecules. Finally, model building and refinements for the ApU-bound Cid1 protein are on-going.

Reference:

Munoz-Tello P., Gabus C., Thore S., Functional Implications from the Cid1 Poly(U) Polymerase Crystal Structure. (2012) Structure *20*, 977-986