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.

ESRF	**Experiment title:** Structural study on Flavoproteins and Glycosyltransferases	**Experiment number**: MX 1335
Beamline: ID23-2 ID14-4	**Date of experiment**: from: 14th to: 16th July	**Date of report**: 9-9-2011
Shifts: 4	**Local contact(s)**: Max Nanao	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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BIFI - Edificio I + D

Report:

We are going to summarize the results obtained in MX 1335 experiment but first I would like to explain why we used two beamlines instead one, when we only got initially two shifts at ID23-2. Please see below.
Our beamline time was on 14th of July and unfortunately for us, DHL did not deliver our samples one day before our working time. On top of that the 14th of July was the national day in France and DHL did not work. Thus we could not have any frozen crystals to be tested in ID23-2. Thanks that we brought some small crystals in a crystal tray, we could use our two shifts in ID23-2. Thanks also to the ESRF staff, we had the opportunity to use another beamline, ID14-4, from 5:00 pm (15th July) to 3:00 am (16th July). Thus, the report of the experiment MX 1335 contained the results obtained in both beamlines.

Results:

- Ferredoxin-NADP(H) reductase (FNR) from pea: We managed to get diffraction data at 2.8 Å of C266M mutation (pointed to be involved in electron transfer during catalysis). The rest of the crystals of mutants localised in C266 and L268 positions diffracted very poorly (higher than 4 Å). X-data from C266M FNR was processed at home and the structure solved. C266M FNR crystals belonged to the $P2_12_12_1$ orthorhombic space group and the unit cell dimensions were a=73.7, b=91.7 and c=139.8 Å. The crystal structures of C266M and L268V mutants (L268V data collected in a previous trip with experiment number MX-1230) have been compiled with biophysical characterisation of these two mutants, and a manuscript is under preparation at the moment. At present we are carrying out new trials of crystallisation of other mutants in order to get more insights into catalysis.

- FAD synthetase: The crystals of FAD synthetase from *C. ammoniagenes* complexed with the cofactor FAD did not diffract well and no good datasets could be obtained.
Unlike the full-length protein, crystals of the C-terminal domain (Riboflavin Kinase module, RFK) cocrystallised with ATP diffracted at 2.6 Å. The crystal was processed and solved by molecular replacement. The location of ATP was determined and now we can compare it with a large number of crystal structures with other complexes that we have previously determined. Soon, we will publish all the structural work together with kinetic data of this domain.

- POFUT1

We had different crystals of catalytic mutants in complex with GDP-fucose, but unfortunately these crystals did not diffract well. We are trying now to get new conditions in order to get the structures of these mutants in complex with GDP-fucose.
We also brought untested crystals of POFUT1 in complex with an EGF-like repeat unit. These crystals diffracted very well and we managed to solve the crystal structure at 2.02 Å. The crystals belonged to P212121 space group and the unit cell dimensions were a=84, b=89 and c=100 Å. The structure showed a complex of POFUT1 with this peptide but in an unproductive conformation. Although it is an interesting data, we are now trying to get different crystallisation conditions in order to get a productive conformation. We are also currently carrying out biophysical experiments in order to understand the significance of the unproductive conformation.

In general we managed to get all the information that we initially wanted despite all the problems. Furthermore we have our first manuscript of POFUT1 crystal structure in press (accepted in PLoS ONE) and two other manuscripts under preparation.