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:** Insights into the catalytic mechanism of an essential eukaryotic glycosyltransferase	**Experiment number**: MX-1230
Beamline: BM16	**Date of experiment**: from: 10th to: 11th December	**Date of report**: 27-05-2011
Shifts: 2	**Local contact(s)**: Daniele de Sanctis	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Ramón Hurtado-Guerrero

University of Zaragoza

BIFI - Edificio I + D

Report:

- POFUT*:* we did UV-RIP experiments with different crystal types. We could not get the structure from these experiments because in general the crystals diffracted with splitted reflections. We tried a large number of crystals but as I said above, we did have many problems with the diffraction pattern of these crystals. The most succesful experiment was that we managed to get a high resolution of a native crystal (apo-form) for the first time. At the end we were also able to solve the structure using a high redundant data set taken at home combined with phase extension of a high resolution data set collected at BM16. We have just submitted a manuscript with the structure of this protein in combination with site directed mutagenesis. These studies describe the first crystal structure of these family of proteins and from this work, we have been able to propose a new exciting catalytic mechanism. We hope that we can publish it soon.
- With the remaining time that we had, we collected data for crystals of the C-terminal of Corynebacterium ammoniagenes FAD synthetase in complex with ADP and Anabaena FNR S80A in complex with FAD and $NADP^+$ (mutant involved in the transfer of electrons in this type of protein). As a brief explanation of these two above proteins: FAD synthetase is an enzyme involved in the synthesis of cofactor FMN and FAD and is essential in a large number of pathogenic organisms; and FNR is another essential enzyme catalysing the reduction of $NADP^+$ to NADPH during photosynthesis. We have already gone to BM16 with crystals of these two proteins We have been able to solve the structures of these two proteins by molecular replacement techniques. Both proteins were complexed with different ligands in order to understand their catalytic mechanism.

The trip to ID23-1 was good in general although the main reason of the trip, to solve the structure of POFUT by UV-RIP, was not achieved. We managed to get better resolution for native POFUT, FAD

synthetase C-terminal in complex with ADP and for *Anabaena* FNR S80A in complex with $NADP^+$ plus FAD.