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:** Structures of posttranslational SUMO modification protein complexes.	**Experiment number**: MX-894
Beamline: ID-29	**Date of experiment**: from: April 24th 2009 to: April 25th 2009	**Date of report**: 22/2/2010
Shifts: 2	**Local contact(s)**: Elspeth Gordon	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

David Reverter Group Leader. Universitat Autonoma de Barcelona.

Dunja Urosev Post-doc researcher. Universitat Autonoma de Barcelona.

Oscar Conchillo Doctorate Student. Universitat Autonoma de Barcelona.

Report:

Three different experiments were performed during this trip to the ESRF. The first one is from the main project in which my laboratory is involved at the "Universitat Autonoma de Barcelona" is the structural and functional characterization of the members of the SENP protease family, the protease family that cleaves the SUMO post-translational modification from protein substrates. The second and third projects are collaborations that my lab has stablished with two different gruops form my University.

Crystals from the complex between Senp6 and SUMO2 diffracted beyond 1.3 A resolution. Unfortunately the protein complex was not there, and only crystals containing SUMO2 were able to grow. This crystal form of SUMO2 has been previously published (Huang et al., 2004. Crystal structures of the human SUMO-2 protein at 1.6 A and 1.2 A resolution: implication on the functional differences of SUMO proteins. *Eur. J. Biochem.* 4114-4122); so there is no need to pursue any longer with those crystals.

The second experiment performed is the diffraction of crystals from the carbamate kinase from *Mycoplasma penetrans.* This protein is involved in the arginine deiminase pathway. Crystals were cristallized with 0.1M HEPES 7.0, 2.0M Am Sulfate and 2% PEG400. One the DATA sets that were collected at the ID29 diffracted beyond 2.4 A. The cystals belonged to the P3221space group (a=53.4, b=53.4, c=175.16, and *a*=*b*=90º *g*=120º). The DATA set was scaled with a final Rmerge of 0.037, I/sigma of 6.6 and 97% of completeness. The structure can be solved by molecular replacement using the CK structures 1B7B (*E. faecalis*) and 1E19 (*P.furiosus*) as a model. After the first cicles of refinement and rebuilding, an atomic model for the structure can be clearly observed with an Rfactor= 0.34 and a Rfree=0.39. The structure is waiting to be finally refined.

The third project that we took to the ESRF are the crystals of NMB1946, an outer membrane lipoprotein from the pathogen *Neisseria meningitidis*. The protein can be potencially used as a target for a drug against this pathogen, this experiment belongs to an european anti-pathogen project. Crystals of NMB1946 were grown at 18 degrees by the hanging drop vapor diffusion method in following conditions 26% PEG8000, 0.1M Na acetate and 0.2M Li Sulfate, pH 4.5. Crystals were cryoprotected in a well solution that additionally contained 12 % glycerol. Data were collected at 100K, at beamline ID 29, ESRF, Grenoble. Crystals diffracted to a resolution of 2A, belonged to I121 (C2) space group, with following unit cell characteristics (a, b, c; angles): 107.32 49.71 58.22; 90.00 122.77 90.00. Some important statistics includes: completness of 99.3%, Rmerge of 0.05 and I/sigma: 16.6. Structure was solved using molecular replacement method, where hypothetical protein PG110 (1p99) and membrane lipoprotein TpN32(1xs5) (Williams et al. 2004 and Deka et al. 2004) were used as search models. Cycles of refinment yielded final R factor =18.6% and Rfree= 23.0%. Elucidation of the structure coincided with NMB1946 structure solved by another group (3gxa) being published (Yang et al. 2009).