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:** LEBS BAG 2002-2 | **Experiment number**: MX 60 |
|---|---|---|
| **Beamline**: ID14 2 | **Date of experiment**: from: 24/02/03-8:00 to: 25/02/03-8:00 | **Date of report**: 28/02/03 |
| **Shifts:** 3 | **Local contact(s)**: Dr. Stephanie Monaco | *Received at ESRF:* |

**Names and affiliations of applicants** (* indicates experimentalists):

**Ronald Melki * (research director, CNRS), L. Bousset* (Post-doc), B. Gollineli* (research assistant, CNRS), Thierry Bizebard*(research assistant, CNRS), Pierre Briozzo (Maitre de conference), Isabelle Llorens* (student), M. Graille*(Post-doc),** LEBS, Bat 34, CNRS UPR9063, 1 av. de la Terrasse, Gif-Sur-Yvette, France

**Report:**

## Ronald Melki *, L. Bousset* (0.78 shift) : Ure2p

The [URE3] phenotype in yeast *Saccharomyces cerevisiae* is due to an altered form of Ure2p, a protein involved in nitrogen catabolism. To understand possible conformational changes at the origin of prion propagation, we previously solved the crystal structure of the Ure2p functional region [Bousset et al. (2001) *Structure 9*, 39-46]. We showed the protein to have a fold similar to that of the, class of glutathione *S*-transferases (GSTs). Here we report crystal structures of the Ure2p functional region (extending from residues 95-354) in complex with glutathione (GSH), the substrate of all GSTs, and two widely used GST inhibitors, namely, *S*-hexylglutathione and *S-p*-nitrobenzylglutathione [Bousset et al. (2001) Biochemistry. 40 :13564-7]. Ure2p assembles into fibrils. The protein looses its function upon assembly. X-ray fiber diffraction measurements allow us to access the packing of Ure2p molecules in the fibrils. Also, to determine the extent of changes in the conformation of Ure2p that lead to the loss of its function upon assembly.

X-ray fiber diffraction patterns of Ure2p fibrils assembled under different experimental conditions were collected. These patterns reveal in some cases cross beta sheet structures. This is part of an ongoing project [L., Bousset et al., (2003) J. Struct. Biol. 141, 132-142].

## P. Briozzo (0.26 shift) : Structural study of UMP kinase

The aim of the experiment was to check the diffracting power of crystals from a soluble variant of uridine monophosphate (UMP) kinase from *E. coli*., which catalyzes the reaction UMP + ATP -> UDP + ADP. Whereas in eucaryotes phosphorylation of UMP and CMP is carried out by a same protein (UMP/CMP kinase), bacteria contain separate UMP and CMP kinase. The primary structure of UMP kinase is divergent from that of other nucleoside monophosphate kinases (AMP-, TMP-, GMP-, bacterial CMP- and eucaryotic UMP/CMP-kinases). UMP kinase is essential for bacterial survival, and is therefore a potential target for new antibiotics. So far there is no published structure of UMP kinase, which is due in part to the low solubility of this family of enzymes.
We have grown small (0.16 x 0.03 x 0.03 mm) needle-like crystals of our variant of UMP kinase.
A frozen crystals diffracted X rays up to 3 Å. A set of data was therefore collected (120° of rotation).
The collect took 2 hours, including preliminary tries of 3 different crystals.
As proteins of known structure which are somewhat homologous in sequence to UPM kinase share less than 20% identity with it, solving the structure of the protein will probably need further MAD experiments.

## B. Gollinelli*, T. Bizebard* (0.9 shift) : Catalytic antibody

First, several crystals of antibody 15A9 that uses pyridoxal-phosphate as a cofactor to catalyze transamination of aminoacids (Gramatikova & Christen, *J. Biol. Chem*., 1997, 272, 9779) have been tested but they were twinned. Finally a 2.5 Å full data set (20s per image, space group P1, a=57.9, b=60.3, c=64.6 $\alpha$=79.4, $\beta$=78.2, $\gamma$=80.8) could be recorded. The completion is 98.5 % (94.4 % in the last shell), Rsym=7.7 % (19.6 % in the last shell). The structure will be solved by molecular replacement.

## I. Llorens*, M. Graille* (1.03 shift) : yeast *Saccharomyces cerevisiae* Structural Genomics project

The systematic names of the genes are used. More details on every orf can be found on http://genomics.eu.org:targets.html

1)YGR205w (target 43).
Spacegroup P43212 a=b=65A; c=140A.
Resolution 2.25A
Completion 100%
Rsym=4.1

The structure of this protein was previously solved by MAD data obtained from Se-Met crystals. The structure revealed an unexpected strong resemblance to the structure of a know enzyme. During this shift, three datasets were collected from crystals soaked with either triphosphate or diphosphate nucleotide. Unfortunately, analysis of the data did not reveal the presence of any nucleotide at the active site.

2)YDR533c (target 155 ).
Spacegroup P21212 a=61A; b=166A; c=48A.
Resolution 1.9A
Completion 97.8%
Rsym=8.1%

The structure and function of this orf are unknown. Structural similarity may exist with a protein from Methanococcus whose structure was recently solved in a structural genomics project at Berkeley. The protein is probably a protease but this has to be confirmed experimentally. As this orf contains no methionine with the exception of the N-terminal one for 237 residues, we constructed a quadruple methionine mutant. We have collected a dataset at 1.9A resolution from a crystal of this quadruple mutant. We need now to collect a full MAD dataset to solve the structure of this orf.

3)YGL148w (target 238)
Spacegroup P1 a=58A; b=76A; c=91A; alpha=114°; beta=108°; gamma=90°.
Resolution 2.9A.
Completion 90%
Rsym=4%

This orf codes for the enzyme chorismate sythase, which is key step in the stynthesis of the essential aromatic amino acids: Tyr and Phe. The crystal used to collect these data was the first one of this project to diffract. Work is underway to produce SeMet crystals of this orf.

4)LicT from Bacillus subtilis.
Spacegroup P3221 a=b=48A; c=162.5A.
Resolution 1.9A.
Completion 100%
Rsym=3.7%.

LicT is involved in the regulation of polyglucane degradation in Bacillus subtilis. LicT functions as a transcriptional antiterminator protein composed of two structural and functional domains: one RNA binding domain and a regulatory domain. From the same crystal, we have collected two datasets: one at low and one at high resolution. These data are currently used for the refinement of the structure.

5)human VpreB receptor complexed to its stromal ligand : human galectin-1.
Spacegroup P212121 a=44A; b=58A; c=112A.
Resolution 2.1 A
Completion 99 %
R-fact_total 0.077

VpreB is a receptor protein which is expressed on the surface of developing B-cells at the preB stage. There has been a longstanding effort in the purification and crystallization of this atypical receptor. Crystals of the V preB receptor were obtained in presence of its natural stromal ligand : galectin 1, a protein of about 120aa belonging to the mammalian lectin family. Analysis of the solvent content of the crystals showed that the whole complex could not be present in the asymmetric unit. The structure was solved by Molecular Replacement showing that indeed only the galectin1 crystallized. The structure is refined and is very similar to previous determined mammalian galectin structures. Studies are underway to characterize biochemically the interaction between VpreB and galectin in order to helps us to get crystals of the complex.