EUROPEAN **S**YNCHROTRON **R**ADIATION **F**ACILITY

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.

	Experiment title: **CNRS-Gif sur Yvette BAG**	**Experiment number**: LS2072
Beamline: BM30	**Date of experiment**: from: 04-07-02 at 8 :00 to:05-07-02 at 8 :00	**Date of report**: 26/08/02
Shifts: 3	**Local contact(s)**: J.-L. Ferrer	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Béatrice Golinelli* (LEBS, CNRS), **T. Bizebard*** (LEBS, CNRS), **Herman Van Tilbeurgh*** (L.E.B.S., Professor at the Orsay University), L.E.B.S., C.N.R.S./U.P.R. 9063, 1 avenue de la Terrasse, Bat. 34, F-91198 Gif-sur-Yvette, France

Nicolas Leuliot* (LURE, Research/Professor assistant), LURE, Bat 209d, Centre Universitaire Paris Sud, BP34, F-91898 Orsay Cedex

Report:

B. Golinelli (1.5 shift) : Structure of the hemic domain of flavocytochrome b2 complexed with an Fab fragment

Flavocytochrome b_2 catalyses the oxydation of lactate to pyruvate. This enzyme is composed of two domains : the flavodeshydrogenase that contains the flavine mononucleotide and the heme domain. In order to understand the mechanism of electron transfer and to know if the mobility of the heme domain is necessary for catalysis **(**Miles et al., *Biochemistry*, 1998, 37, 3340), we crystallized the heme domain in complex with the Fab fragment of an antibody that hinders the intramolecular electron transfer between the flavin and the heme b_2**.**

A full data set (95.9 % complete) of the complex has been recorded at 3.2 Å resolution. The space group is P21 with a unit cell of a=73.0 Å, b=85.6 Å, c=91.5 Å, α=90 β=97.4 γ=90, R_{sym}=16.3 % (42.4 % in the 3.31-3.2 Å resolution shell). The molecular replacement search positions the constant domain and the variable domain for the two Fabs in the asymmetric unit. Refinement is in progress to locate the two copies of the heme domain.

T. Bizebard (0.5 shift) : Structural study of Ribonuclease E

Ribonuclease E (RNase E) is an essential *E.coli* endoribonuclease and is evolutionarily conserved in many other bacteria. In *E.coli* the protein fulfils at least two important functions: it is necessary for the maturation of stable RNAs (5S ribosomal RNA and tRNAs) and it has a key role in the degradation of most messenger RNAs -thus making a very important contribution to the overall regulation of genetic expression.

Although this enzyme has been extensively studied, nothing is known about its structure and enzymatic mechanism. In the past, structural studies of RNase E have been hampered by the lack of stability and solubility of this protein; to try to overcome these problems, we have decided to work with a fragment of the

protein which retains the catalytic activity of the full-length enzyme and is much easier to manipulate. This fragment has been overexpressed, purified, submitted to crystallisation trials and small crystals have recently been obtained.

These crystals tested showed no diffraction spots were observed beyond 7 Å resolution; this crystalline form is thus unsuitable for structural studies; additional crystallisation trials are underway.

Herman Van Tilbeurgh, Nicolas Leuliot (1 shift) : Structure determination of proteins from the yeast *Saccharomyces cerevisiae* genome

During the last shift of the proposal number LS2072 on BM30 on 05/07/07 we started to collect data sets for the structural genomics project on yeast target proteins. The data collection was continued on 05-06/07/02 on BM30 with the french CRG beam time allocation (to see the BM30 experiment report corresponding to the proposal number 30-01-514). The systematic names of the genes are used. More details on every orf can be found on http://genomics.eu.org/targets.html. We started during the LS2072 shift with orf YDR435c (target 182).

Spacegroup P6122 or P6522 a=112.7 c= 162.7
RESOLUTION = 2.0 A

This orf codes for a N-terminal methyl transferase involved in the regulation of a phosphatase complex. There are no structural models for this protein, so the structure has to be solved by MAD or MIR. Se met data were collected on beamline ID14 one week before and we are in the stage of phasing the protein. During our time on BM30, complete data sets on the native protein with and without a potential cosubstrate were collected. The good quality of the native data at 2.0A resolution will allow to refine the structure accurately.