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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:** **CNRS-Gif sur Yvette BAG**	**Experiment number**: LS 2072
Beamline: ID29	**Date of experiment**: from: 23/2/02 -8 :00 to: 24/2/02 – 8 :00	**Date of report**: 26/08/02
Shifts: 3	**Local contact(s)**: A. Royant	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

S. Fieulaine* (LEBS, PhD student), **S. Nessler*** (LEBS, Research/Professor Assistant), **Luc Bousset*** (LEBS, PhD student), **Nicolas Fay*** (LEBS, PhD student), **M. Knossow***(LEBS, CNRS), **B. Gigant***(LEBS, CNRS), 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

Report:

Sonia Fieulaine, Sylvie Nessler (0.75 shift): structure of HPr Kinase/Phosphorylase (HprK/P)

In year 2000, a first native data set had been collected on **ID14-1 of 24/02/00** to 2.8Å resolution with crystals of the catalytic domain of *L. casei* HprK/P. A MAD data set collected on **ID14-4 of 22/09/00** using the selenomethionine modified form of the protein allowed us to solve the first structure of HprK/P.
Fieulaine et al. (2001) EMBO J., 20, 3917-3927.
PDB ID code: 1jb1

Latter on, a data set collected on **ID14-1 of 07/04/02** allowed us to solve by molecular replacement the structure of the complex between the catalytic domain of *L. casei* HprK/P and *B. subtilis* HPr.
Fieulaine et al. (2002) PNAS, (accepted).
PDB ID code: 1kkl

During the run **ID14-4 of 14/09/01** we collected data that allowed us to solve the structure the complex between the phosphorylated form of *B. subtilis* HPr and the catalytic domain of *L. casei* HprK/P
Fieulaine et al. (2002) PNAS, (accepted)
PDB ID code: 1kkm

During **this run**, we tested crystals of the complex obtained in presence of PPPi, an alternative phosphate donor for HprK/P. In the complex cocrystallized with PPi (PDB code 1kkm) one phosphate was transferred to Ser-46 of HPr while the second phosphate remained in the active site of the enzyme, at the position usually occupied by beta phosphate of nucleotide bound to P-loop. We hope that the structure

obtained with PPPi could show changes induced in the kinase by the phosphate equivalent to the alpha phosphate of the nucleotide. The crystals diffracted to 3Å resolution but were twisted.

Luc Bousset, Nicolas Fay (0.75 shift): Structures of Ure2p with inhibitors

Ure2p is a protein involved in the regulation of nitrogen catabolism in the yeast *Saccharomyces cerevisiae*. It has prion properties in that it adopts at least two conformations one of which converts the normal form into an abnormal form that assembles into protein fibrils. The crystal structures of Ure2p domain that extends from amino acid residue 94 to 354 alone and in complex with glutathione and related compounds were solved recently at the ESRF. We synthesised a novel set of drugs that affect the assembly of Ure2p into fibrils. To better understand the mechanism of action of these ligands we crystallised Ure2p 95-354 in the presence of the ligands and tested the crystals for diffraction on ID29 on February 23 using 0.75 of a shift. The crystals of Ure2p95-354 made in the presence of an inhibitor of assembly diffracted to 2.8Å but lacked the inhibitor indicating either that the inhibitor is not bound at all or not in a stable manner to Ure2p95-354. Thus, we need to test Ure2p95-354 crystals made in the presence of higher concentrations of the inhibitor of assembly or to find conditions where full-length Ure2p crystals are produced in the presence of the ligand.

Benoit Gigant, Marcel Knossow (1.5 shifts) : Structure of the tubulin-stathmin complex

During this session a di (Pt)-ethylene diamine derivative crystal was used to collect anomalous diffraction data at the Pt peak wavelength (λ = 1.072 Å), using a very narrow beam to diminish the effect of mosaicity on the diffraction pattern as much as practically possible. The crystal diffracts to 3.8 Å (Rmerge : 0.077) and data are useful to 4.5 - 4 Å. This, together with other derivatives mostly collected on ID14-4, is used to calculate an experimental map from which the molecular replacement model is being rebuilt. The status of this is described together with the report on our 15/6-16/6 visit on ID14-4.