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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: ID14-EH4	**Date of experiment**: from: 21/06/02-8 :00 to: 22/06/02-8 :00	**Date of report**: 26/08/02
Shifts: 3	**Local contact(s)**: Raimond Ravelli	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Sonia Fieulaine* (LEBS, PhD student), **Sylvie Nessler*** (LEBS, Research/Professor Assistant), **P. Briozzo*** (LEBS, Research/Professor Assistant), **Ines Li De La Sierra***(LEBS, Post-Doc), 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, PhD student), LURE, Bat. 209D, Centre Universitaire Paris Sud, BP34, F-91898 Orsay Cedex, France

Report:

Ines Li De La Sierra, Nicolas Leuliot (2 shifts) : yeast *S. 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=65.2 c=140.6
Resolution 2.15 A
Completion= 98%
R-fact = 0.089

The structure of this protein was previously solved by MAD data obtained from Se-Met crystals. The structure revealed an unexpected strong resemblance to a structure of a known enzyme. Se-Met protein crystallized in this case more easily than the native protein, and structure determination was in this case well ahead of native data collection. Considerable effort was put into the crystallization of the native protein. The refinement of the 2.1A data is underway.
Cocrystallization with potential substrates proved also very difficult. Data collected in presence of nucleotides did not reveal any bound in the active site.

2) YER010c (target 154)

Spacegroup C2 a=114.9, b=132.97, c=48.6, β=97.23
RESOLUTION = 2.2 A

This protein is of unknown structure and function. Crystals of the Se-Met substituted protein were obtained under the same crystallization conditions as the native protein. Analysis of the fluorescence scan spectrum revealed that Se was partially oxidised in the crystals. Data were therefore collected at 4 different wavelengths
The protein contains only two methionines for 234 residues and the corresponding anomalous signal was too weak to determine protein phases. Site directed mutagenesis is carried out to incorporate more methionines into the protein.

3) YDR435c (target 182)

Spacegroup P6122 or P6522 a=112.7 c= 162.7
RESOLUTION = 2.3 A

This orf codes for a N-terminal methyl transferase involved in the regulation of a phosphatase complex. A four wavelength data set was collected on the Se-met substituted crystals. The protein contains 14 methionines and phasing is underway.
Complete data sets on the native protein with and without a potential cosubstrate have also been collected.

Sonia Fieulaine, Sylvie Nessler, Pierre Briozzo (1 shift) : Structural studies of the *Lactobacillus casei* HPr Kinase / Phosphorylase FBP complex and of the Deoxyribose-induced protein (Deox)

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

The **14/09/02 on ID14-4** we also collected the first data of the complex between HPr and HprK/P in presence of the allosteric effector fructose-1, 6 bisphosphate (FBP). The crystals diffracted to 2.5Å resolution but the both data sets were incomplete and the resulting density was of bad quality.

During **this run**, we collected two data sets to 3.5Å resolution with a new crystal form of the complex with the catalytic domain of *L. casei* HprK/P in presence of FBP. These new data helped us to improve our model of the allosteric transition induced by FBP in HprK/P but we need higher resolution to describe its interaction mode.

Deoxyribose-induced protein (Deox):

DeoX, a protein from *Salmonella typhimurium*, is possibly related to the growth of the bacteria when deoxyribose is the only source of carbon in the medium. Structure and function of this protein were unknown. We collected a native dataset on the beamline BM14. Crystals diffracted to 2.4 A resolution in space group $P2_1$. Because the aminoacid sequence of DeoX shared no homology with anything known, we decided to solve its structure by MAD phasing. A good dataset could be collected on the beamline BM30A (FIP) at 2.6 A resolution in space group $P2_1$. However, the low symmetry and the high number of Se atom in the asymetric unit (90) did not allow us to find good position of the Se sites and therefore solve the structure (neither with SOLVE, SnB nor ShelxD). Recently, a function of deoxyribose mutarotase has been attributed to the enzyme. The only known structure of a mutarotase is the galactose mutarotase from *E. coli*. Molecular replacement with this model was not successful. We found different crystallisation conditions, where the ASU only contained 2 monomers in the P321 space group. A MAD dataset has been collected on the ID14-4 with these crystals. Diffraction patterns reached 2.2 A reolution. But the 30 sites were not found because this time, merohedral twinnng occured (twin fraction of 22%) and the anomalous signal was lost.