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

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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: BAG-LEBS 2004-1	**Experiment number**: 30-01-627
Beamline: BM30A	**Date of experiment**: from: 07/02/2004 at 8:00 to: 09/02/2004 at 8:00	**Date of report**: 1/6/04
Shifts: 6 (16 bunch)	**Local contact(s)**: **Dr. Philippe CARPENTIER**	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Nicolas Leulliot, Maitre de Conference (Prof. Assistant), M. Graille, Post-doc., IBBMC, Orsay

Pierre Briozzo*, Maitre de Conference (Prof. Assistant), Cecile Evrin, PhD ,

Stephane Mouilleron, PhD, Thierry Bizebard, CR1 (Research Assistant), CNRS,

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Nicolas Leulliot, M. Graille (2.75 shifts): 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) YPL152157w (target 228).
Spacegroup C2221 a=157A; b=172A; c=53A.
Resolution 3.5A
R_{sym}=0.18

This protein is an activator of the phosphotyrosyl phosphatase activity of protein phosphatase 2A (PP2A) in *S. cerevisiae* and hence is important for cell cycle progression and microtubule dynamics. Rod shaped crystals grown from a SeMet labeled protein have been obtained just before this run and have diffracted to 3.2A. This protein contains 12 methionine out of 358 residues. We have collected 210 degrees at the Selenium edge but the resulting dataset was of relatively bad quality (3.5A resolution with a Rsym of 0.18). More data could not be collected on this crystal as it died after the first dataset and phasing was not possible.

2) RRM. (SET-1 domain)
Spacegroup $P4_332$
Resolution 3.0 A.
Completion 100%.
This domain from the Set1 protein of *S. cerevisiae* plays a key role in methylation of histones thus in chromatin

remodeling. During this session, we have tested several crystals and have collected 3 datasets from crystals soaked in different heavy metals. More data are needed to solve the structure of this protein.

3) Neocarzinostatin mutants.
Spacegroup: $P6_5$.
Resolution: 1.9A.
Completion: 100%
Neocarzinostatin (NCS) is an antitumour antibiotic protein isolated from the actinomycetes *Streptomyces carzinostaticus* whose structure has been previously solved to 1.5Å resolution. A directed evolution strategy was used to confer to this protein the ability to bind a human hormone. Among the selected mutants, some have very different side chains at the mutated positions and we want to solve the structures of each of these mutants complexes to the hormone. We have already solved the structure of two mutants complexed to the steroid. During this shift, we have collected a full dataset from a third mutant which differs greatly in sequence from the previous ones. However, the crystal belongs to space group $P6_5$ and present a perfect twin which is problematic for refinement.

Pierre Briozzo*, Cecile Evrin (0.75 shift): Structural study of Uridine monophosphate kinase from *Escherichia coli* and of Cytokinin oxidase from *Zea mays*

We used the beamtime from 5h30 am to 12h30 am. No problem with the beam, but a there was a problem with the camera allowing centering of the crystals (magnification too weak).

1) Uridine monophosphate kinase from *Escherichia coli* (UMPKeco):
Uridine monophosphate kinase from *Escherichia coli* (UMPKeco) catalyses the reaction UMP + ATP -> UDP + ADP. This enzyme is essential for bacterial survival, and its primary structure is divergent from that of eukaryotic UMP/CMP kinases. It 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 kinases. We have grown small needle-like crystals (0.2 x 0.025 mm) of a soluble variant of UMPKeco. On 01/02 August 2003 we had collected data on the BM30A/FIP beamline using the anomalous diffraction of selenium. We then solved and refined the structure at 2.45 A resolution using SAD method (manuscript in preparation).

On 8 February we have collected data for a crystal of the complex between UMPKeco and UDP. The time remaining for data collection was rather short and the crystal quality poor. Anyway, we collected a complete set of data at 2.6 A resolution. The structure of the complex has been solved and is currently at the final steps of refinement.

2) Cytokinin oxidase from Zea mays:
Cytokinins are hormones that control plant growth and development. The flavoenzyme (with FAD cofactor) cytokinin oxidase irreversibly inactivates these hormones. Up to now, there is no known structure for a cytokinin oxidase. We have grown yellow crystals of the enzyme. We have collected a complete diffraction data set of the protein at 1.95 angstroms resolution.

Stephane Mouilleron, Thierry Bizebard (2.5 shifts): Structural studies of ribonuclease E and Glucosamine Phosphate Synthase

The diffraction of crystals of *Escherichia coli* CafA protein (a protein highly homologous –30% identity- to *E. coli* ribonuclease E) was tested on BM30A. The best crystals diffracted up to 6 Å, with a trigonal or hexagonal lattice. The diffraction thus obtained is insufficient to be able to determine the three-dimensional structure of the protein. For this reason, we have undertaken to overexpress and crystallise truncated forms of this protein (which is highly susceptible to proteolysis), with the hope that some of these truncations could lead to better ordered and well diffracting crystals.

Elsewhere, several crystals of Glucosamine Phosphate Synthase were tested and one dataset was collected, up to 3.5 Å resolution. It was found subsequently that this crystal was twinned, and thus, the diffraction dataset was not usable.