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: BAG-LEBS FIP 2003-1**	**Experiment number**: 30-01-607
Beamline: BM30A	**Date of experiment**: from: 12/05/2003 to: 13/05/2003	**Date of report**: 10/06/2003
Shifts: 3	**Local contact(s)**: Dr. Philippe Carpentier	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Guillaume Hible (Ph. D. student; LEBS); Louis Renault (CNRS; LEBS; CR1); Béatrice Golinelli (CNRS; LEBS; CR1); Clotilde Husson (Ph. D. student; LEBS); Stéphane Mouilleron (Ph. D. student); Pierre Briozzo (Maitre de Conference, LEBS).

Report:

G. Hible, A. Ghosh, L. Renault : Structural studies of the Arf and GBP1 regulatory GTP-binding proteins and of a bacterial Guanylate Mono-Phosphate Kinase (GMPK) (1 shift):

We used 1 shift beam time on BM30A to test small crystals of different protein-protein or protein-ligands complexes, but could not collect any data sets due to the poor quality of crystals combined to the weak beam intensity (16 bunch mode).

Structural study of a bacterial Guanylate Kinase:

Guanylate Kinase (GMPK) is a nucleoside monophosphate kinase that is essential for the biosynthesis of GTP and dGTP by catalyzing the reversible phosphoryl transfer from ATP to (d)GMP to yeld ADP and (d)GDP. In addition, antiviral prodrugs like Aciclovir, and anticancer prodrugs, are dependent of this enzymze for their activation. Our aim is to characterize the catalytic intermediates and use the structures as models for the activation of antiviral prodrugs.

We tested several crystals of different complexes of GMPK bound to nucleotide. A crystal of GMPK-GMP-ADPnP diffracted at 3,9Å resolution and had identical parameters with the crystal on wich we collected a dataset on the 26/04/2003 on BM30-A (space group P4 with unit cell parameters : a=b=108Å c=280Å, $\alpha=\beta=\gamma=90°$). Given that the crystal has a big unit cell, exhibit a high mosaïcity and that the beam intensity mode was only 16 bunch, we didn't have enough time to collect a complete dataset with suitable small oscillations. It seems that the different complexes of GMPK with nucleotides or analogues give crystals with parameters which are not favorable with the use of molecular replacement (estimation of the Matthews parameters give 10 to 20 molecules per asymmetric unit and the only avaible model is monomeric). So we are thinking of producing heavy-atom derivatives in order to solve the structures by MAD or MIRAS.

Structural study of the GEF-catalysed activation of Arf small GTP-binding protein:
Arf G proteins functions as binary switches in regulating transport vesicle budding in endocytosis and exocytosis and phospholipase D activation by cycling between inactive cytosolic GDP-bound and active membrane-anchored GTP-bound states. Like many other regulatory G proteins, the conversion of Arf-GDP to Arf-GTP is intrinsically very slow and is catalyzed by a guanine nucleotide exchange factor (GEF) along a complex multi-step reaction which is poorly understood at the molecular level. This reaction involves transient binary and ternary complexes between G protein, guanine nucleotide, and GEF. We have crystallized full length Arf1 in presence of the Sec7 domain of ARNO GEF.
Crystals were too small and too weakly diffracting (3.5 Å at the most) to collect within 1 shift in the beam mode a complete data set in P1 with high mosaicity and a longer unit cell parameter of 205 Å.

Béatrice Golinelli, Clotilde Husson, Stéphane Mouilleron (1.5 shifts) : tRNA methyltransferase and glucosamine-6-P synthase and pyridoxal-P dependent catalytic antibody

Several very small crystals of dimethylguanine tRNA methyltransferase were tested but they did not diffract.

Crystals of a Cys-Ala mutant of glucosamine-6-P synthase in complex with glutamine and fructose-6-P were tested in different cryoconditions. In the best cryoprotectant, ethylene glycol, the crystals diffracted to 3.5 Å resolution. The space group seems to be primitive rhombohedral with a unit cell of a=b=c=250Å, $\alpha=\beta=\gamma=60$. The minimum exposure was 2 min per image. As we were running out of time, we tried to collect a 90° data set with a $\Delta\phi$ of 1° (instead of 0.5°), although there were too many overlaps. However, the dataset could not be processed.
Different crystals, grown in two different condtions, of wild type glucosamine-6-P synthase in complex with inhibitors of the two binding sites were tested in different cryoconditions. The best crystal diffracts to 2.7 Å resolution and belongs to a primitive monoclinic space group with a unit cell of a=70.45, b=59.37, c=109.2, $\alpha=\beta=90$, $\gamma=100$.

Very small crystals of a pyridoxal-P dependent catalytic antibody in complex with a phosphopyridoxylD-alanine sybstrate analog were tested but did not diffract.

Pierre Briozzo (0.5 shifts) : Cochaperonine and uridine monophosphate kinase

On 13 May, on the FIP beamline, two sort of crystals were tried :
- Of a cochaperonin from mouse (HIP), which unfortunately did not diffract.
- Of uridine monophosphate kinase from Escherichia coli. Several crystals were tried, but none of them diffracted to a better resolution, or with less apparent mosaicity than the data previously collected on ID14-2 on February.