EUROPEAN SYNCHROTRON RADIATION FACILITY

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

婕 fill in a separate form for each project or series of measurements.

婕 type your report, in English.

婕 include the reference number of the proposal to which the report refers.

婕 make sure that the text, tables and figures fit into the space available.

婕 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.

<table>
<tr><td> </td><td>Experiment title: BAG-LEBS FIP 2003-1</td><td>Experiment number:
30-01-607</td></tr>
<tr><td>Beamline:
BM30A</td><td>Date of experiment:
from: 26/04/2003 to: 28/04/2003</td><td rowspan="2">Date of report:
10/06/2003
Received at ESRF:</td></tr>
<tr><td>Shifts:
6</td><td>Local contact(s): Dr. Philippe Carpentier
Dr. Michel Pirocchi; Dr. Jean-Luc Ferrer</td></tr>
<tr><td colspan="3">Names and affiliations of applicants (* indicates experimentalists):
Isabelle Llorens (Orsay University; Ph.D. student); Marc Graille (CNRS; LEBS; Post-doc) ;
Nicolas Leulliot (Orsay University ;
Assistant professor); Laurent Lariviere (LEBS; Ph.D. student);
Guillaume Hible (Ph. D. student; LEBS); Agnidipta Ghosh (Ph. D. student; Mac-Planck Institut of Dortmund, Germany); Louis Renault (CNRS; LEBS; CR1); Philippe Meyer (CNRS; LEBS; Post-doc); Franck Coste (CNRS Orléans; CR2).</td></tr>
</table>

Report:

This file contains experimental reports for experiments on 06 February 2003 (1 shift), 10-11 February 2003 (4 shifts) and 26-28 April 2003 (6 shifts).

1 shift of experiment on 06 February 2003:

Date of experiment: from: 06/02/2003-7:00 to: 06/02/2003-15:00

Local contact**:** Dr. Michel Pirocchi

I. Llorens, M. Graille (1 shift) : DNA topoisomerase VI from *Sulfolobus shibatae*.

DNA topoisomerases are enzymes that catalyze changes in the topological state of DNA molecules during biological processes as important as replication, transcription and DNA repair. We are interested in DNA topoisomerase VI produced by the archaebacteria *S. shibatae*. Strikingly, this bacterial protein is the target of different drugs currently used in cancer treatment. Crystals of this enzyme under its free form or complexed to a cofactor have recently been obtained but diffract poorly at our rotating anode. The use of a powerful synchrotron beam line is then necessary to solve the structure of this protein, which should be the first one of an intact DNA topoisomerase. This is the first step towards the resolution of enzyme-inhibitor complexes that should improve the understanding of anti-cancer drugs action on their cellular targets. This extra-beamtime was specially dedicated to test crystals of the free form of the intact enzyme. Unfortunately, as the best diffraction observed was only to 6A resolution, no data set was recorded during this session.

4 shifts of experiment on 10-11 February 2003

Date of experiment:

from: 10/02/2003-7:00 to: 11/02/2003-7:00

and

from:11/02/2003-15:00 to: 11/02/2003-23:00

Local contacts: Dr. Jean-Luc Ferrer; Dr. P. Carpentier

N. Leulliot (1.5 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)YML079W (target 136).
Spacegroup I432 a=b=c=208A.
Resolution 2.8A
Completion 100 %

This orf codes for a protein of unknown function. We have collected a highly redundant SAD dataset that allowed phasing and refinement to 2.8A resolution (Rf=28.4%; R=22.3%). The structure of the protein is a jelly roll fold consisting of ten b-strands organised in two parallel packed b-sheets. The protein has strong structural resemblance to the plant storage and ligand binding proteins (canavalin, glycinin, auxin binding protein) but also to some plant and bacterial enzymes (epimerase, germin). The protein forms homo-dimers in the crystal, confirming measurements of its molecular mass in solution.

2)YGL148w (target 238).
Spacegroup I222 a=58A; b=75A;c=153A.
Resolution 3A
Completion 70 %

This orf codes for a chorismate synthase, an enzyme that catalyzes the last of the seven steps in the shikimate pathway, which is used in prokaryotes, fungi and plants for the biosynthesis of aromatic amino acids. Yeast chorismate synthase is a bifunctional enzyme: it first reduces flavin mononucleotide by NADPH and then catalyzes the 1,4-trans elimination of the phosphate group from 5-enolpyruvylshikimate-3-phosphate (EPSP) to form chorismate which can then be used in phenylalanine, tyrosine or tryptophan biosynthesis. An incomplete SAD dataset has been collected during this beamtime but phasing was not achieved with these data.

L. Lariviere (1.5 shifts) : T4 phage β-glucosyltransferase

I collected two crystal forms of the T4 phage β-glucosyltransferase D100A mutant, in complex with its sugar donor, UDP-glucose. WT enzyme cleaves UDP-glucose during crystallisation and till now only the UDP moiety was present in the structures. D100A mutant prevents this cleveage. These data sets has provided us the first structures of T4 phage β-glucosyltransferase in complex with its complete sugar donor.

Coordinates have been deposited in the RCSB under accession codes 1NZD and 1NZF. These results are described in the following publication :
Lariviere L., Gueguen-Chaignon V., Morera S. *Crystal structures of the T4 phage β-glucosyltransferase and the D100A mutant in complex with UDP-glucose : glucose binding and identification of the catalytic base for a direct displacement mechanism.* **(2003) J. Mol. Biol. in press.**

L. Renault (1 shift) : 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 binary and ternary complexes between G protein, guanine nucleotide, and GEF, that we try to trap for structural studies by mutations.
We obtained small crystals of myristylated and not myristylated Arf5 in complex with the Sec7 domain of Arno in different crystallization conditions but we could not collect any data set on the beam line since most of the crystals were not diffracting or diffracting only to very low resolution.

6 shifts of experiment on 26-28 April 2003

Date of experiment:

from: 26/04/2003-8:00 to: 28/04/2003-8:00
Local contact: Dr. P. Carpentier

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) (3 shifts):

We used a large part of 3 shifts beam time on BM30A to test small crystals of different protein-protein or protein-ligands complexes. We could collect two data sets: one to 1.8Å on a crystal of the small GTP-binding protein Arf1 bound to its Sec7 domain Exchange factor, the other to 3.5Å on a bacterial GMPK bound to adpNHp/Mg and a drug.

Structural study of human Interferon-induced guanylate-binding protein 1 (GBP1):
GBP1 is a large multidomain GTP-binding protein of 67 kDa. The protein is induced by interferon-gamma, an immuno-modulatory substance and has been shown to inhibit viral replication when overexpressed (Anderson S. et al. (1999), Virology 256, 8-14) but its regulation mechanism is not understood. Biochemically the protein has the peculiarity among regulatory GTP-binding protein to undergo oligomerisation with a high concentration-dependent GTPase activity (Prakash B. et al. (2000), Nature 403, 567-71) and to hydrolyse GTP into GDP *and* also GMP.
We have crystallized the protein in presence of a non-hydrolysable GDP analogue to elucidate the molecular mechanism of GDP hydrolysis into GMP, unique by a regulatory GTP-binding protein, and the

basis of co-operativity. The crystals were obtained in different conditions but all were twinned and diffracted to a resolution lower than 3.5 Å. No suitable data sets could therefore be collected.

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 have investigated different nucleotides and analogues in complex with a bacterial GMPK (23,5kDa) but at that time crystals diffracted poorly. We have collected one single dataset on a crystal of GMPK-ADPnP-drug at 3,5Å resolution. The crystal belongs to space group P4 with unit cell parameters : a=b=108Å c=280Å, $\alpha=\beta=\gamma=90°$, with high mosaïcity. Furthermore estimation of the Matthews parameters give 10 to 20 molecules per asymmetric unit. Since the only avaible model is monomeric, the resolution of the structure by molecular replacement will be very difficult. Further improvements of the crystals quality are necessary to solve the structure.

Structural study of the 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 Arf1Δ17 in presence of the Sec7 domain of ARNO GEF and collected a complete data on BM30 to 1.8 Å, with good statistics to 1.88 Å (table 1).

Table 1: **Data Collection statistics**

Data set	Native
Data collection	
X-ray source (wavelength in Å)	BM30A/ESRF (0.98)
Space group	$P4_12_12$
Unit cell parameters	a=111.5 Å, c=74.5 Å
Resolution range (Å)	20 – 1.88
Highest resolution shell (Å)	1.90 – 1.88
Unique reflections	38616
Multiplicity *	12 (12.1)
Completeness (%) *	99.0 (99.2)
R_{meas} *	0.049 (0.329)
I/σ *	29 (7.7)

*: statistics for the hisghest resolution shell are given in parenthesis

A solution of molecular replacement has been obtained and model refinement is currently in progress.

P. Meyer, M. Graille, F. Coste (3 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)YGL148w (target 238).
Spacegroup I222 a=58A; b=75A;c=153A.
Resolution 2.2A
Completion 100 %

This orf codes for a chorismate synthase, an enzyme that catalyzes the last of the seven steps in the shikimate pathway, which is used in prokaryotes, fungi and plants for the biosynthesis of aromatic amino acids. Yeast chorismate synthase is a bifunctional enzyme: it first reduces flavin mononucleotide by NADPH and then catalyzes the 1,4-trans elimination of the phosphate group from 5-enolpyruvylshikimate-3-phosphate (EPSP) to form chorismate which can then be used in phenylalanine, tyrosine or tryptophan biosynthesis. A highly redundant SAD dataset has been collected and allowed phasing. This structure is currently under refinement.

2)YHR049w (target 91).
Spacegroup $P2_1$ a=77A; b=45A; c=78.8A; b=90.153°.
Resolution 2.8A
Completion 93 %

This protein is of unknown structure and function, despite a similarity with *S. pombe* dihydrofolate reductase. This protein contains 5 methionines out of 243 amino acids. A SAD dataset has been collected during this session but phasing was not possible.

3) Colicin D from *E. coli*.
Space group $P4_12_12$; a=b=63A; c=149A.
Resolution 3A.
Completion 100%.

The secreted toxin Colicin D (ColD) kills competing bacteria through an RNase activity. It is composed of 3 domains, involved either in translocation of the protein across the outer-membrane of the target bacteria, in binding to a cell-surface receptor or in the lethal function (specific cleavage of all bacterial arginyl tRNAs). In *E. coli*, this catalytic domain is inhibited by an immunity protein (ImmD), which is released upon entry into the cytoplasm of the target bacterium. As very few are known about colD RNase active site and its inhibition by ImmD, we have crystallized the complex between colD lethal domain and ImmD. Native crystals diffracted to 2Å. A unique and small crystal from the SeMet protein has been obtained. During this session, a SAD dataset has been collected to 3Å. These data allowed phasing to 3.5 Å and model building is currently in process.