EUROPEAN SYNCHROTRON RADIATION FACILITY

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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:** LEBS BAG 2002-2	**Experiment number**: MX 60
Beamline: ID14 1	**Date of experiment**: from: 9/11/02-8:00 to: 10/11/02-8:00	**Date of report**: 28/02/03
Shifts: 3	**Local contact(s)**: Dr. Joanne MCCARTHY	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Laurent Lariviere* (Ph.D. student), S. Pasqualato*(Post-doc), L. Renault*(research assistant, CNRS), M. Graille*(Post-doc), LEBS, Bat 34, CNRS UPR9063, 1 av. de la Terrasse, Gif-Sur-Yvette, France
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Report:

L. Lariviere (1 shift) : β-Glucosyltransferase (BGT)

β-Glucosyltransferase (BGT) is a DNA-modifying enzyme encoded by bacteriophage T4 which catalyses the transfer of glucose from uridine to 5-hydroxymethylcytosine in double-stranded DNA. We have previously solved two ternary complexes of BGT with UDP and a 13-mer DNA analog. This time, we have investigated the DNA binding of different oligonucleotides. Unfortunately, several crystals diffracted poorly or did not contain any DNA. We collected one single dataset of a new ternary complex of BGT-UDP-DNA at 1.9 Å resolution. The refined model shows an unexpected bound DNA. Further studies are needed to valid the structural result.

S. Pasqualato, L. Renault, Agnidipta Ghosh (1 shift) : Structural studies of G proteins

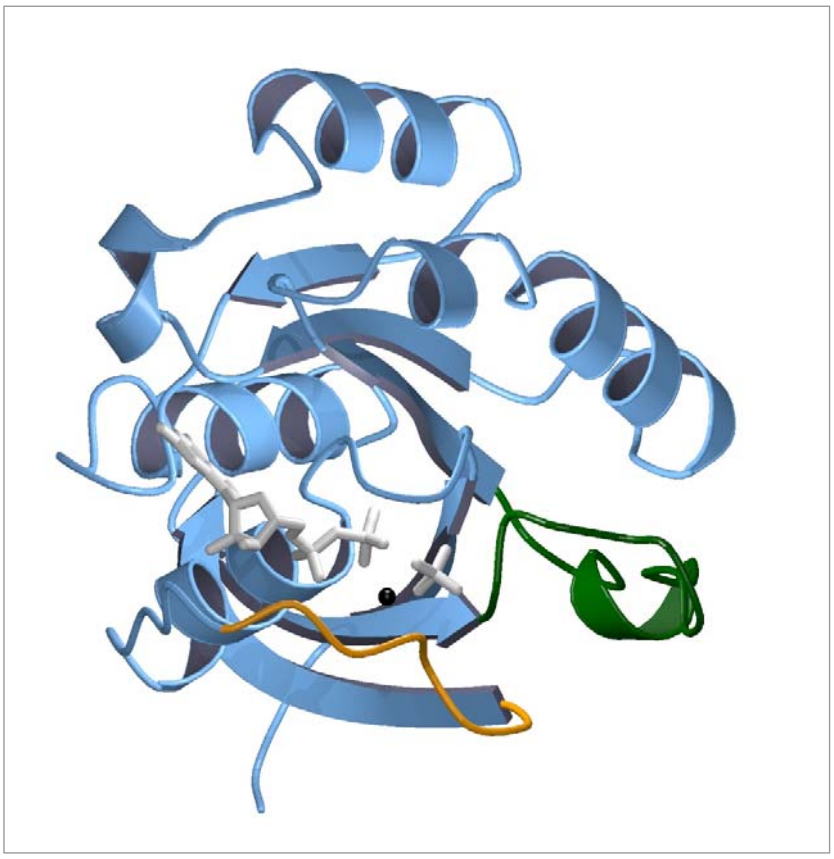
We used 1 shift of beam time on the beam line ID14-1 to collect data sets on protein crystals of two regulatory GTP-binding proteins : the human small GTP-binding protein Rab11 (24.4 kDa) and the human Interferon-induced guanylate-binding protein 1 (GBP1 – 67.9 kDa).

Structural study of the human small GTP-binding protein Rab11

Rab proteins are small GTP binding proteins involved in tethering and specific sorting between vesicles and membranes, allowing the vesicles to specifically address to the target membranes and fuse with them in order to release their cargo. Rab11a is implicated in the regulation of the trafficking events in the endocytic pathway at the recycling endosome. As all other small nucleotide binding proteins it cycles between an 'inactive' GDP-bound conformation and an 'active' GTP-bound form that can bind effectors proteins and thus transmits a signal or trigger an event. Given the high number of Rab proteins in the cell (as high as 60 in human), understanding the structural mechanism by which each Rab protein is specifically associated to a given pathway, in which it plays a key role, remains a major issue.

To elucidate the structural GDP/GTP cycle of Rab11a we undertook its crystallization in complex with GDP, the non-hydrolysable analog of GTP, GTPγS, and GTP. The structures of Rab11a in complex with GDP and GTPγS have been solved by virtue of data collected at the ESRF in late 2001 and early 2002. In November 9th, 2002, we have collected a complete dataset, at 1.7 Å of Rab11a(Q70L) in complex with GTP, on beamline ID14-1 (see table for dataset statistics). The structure is currently under refinement. The current model (R_{cryst} = 22.0%, R_{free} = 24.0%) displays a GDP molecule plus an inorganic phosphate in the nucleotide-binding site, and is represented in figure.

Data collection	
resolution limits (Å)	30 – 1.70
space group	I422
unit cell parameters	
a = b (Å)	73.73
c (Å)	124.905
α = β = γ (°)	90
Reflections	
Measured	356479
Unique	19517
Completeness (%)	99.2
Rsymm (%)	4.6
I/σ	13.2
Structure refinement	
Resolution limits (Å)	30 – 1.70
reflections for R_{cryst}/R_{free}	17753/1620
Rcryst/Rfree (%)	22.0/24.0



Structural study of human Interferon-induced guanylate-binding protein 1 (GBP1)

We used beam time on the beam line ID14-1 to collect data sets on protein crystals of the human Interferon-induced guanylate-binding protein 1 G-domain (hGBP1 amino acid 1-317 38.2kDa) in complex with GppNHp (a non hydrolysable GTP analogue).

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 GppNHp to elucidate the molecular mechanism of GTP hydrolysis & the basis of co-operativity.

On Nov11, 2002 we have collected a complete data set on ID14-1 to a 2.75Å resolution on GBP1G-doamin crystals obtained with GppNHp. The crystals belong to space group I212121 with the unit cell constant a=54.903 Å,b=101.144Å,c=149.267 Å. Statistics on this data set from ID14-1 gave an overall Rmeas=6.9% (I/sI=13.85) for a resolution range from 20 to 2.74Å and an Rmeas=43.3% (I/sI=5.66) in the last resolution shell 2.91-2.74 Å. The molecular replacement solution has confirmed the presence of GppNHp in the crystal structure. Model building and refinement are finished with a final Rfree of 28% and Rfactor of 24%.

M. Graille, N. Leuliot (1 shift) : 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)YGR205w (target 43).
Spacegroup P43212 a=b=65A; c=140A.
Resolution 2.25A
Completion 100%
Rsym=4.1

The structure of this protein was previously solved by MAD data obtained from Se-Met crystals. The structure revealed an unexpected strong resemblance to the structure of a know enzyme. During this shift, three datasets were collected from crystals soaked with either triphosphate or diphosphate nucleotide. Unfortunately, analysis of the data did not reveal the presence of any nucleotide at the active site.

2)YDR533c (target 155).
Spacegroup P21212 a=61A; b=166A; c=48A.
Resolution 1.9A
Completion 97.8%
Rsym=8.1%

The structure and function of this orf are unknown. Structural similarity may exist with a protein from Methanococcus whose structure was recently solved in a structural genomics project at Berkeley. The protein is probably a protease but this has to be confirmed experimentally. As this orf contains no methionine with the exception of the N-terminal one for 237 residues, we constructed a quadruple methionine mutant. We have collected a dataset at 1.9A resolution from a crystal of this quadruple mutant. We need now to collect a full MAD dataset to solve the structure of this orf.

3)YGL148w (target 238)
Spacegroup P1 a=58A; b=76A; c=91A; alpha=114°; beta=108°; gamma=90°.

Resolution 2.9A.
Completion 90%
Rsym=4%

This orf codes for the enzyme chorismate sythase, which is key step in the stynthesis of the essential aromatic amino acids: Tyr and Phe. The crystal used to collect these data was the first one of this project to diffract. Work is underway to produce SeMet crystals of this orf.

4)LicT from Bacillus subtilis.
Spacegroup P3221 a=b=48A; c=162.5A.
Resolution 1.9A.
Completion 100%
Rsym=3.7%.

LicT is involved in the regulation of polyglucane degradation in Bacillus subtilis. LicT functions as a transcriptional antiterminator protein composed of two structural and functional domains: one RNA binding domain and a regulatory domain. From the same crystal, we have collected two datasets: one at low and one at high resolution. These data are currently used for the refinement of the structure.

5)human VpreB receptor complexed to its stromal ligand : human galectin-1.
Spacegroup P212121 a=44A; b=58A; c=112A.
Resolution 2.1 A
Completion 99 %
R-fact_total 0.077

VpreB is a receptor protein which is expressed on the surface of developing B-cells at the preB stage. There has been a longstanding effort in the purification and crystallization of this atypical receptor. Crystals of the V preB receptor were obtained in presence of its natural stromal ligand : galectin 1, a protein of about 120aa belonging to the mammalian lectin family. Analysis of the solvent content of the crystals showed that the whole complex could not be present in the asymmetric unit. The structure was solved by Molecular Replacement showing that indeed only the galectin1 crystallized. The structure is refined and is very similar to previous determined mammalian galectin structures. Studies are underway to characterize biochemically the interaction between VpreB and galectin in order to helps us to get crystals of the complex.