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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-EH1	**Date of experiment**: from: 17/05/02-8 :00 to: 18/05/02-8 :00	**Date of report**: 26/08/02
Shifts: 3	**Local contact(s)**: Joanne Mac Carthy	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Sebastiano Pasqualato* (LEBS, PhD student), **Louis Renault*** (LEBS, CNRS), **Sonia Fieulaine*** (LEBS, PhD student), **Sylvie Nessler*** (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:

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

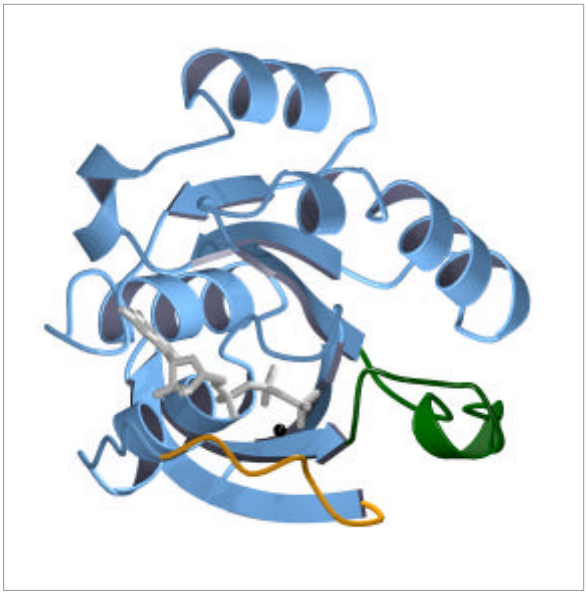
We used 1 shift of beam time on the beam line ID14-1 to collect data sets on protein crystals of two regulatory GTP-binding proteins : the human small GTP-binding protein Rab11 (24.4 kDa) and the human Interferon-induced guanylate-binding protein 1 (GBP1 – 67.9 kDa).

Structural study of the human small GTP-binding protein Rab11

Rab proteins are small GTP binding proteins involved in several different substeps of the intacellular trafficking machinery. Vesicles bud from donor compartments and reach target organelles to which membranes they fuse, releasing their cargoes. Rab proteins regulate specifical targetting and tethering of vesicles to acceptor compartment membranes. Rab11 is implicated in the regulation of the trafficking events in the endocytic pathway at the recycling endosomes. 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 trasmit a signal or trigger a cellular event. Given the high number of Rab proteins in the cell (more than 40) understanding the structural mechanisms 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 these mechanisms we undertook the structural study of the complete GDP/GTP cycle of human Rab11. Structure of Rab11 in complex with GDP have been resolved by virtue of data collected at the ESRF in 2001. Crystals of the GTP-bound form of Rab11 have

been obtained and a complete dataset at 1.9 Å was collected on May 17th, 2002 at beamline ID14-2 (see table for dataset statistics). These data permitted to solve the structure, that is actually under refinement. The current model displays R_{cryst} = 26.4 and R_{free} = 28.6 and is represented in figure.

Data collection	
resolution limits (Å)	30 – 1.90
space group	I422
unit cell parameters	
a = b (Å)	73.73
c (Å)	125.15
a = ß = ? (°)	90
Reflections	
Measured	207365
Unique	13984
Completeness (%)	96.5
Rsymm (%)	9.6
I/s	22.2
Structure refinement	
Resolution limits (Å)	30 – 2.05
reflections for R_{cryst}/R_{free}	10214/919
Rcryst/Rfree (%)	26.4/28.3



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

The protein GBP1 is induced by interferon γ which is an immuno-modulatory substance and has an antiviral activity (Anderson S. et al. (1999), Virology 256, 8-14). Its cellular function is related at the biochemical level to the ability to undergo oligomerisation with a high concentration-dependent GTPase activity (Prakash et al., (2000), Nature 403, 567-571). The protein is further characterised among regulatory GTPases by its distinctive ability to hydrolyse GTP to GDP *and* GMP. To understand at the atomic level the high concentration-dependent and unique GTPase activity properties of the protein, we have have cristallized the protein in presence of GMP and AlF to obtain a state mimicking the intermediate state of the GDP hydrolysis.

On May 2002 we have collected a first complete data set on ID14-1 to a 2.95Å resolution on these GBP1 crystals. Based on this experience we have been able to collect a second higher resolution data collected on ID14-4 to 2.45Å on June 2002. The crystals belong to P212121 and the orthorhombic unit cell of the ID14-1 data set is a=149.043 Å,b=101.164 Å,c=54.881 Å. Statistics on this 2.95Å data set gave an overall Rmeas=7% (I/σI=25.7) for a resolution range from 30 to 2.95 Å and an Rmeas=30.6% (I/σI=8.8) in

the last resolution range 3.0-2.95 Å. We have obtained a molecular solution replacement solution with as search model the GMPPnhP-bound GBP1 structure (Prakash et al., (2000), EMBO 19, 4555-64). Model building and refinment are currently in process with a current Rfree at 37% and Rfactor at 34%. Current electron density maps suggest that GMP and AlF are bound to the protein in the crystal structure and that several regions of GBP1 undergo conformational changes in addition to the usual conformational changes found in switch 1 and switch 2 common to all regulatory GTP-binding proteins.

Sonia Fieulaine, Sylvie Nessler (1 shift) : Structural study of the *Lactobacillus casei* HPr Kinase / Phosphorylase FBP complex

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 **11/02/02 on ID14-1**, we tested crystals of complexes between a full-length *S. salivarius* HprK/P in complex with *B. subtilis* HPr.

During **this run** we collected a low resolution data set with a new kind of crystals obtained with full length *S. salivarius* HprK/P in complex with HPr. We try to find a molecular replacement solution at 7Å resolution in the hope to see if HPr binding induces a large conformation change in the global structure of HprK/P, in particular in the position of the N-terminal regulatory domains.

We also tested the diffraction of small crystals obtained with the full length *Neisseria* HprK/P in presence of GDP but here again, we were not able to see spots above 8Å resolution. We now try to increase the dimensions of these crystals.

Ines Li De La Sierra, Nicolas Leuliot (1 shift) : Structural studies of the complex VPREB receptor, of COLLICIN D from E. coli and of the yeast protein orf YML079w (yeast *Saccharomyces cerevisiae* Structural Genomics project)

1) COMPLEX VPREB receptor,

Spacegroup P21 ; a=48.7 b=71.2 c=65.9 ; β= 100°
Resolution = 3.0 A
completion = 89.9 %
R-fact_total = 0.104

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 purufucation and crystallization of this atypical receptor. Crystals of the V preB receptor were obtained in presence of a Fab fragment directed against the receptor in order to help the crystallization process. 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 Fab fragment crystallized. This structure will be useful since the same strategy of cocrystallization with the Fab, is now being used in other combinations : VpreB and effector ligands. Better crystals of the Fab are being prepared to allow more accurate refinement of the Fab structure.

2) COLLICIN D from E. coli

spacegroup P41212 a=62.8 c=148.7
resolution = 2.6 A
completion = 99.8%
R-fact_total = 0.079

Collicins are toxins that are used by bacteria to protect themselves against other microorganisms. They are secreted in the culture medium as a complex with the immunity protein, which protects the producing organism against toxic effects. Colicin D has a tRNA hydrolysing toxic activity. It was shown that ColD is proteolytically cleaved during entrance in the target cell. ColD has no sequence identity with colicins of known structure. We obtained crystals of the complex of the E. Coli ColD and its corresponding immunity protein. Native data have been collected at 2.6A and improvement of the crystals has now yielded data at 2.0A resolution (collected on the French CRG BM30). Biochemical analysis of the crystals revealed that they contain a proteolytic fragment of ColD that is generated during the crystallization process. Mass spectrometric analysis has been carried out to determine the exact sequence of the fragment. Se-Met production of these fragments is on the way to solve the structure of the fragment.

3) Yeast Orf YML079w (target 136) – 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

Spacegroup P212121 a=56.4 b=69.3 c=108.67
Resolution = 2.2 A
completion = 93.7 %
R-fact_total = 0.123

This orf of unknown function crystallizes as thin plates. Crystals suffer from severe anisotropy but useful data could be collected on native crystals during this run. Unfortunately , anisotropy gets beyond

acceptable levels for the Se-Met substituted crystals. Efforts are being oriented towards improvements of the quality of the Se-Met crystals.