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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.

ESRF	**Experiment title:** CNRS Gif sur Yvette BAG (LEBS)	**Experiment number**: LS 2072
Beamline: ID14-2	**Date of experiment**: from: 10/11/2001 to: 12/11/2001	**Date of report**: 22/02/2002
Shifts: 6	**Local contact(s)**: Dr. Cecile Jamin	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Yuxing CHEN*, PhD student, Laurent Lariviere*, PhD student, Joel Janin, Professeur Université Orsay, Laboratoire d'Enzymologie et Biochimie Structurales (LEBS), CNRS, Gif-sur-Yvette

Sebastiano Pasqualato*, PhD student, Louis Renault*, CNRS, Jacqueline Cherfils, CNRS, same than above

Julie Menetrey*, Post-Doc, Marc Graille*, PhD student, Enrico Stura, CEA, Dépt. Ingéniérie et Etude des Protéines-CEA, Saclay

Report:

Yuxing CHEN, Laurent Lariviere (2.25 shift)

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 shown that BGT cleaves the glucose of UDPG. To identify the glucose position, inhibitors of BGT mimicking UDPG were diffused in native BGT crystals. We collected 5 datasets. Another dataset was collected from a crystal of human NDP kinase A complexed with ATP. All these structures are currently at late stages of refinement.

Sebastiano Pasqualato, Louis Renault (1.5 shift): Structural studies of small G proteins and of their partners

Structural study of the small G protein Rab11 in complex with GDP

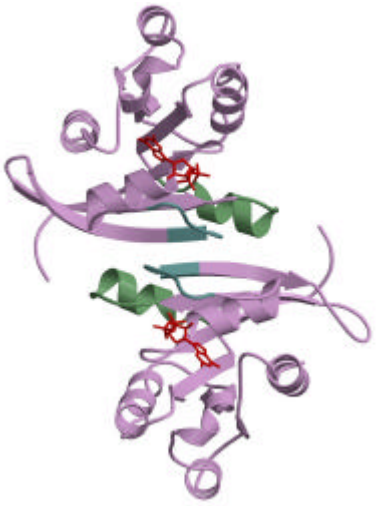
Rab proteins are small GTP binding proteins involved in tethering and specific sorting between vescicles and membranes, allowing the vesicles to specifically address to the target membranes and fuse with them in order to release their cargo. Rab11 is implicated in the regulation of the trafficking events 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 trasmit a signal or trigger an event.

Given the high number of Rab proteins in the cell (more than 40) 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 Rab11 we undertook its crystallization in complex with GDP and the non-hydrolysable analog of GTP, GTPγS.

On September 11th, 2001 we collected two datasets on crystals of Rab11-GDP, at 2.4 and 2.1 Å. As the preliminary refinement of the structure seemed to rule out the presence of a Mg ion in the nucleotide binding site of the small GTP binding protein, we produced crystals in the presence of higher $MgCl_2$ concentration. The crystals belonged to the same spacegroup as the ones grown in lower $MgCl_2$ concentration ($P2_12_12_1$ with a = 47.3 Å, b = 69.7 Å, c = 108.3 Å, $\alpha = \beta = \gamma = 90$ °) and permitted to collect a complete 1.98 Å dataset on November 11th, 2001. This dataset permitted to solve the structure of Rab11-GDP, that is now refined at R = 21.0, R_{free} = 24.6 after molecular replacement with the model obtained with the datasets collected on September 13th, 2001. Rab11-GDP crystallizes as a dimer in the asymmetric unit, and no Mg ion is visible in the electron density map to coordinate the GDP. Experiments are actually on progress in the lab to elucidate wether this dimeric arrangement reflects an *in vivo* status of the protein.



Crystals of Rab11 crystallised in the presence of the non-hydrolysable analog of GTP, GTPγS, were tested for diffraction on November 11th, 2001. They all diffracted and belonged to the same space group as the GDP ones. Indeed, they all revealed to be Rab11-GDP crystals.

Structural study of the GEF ARNO isolated and in complex with its cognate small G protein Arf1

We tested also small and rare crystals of fragments of the Arf guanine nucleotide exchange factor ARNO alone or in an abortive complexe with Arf.

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 or by the use of an inhibitor brefeldin A. In parallel since Arf exchange factors are always multi-domain proteins containing a GEF catalytic Sec7 domain whose activity is modulated by neighbouring domains, we investigate also Arf-GEF proteins alone to understand the three-dimensional arrangement and regulation of their domains.

We tested therefore in november 2001 on the ID14-2 beam line small and rare crystals of a putative Sec7-ARNO•Arf1•GDP complex of low affinity blocked by Brefledin A (size of 0.05x0.04x0.03 mm) and of a fragment of ARNO containing both its Sec7-domain and PH domain (size of 0.2x0.05x0.05 mm). Both crystal forms did not diffract below 10 Å.

Julie Menetrey, Marc Graille, Enrico Stura (2.25 shift)

The C3 exoenzyme is an ADP-ribosyltransferase toxin that has been extensively used as molecular tool for study the small G protein Rho functions. Understanding molecular properties of C3 by structural studies will be helpful to modify C3 and improve its selectivity for Rho protein. Previously, we have solved the structures of C3, free of dinucleotide (2.4 Å) and in complex with its co-factor the NAD (1.95 Å). To complete this structural study, we have undertaken the resolution of the structure of C3 in complex with a NAD inhibitor which blocks the activity. Data set of C3-free crystals soaked with the NAD analog have been collected at 2.0 Å resolution. The structure show that the NAD analog is only partially bound to the toxin, the complete binding being hindered by the crystal packing environment. Further experiments, including co-crystallisation should be undertaken to obtain the structure of C3 in complex with an inhibitor.

The parasite *Toxoplasma gondii* is responsible for toxoplasmosis, a worldwide infectious disease that can be life-threatening in immunocompromised individuals and in pregnant woman. The protein P30, present at the surface of the invasive form of the parasite plays a major role in parasite attachment to the host cell. Then, the crystal structure of the complex between P30 and the Fab of a neutralising antibody is of biological importance to map the epitope responsible for parasite entry in the host cell. Previous crystals of the P30-Fab complex had diffracted to 4.3 Å resolution. During this session on ID14-EH2, we have collected a native data set to 3.9 Å (Space group C222; R_{sym} of 0.099 and 88.4% complete) and to 3.1 Å resolution (Space group $P2_12_12_1$; R_{sym} of 0.10 and 100% complete). However, the low sequence homology of P30 with any protein of known structure combined with the low phasing power of the Fab imply the use of *ab initio* phasing methods. Further experiments using heavy atom derivatives are necessary to obtain phase informations to solve the structure.

The human urokinase receptor plays an important role in cancer and cell invasion. The elucidation of the structure will be of great importance in the diagnosis and therapy in cancer. This human receptor is a highly glycosylated protein that crystallised in a cubic space group, diffracting to 6.8 Å in BM 30 for the native form. Crystals of the native form were improved and then tested in ID 14-2 in order to attain a better resolution. We could collected two data sets to 4.5 Å and 5 Å. We succeed in the improvement of the attained resolution but it still not enough to solve the structure of the receptor.

The alkaline phosphatase from human placenta (PLAP) hydrolyzes the phosphomonoesters to produce phosphate and alcohol. It is involved in fœtal growth and probably also in cancer cell proliferation but its precise function remains to determine.The structure of PLAP has been solved at 1.8 Å resolution (*J. Biol. Chem.* , **276**, 9158-9165 (2001)), and we are now trying to determine its precise physiological role. We have therefore collected to data set of PLAP in complex with inhibitors. Unfortunately, the ligand was absent in the electronic density corresponding to both data sets.