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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:**<br>CNRS Gif sur Yvette BAG (LEBS) | **Experiment number**:<br>LS 2072 |
| **Beamline**:<br>ID29 | **Date of experiment**:<br>from: 11/02/2002 to: 12/02/2002 | **Date of report**:<br>22/02/2002 |
| **Shifts:**<br>3 | **Local contact(s)**:<br>Dr. Andy THOMPSON | *Received at ESRF:* |

**Names and affiliations of applicants** (* indicates experimentalists):

Sebastiano Pasqualato*, PhD student, Jacqueline Cherfils, CNRS, Laboratoire d'Enzymologie et Biochimie Structurales (LEBS), CNRS, Gif-sur-Yvette
Benoit Gigant**, CNRS, Thierry Bizebard*, CNRS, Marcel Knossow, CNRS, same than above
Sonia Fieulaine*, PhD student, Sylvie Nessler**, CNRS, Joel Janin, Professeur Université Orsay, same than above

**Report:**

**Sebastiano Pasqualato (0.4 shift): Structural study of the RabGEF Rab3IP**

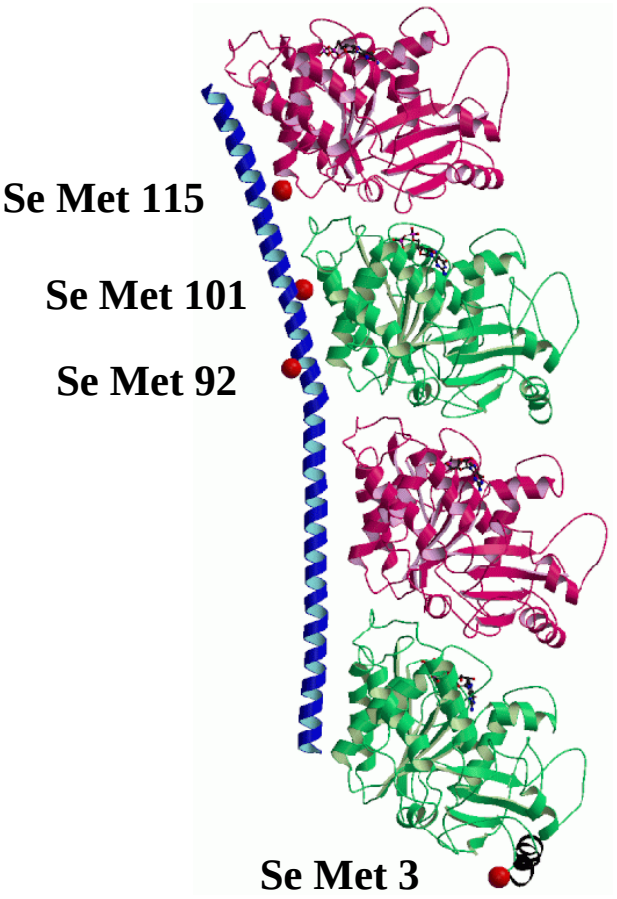
Rab proteins are small GTP binding proteins involved in tethering and specific sorting between vescicles and membranes, allowing the vesicles to specifically address to the target membranes and fuse with them in order to release their cargo. Rab11 is implicated in the regulation of the trafficking events 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 mechanisms by which this subtle regulation is accomplished we overtook the structural study of a protein (Rab3IP) that has been shown to interact with Rab11 and is supposed to be a nucleotide exchange factor (GEF), that is a protein that catalyses the release of GDP form the protein and permits thus its activation.
Crystals of a fragment of Rab3IP have been obtained and tested for diffraction on ID29 on February 11$^{th}$, 2002. The crystals diffracted up to 9 Å in the best of the case and a diffraction pattern of a fiber-like structure was always present. We are currently working on enhancing the crystal quality, namely working on different crystallisation conditions.

**Benoit Gigant, Thierry Bizebard (1.7 shifts): Structural strudy of the stathmin – tubulin complex**

During this visit, a data set was collected at two wavelengths (peak and inflection point) with a platinum derivative, using a crystal that diffracts to 4.0 Å. The crystal suffered very much from radiation damage (scale factor varying from 1 to 0.25 in scalepack) and, probably as a consequence, the data quality is not very good (Rsym overall : 0.11; Rsym in the 4.15 Å – 4.0 Å shell: 0.53). Although platinum sites have previously been identified in this derivative, the heavy atoms could not be located using the two data sets we collected during this visit. Radiation decay is a major problem as it has always been with these crystals. Apparently the attenuators we introduced did not compensate for the additional brilliance of the beam on ID29 since the vacuum ondulator has been installed.

Data collected in previous visits, during the first half of this BAG and the one that precedes it (LS 1928) have now been fully analysed. There are two major results :

- a SAD data set collected on ID14-4 at the Se peak wavelength has allowed us to locate four peaks in an anomalous difference map (in a complex that comprises ca 2000 amino acids); their spacing coresponds to that of the four methionine residues of stathmin. Three of the methionines are located in the stathmin long helix that connects the two tubulin molecules of the complex. Their identification allows us to orient this stathmin helix in the complex, which had not been possible before from the molecular replacement phases.




The fourth methionine is the third stathmin residue (counting from the Nterm) and is located close to helix H10 of a tubulin α subunit. This confirms results obtained by others using a cross-linking reagent and is a direct experimental indication of the location of the N-term domain of stathmin in the complex. Unfortunately, the phases deduced from these data are not of sufficient quality to calculate an experimental map.

**Figure** : the stathmin-tubulin complex superimposed to 4 Se peaks (red balls) identified in a single SAD experiment. Green and red : alpha and beta tubulin subunits. Blue : stathmin α-helix

1) two derivatives have been identified, one with Yb, the other with Platinum, both in SAD experiments. They have allowed us to calculate experimental phases to 5.5 Å (with a figure of merit larger than 0.2, before any solvent flattening; figure of merit at 6.5Å: 0.4). More data should now be collected on these derivatives to extend the resolution of experimental phases to the highest resolution possible. It should in particular be possible now to exploit the length of our crystals (more than .3 mm in most cases) to collect MAD data sets in wedges at different points, as has been demonstrated successfully with ribosome crystals.

**Sonia Fieulaine, Sylvie Nessler (0.9 shift) : structural study of HPr Kinase/Phosphorylase (HprK/P)**

HPr-kinase/phosphorylase is a bacterial Ser/Thr kinase which has no sequence similarities with other known protein kinases. Sugars enter the cell via the PTS system, then glycolysis produces FBP which activates phosphorylation of the small protein HPr on residue Ser-46, catalysed by HPr-kinase. Further interaction of P-Ser-HPr with the transcription regulator CcpA activates the carbon catabolite repression signalisation pathway. The aim of this study is to understand the catalytic mechanism of the enzyme HPr-kinase/phosphorylase.

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 structure of the full length *S. xylosus* HprK/P (PDB ID code 1ko7) has been solved by K. Scheffzek at the EMBL of Heidelberg (Marquez et al. (2002)PNAS,19, 3458-3463) using the catalytic domain of *L. casei* HprK/P as model for molecular replacement.

We now try to obtain the structure of a full-length HprK/P in complex with HPr. During **this run**, we tried to collect data with two crystal forms of the complex with the full length *S. salivarius* HprK/P. The first one, in presence of magnesium, did not diffract at all whereas the second form containing calcium diffracted to low resolution. We try to improve the quality of these crystals.