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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-EH4	**Date of experiment**: from: 15/06/02-8 :00 to: 16/06/02-8 :00	**Date of report**: 26/08/02
Shifts: 3	**Local contact(s)**: Raimond Ravelli	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Louis Renault*(LEBS, CNRS), **Benoit Gigant*** (LEBS, CNRS), **Marcel Knossow*** (LEBS, CNRS), 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

Report:

L. Renault (1 shift) : Structural study of GBP1 in different intermediate states of the GTP hydrolysis reaction

I used 1 shift of beam time on the beam line ID14-4 to collect data sets on protein crystals of the human Interferon-induced guanylate-binding protein 1 (GBP1 – 67.9 kDa) putatively trapped in different states.

The protein GBP1 is a GTP-binding proteins whose cell regulation is not properly understood. The protein 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/GDP and AlF to obtain a state mimicking the intermediate state of GDP/GTP hydrolysis.

On May 2002 we have collected a first complete data set on ID14-1 to a 2.95Å resolution on GBP1 crystals obtained with GMP and AlF. Based on this experience we were able first to collect a second higher resolution data collected on ID14-4 to 2.45Å on June 2002. The crystals belong to P212121 and the unit cell of the higher data set is a=149.617 Å,b=101.927 Å,c=54.121 Å. Statistics on this data set from ID14-4 gave an overall Rmeas=7.2% (I/σI=25.0) for a resolution range from 30 to 2.43 Å and an Rmeas=31.3% (I/σI=8.7) in the last resolution range 2.47-2.43 Å. The molecular solution replacement solution obtained with the previous 2.95Å resolution data sets was confirmed with the new data set and current electron density maps suggest that GMP and AlF are bound to the protein in the crystal structure. Model building and refinment are currently in process with a current Rfree at 37% and Rfactor at 34%.

I have also collected on ID14-4 two complete data sets of GBP1 crystals obtained with GDP and AlF in different crystallization conditions. Crystals belong to P212121 space group and difffract around 3.5Å

resolution. The best data set has an orthorhombic unit cell of a= 148.820 Å,b=103.921 Å,c=55.485 Å. Statistics on this data set gave an overall Rmeas=8.4% (I/σI=19.2) for a resolution range from 30 to 3.33 Å and an Rmeas=30.1% (I/σI=8.8) in the last resolution range 3.44-3.33 Å. We have obtained a molecular solution replacement solution with 2 molelucles/assymetric unit with as search model the GMP/AlF-bound GBP1 structure still in refinement. Model building and refinment for this second hydroslysis state of GBP1 are currently in process with a current Rfree at 43% and Rfactor at 39%.

Benoit Gigant, Marcel Knossow (2 shifts) : Structure of the tubulin-stathmin complex

During this session, anomalous data on three new potential derivatives (obtained by soaking with Ir hexamine, Methyl Re oxyde and potassium Osmate) were collected at the peak of anomalous diffusion, after the fluorescence of the heavy atom had been observed from the crystals. No heavy atom was present in the anomalous Patterson. Since Se peaks that correspond to the Se-methionine residues of the stathmine-like moiety of the complex were detected in these maps (though data were not collected at the optimum wavelength for Se), the conclusion is that the quality of the data is adequate but that the derivatives tested are not useful for phasing.

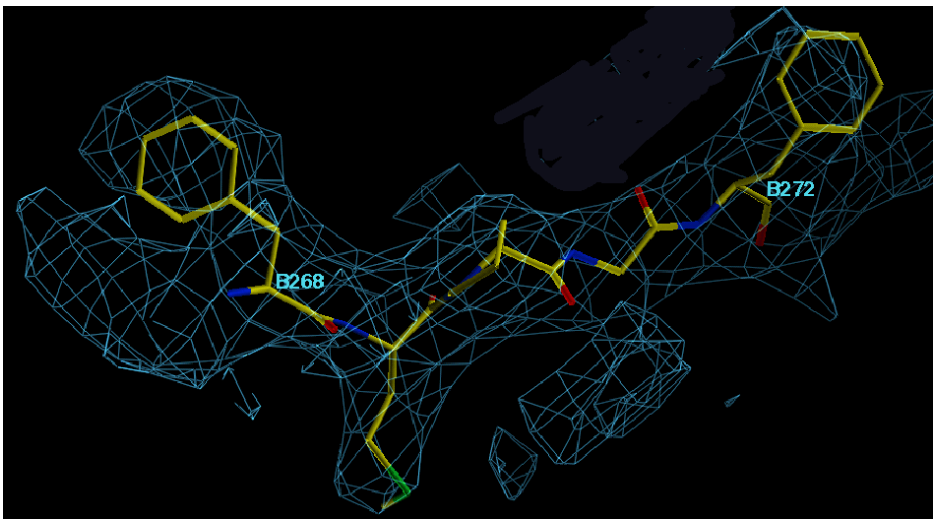

Figure : Sharpened Fobs Φexp map in the 268-272 region of one of the beta tubulin subunits of the stathmin-like: tubulin complex. Highest resolution of the data: 3.7 Å

Using the anomalous data collected on previous visits to ID14-4 from Lu, Se, Pt and some mercury derivatives, we have calculated (in collaboration with R. Ravelli) a map after solvent flattening, multi-crystal and non-crystallographic symmetry averaging. This was then sharpened (Bsharp = 100.). The resulting experimental map (see Figure) shows for the first time side-chains for most tubulin residues, alowing the model to be rebuilt. At the time the report is written, one complete alpha subunit and two thirds of a beta subunit out of the two alpha and two beta subunits of the complex have been rebuilt.