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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 (LEBS)	**Experiment number**: LS 2072
Beamline: ID14-1	**Date of experiment**: from: 13/09/2001 to: 14/09/2001	**Date of report**: 22/02/2002
Shifts: 3	**Local contact(s)**: Dr. Elspeth Gordon	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Beatrice Golinelli*, CNRS, Marcel Knossow, CNRS, Laboratoire d'Enzymologie et Biochimie Structurales (LEBS), CNRS, Gif-sur-Yvette
Sebastiano Pasqualato*, PhD student, Ludovic Guigou*, PhD student , Marc Mirande, CNRS, Jacqueline Cherfils, CNRS, same than above

Solange Morera*, CNRS, Laurent Lariviere*, PhD student, Joel Janin, Professeur Universite Orsay, same than above

Report:

Beatrice Golinelli (1 shift) : Structural studies of catalytic antibodies

1) 2 hours were lost with problems on the beam line (there was a problem with the detector and the beam was not correctly aligned at the beginning of the experiment and the local contact was late; the camera was not fixed properly and had to be frequently repositioned)

2) Time left was used to test infiltrated crystals that either did not diffract properly or were twinned.

S. Pasqualato (0.66 shift): Structural study of the small G protein Rab11

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.
Crystals of Rab11-GDP were obtained and two complete datasets were collected on ID14-1 on September 13^{th}, 2001. Crystals belong to space group $P2_12_12_1$ with a = 47.3 Å, b = 69.7 Å, c = 108.3 Å, $\alpha = \beta = \gamma = 90$

°. The poor quality of crystals reflected on the scarce quality of the low resolution data collected, and a molecular replacement solution appeared clearly only merging the two datasets and using Sec4-GDP (pdb entry 1G16) as a searching model. The structure was under refinement when better quality dataset was collected on ID14-2, on November 11th, 2001. (see 11.11.01 report for details).

L. Guigou (0.33 shift): Structural study of the tRNA-binding protein p43

In mammals, nine aminoacyl-tRNA synthetases are associated within a multi-enzyme complex also comprising three additional components, p18, p38 and p43. The p43 protein is an ubiquitous, auxiliary component of this multisynthetase complex. It is an α_2 dimer of 2×35 kDa. Dimerization and association with other components of the complex is mediated by the N-terminal segment of the polypeptide chain. In the native state, p43 binds tRNA with a K_d of about 0.2 µM and may play the role of a cofactor of aminoacyl-tRNA synthetases in the aminoacylation reaction.

The 166 C-terminal residues of human p43 is a monomer of 18 kDa, $p43^{18}$. It corresponds to EMAPII (Endothelial-Monocyte-Activating Polypeptide II), an inflammatory cytokine released under apoptotic conditions. It is produced during the late steps of apoptosis and stimulates chemotactic migration of phagocytes. The crystal structure of $p43^{18}$ revealed an OB-fold β-barrel domain.

We recently established that p43 is processed in a variety of proteolytic fragments at different stages of the development of apoptosis in cultured human cell lines. One of these derivatives, $p43^{23}$, is produced early and may play a triggering role in the irreversible cell growth/cell death transition induced under apoptotic conditions. The $p43^{23}$ derivative still binds tRNAs but, due to its release from the multi-enzyme complex, loses its ability to deliver tRNA to the synthetases. In order to determine the mode of binding of tRNA to p43, and to characterize the structural features that may explain the cellular role of $p43^{23}$ and its ability to be secreted in the culture medium, we obtained crystals ($150 \times 150 \times 50$) of the recombinant protein expressed in E. coli and collected a complete data set. Crystals (space group I2,2,2, cell $44 \times 95 \times 96$) diffracted at 2.0 Å resolution. This structure was solved by molecular replacement using the $p43^{18}$ model (1FLO). The structure is being to be refined. The 30-40 N-terminal residues are not visible in the electron density map. Because this could be due to the mobility of this peptide in the crystal, we thought to grow crystals in the presence of RNA. Crystal growth and analysis are in progress.

Solange Morera, Laurent Lariviere (1shift): Structural study of 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 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 6 data sets. All the structures are under refinement.