EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

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:** LEBS BAG 2002-2	**Experiment number**: MX 60
Beamline: ID14 4	**Date of experiment**: from: 02/12/02-8:00 to: 03/12/02-8:00	**Date of report**: 28/02/03
Shifts: 3	**Local contact(s)**: Dr. Raimond Ravelli	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Marcel Knossow* (Research director, CNRS), Maud Hertzog* (Ph.D. student), LEBS, Bat 34, CNRS UPR9063, 1 av. de la Terrasse, Gif-Sur-Yvette, France
Nicolas Leuliot*(Maitre de Conference, Orsay University), Institut de Biochimie et de Biophysique Moléculaire et Cellulaire (IBBMC), Equipe Génomique Structurale, CNRS UMR8619, Université Paris-Sud, Orsay, France

Report:

M. Knossow, M. Hertzog (2.5 shifts) : Cytoskeleton dynamics

Structural study of Tubulin:

Stathmin is a 17 kDa ubiquitous phosphoprotein that has been proposed to be a relay integrating diverse intracellular signaling pathways. This protein has also been identified as a microtubule destabilizing factor likely to be implicated in various microtubule dependent cellular functions in interphase or mitosis. Stathmin influences microtubule dynamics *in vivo* and *in vitro* either by preventing assembly or promoting disassembly of microtubules in a concentration-dependant manner.

Stathmin interacts with the αβ tubulin heterodimer to form a ternary complex comprising one stathmin and two tubulins. This complex has proven to be stable enough to be cristallized, whereas no well-diffracting 3D-crystal of tubulin has been reported up to now. The only near-atomic structural information on tubulin comes from electron microscopy of tubulin protofilaments. We have determined the 4 Å x-ray structure of a complex of GDP-tubulin with the stathmin-like fragment of RB3, a stathmin family protein (PDB ID code : 1FFX). This structure was solved by molecular replacement using the electron microscopy model (Cell (2000) **102**, 809-816).

Informations on the tubulin-stathmin interactions and on the way stathmin sequesters tubulin were gained from this structure but, in order to define the interactions of tubulin with ligands and nucleotides, a more precise structure is required. Experimental phases have been deduced from experiments performed prior to this visit at ESRF. During this visit 8 cryobuffers were tested in order to improve the resolution limit of our data. Glucose was found to perform best. This has allowed us to measure data to 3.5 Å resolution (Rmerge: 8.2 %). Together with the phases we have previously determined, these data are used in the refinement of the stathmin-tubulin structure which is now nearly complete (R: 0.23, Rfree: 0.26). This is being prepared for publication.

Structural study of Actin:

The assembly of actin filaments is regulated by several proteins. Thymosin β4 sequesters actin whereas proteins such as Ciboulot participate in the assembly of actin at the barbed end of filaments; all these proteins are members of the actobindin familly and share an actin-binding domain for which no crystal structure is known, either alone or in complex with actin. Using one single crystal (the only one we have ever obtained) of an actin-ciboulot complex, we collected a 2.5 Å data set (Rmerge: 9%) and determined the structure. Refinement is now complete (R: 0.23). This work is being prepared for publication.

M. Graille, N. Leuliot (1 shift) : yeast *Saccharomyces 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

1)YDR533c (target 155).
Spacegroup P21212 a=61A; b=166A; c=48A.
Resolution 2.5A
Completion 50%
Rsym=8%

The structure and function of this orf are unknown. Structural similarity may exist with a protein from Methanococcus whose structure was recently solved in a structural genomics project at Berkeley. The protein is probably a protease but this has to be confirmed experimentally. As this orf contains no methionine with the exception of the N-terminal one for 237 residues, we constructed a quadruple methionine mutant. We planned to collect a full MAD dataset but crystals died just in the middle of the SeMet peak wavelength. We couldn't do anything with this partial dataset.

2)YFL030w (target 241).
Spacegroup P3221 a=b=58A; c=185A.
Resolution 3A.
Completion 99%
Rsym=6.8%

This protein of unknown structure has been annotated as a putative alanine glyoxylate aminotransferase, probably PLP-dependent. We have crystallised this orf in the presence of PLP, a putative cofactor. One dataset has been collected during this shift but it will only be usefull when experimental phases will be obtained from SeMet crystals.