EUROPEAN **S**YNCHROTRON **R**ADIATION **F**ACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

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.

	Experiment title: **BAG-LEBS-ESRF-2003-1**	Experiment number: MX-60
Beamline: ID29	**Date of experiment**: from:05/04/2003-7:00 to:06/04/2003-7:00	**Date of report**: 29/08/03
Shifts: 3	**Local contact(s)**: B. Sheppard	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

M. Knossow, B. Gigant and F. Eghian (1 shift): Structural study of complexes of Tubulin with drugs

Tubulin is the basic building block of microtubules, one of the key components of the cytoskeleton. In particular, and as an example, during cell division microtubules form the mitotic spindle. Tubulin, either free or microtubule-polymerized, is therefore one of the best characterized targets of anticancer drugs.

We have obtained crystals of tubulin in complex with the stathmin-like domain of the neural protein RB3. The structure was solved initially by molecular replacement using an electron microscopy model of tubulin, and subsequently by the SAD method using a seleno-methionine derivative of RB3 (see previous reports). The structure has allowed us to determine the binding site of cocristallized ligands of the colchicine binding site of tubulin (manuscript in preparation). The experiment performed at ESRF on ID14eh4 on july 3 /4 were devoted to characterize the binding mode of other ligands of tubulin, with soaked crystals. The soaking technique was previously used succesfully with vinblastine (refinement in progress).

Three different ligands were tested and 3 datasets collected. For one ligand, the electron density maps are devoided of specific signal. The last two ligands are thought to bind to the vinblastine site of tubulin. In these cases, data were collected to 4.3 and 4.4 Å resolution. The site of the second ligand (weak signal) does not appear to overlap with the vinblastine site,

whereas the site of the third one overlaps partially with this site; in this case data should be collected to higher resolution to confirm our finding and allow meaningful refinement.

N. Leuliot, F. Coste (2 shifts) : 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) DNA topoisomerase VI from Sulfolobus shibatae complexed to a cofactor.
Spacegroup $P2_12_12_1$ a=315A; b=112A; c=192A.
Resolution 4.4A.
Completion 94.1%
Rsym=13,4%

DNA topoisomerases are enzymes that catalyze changes in the topological state of DNA molecules during biological processes as important as replication, transcription and DNA repair. We are interested in DNA topoisomerase VI produced by the archaebacteria S. shibatae. Strikingly, this bacterial protein is the target of different drugs currently used in cancer treatment. We have collected a 4.4A dataset from crystals of DNA topoisomerase VI grown in the presence of a cofactor. Molecular replacement trials are currently carried out to try to solve the structure.

2)YHR029c (target 48).
Spacegroup $P4_32_12$ a=b=81A; c=98A.
Resolution 2A.
Completion 100%
Rsym=6%

This orf codes for a protein, which belongs to the Phzf family involved in the synthesis of the broad spectrum antibiotic phenazine-1-carboxylic acid (PCA). Phzf is involved in dimerization of two 2,3-dihydro-3-oxo-anthranilic acid molecules to create PCA in P. Fluorescens. It is is similar to 3-deoxy-D-arabino-heptulosonate-7-phosphate synthases of solanaceous plants and distantly related to diaminopimelate epimerases. We have collected a 3 wavelength MAD SeMet dataset which allowed the resolution of the structure to 2.0A resolution. This protein composed of 294 amino acids is composed of 2 structural domain that have the same fold, i.e. a β-barrel with an α-helix running inside the barrel.

3) YIR029w (target 205)
Spacegroup $P3_221$ a= b=109A; c=190A.
Resolution 2.6A.
Completion 100%
Rsym=6.7%

This orf codes for an allantoicase, an enzyme associated with the purine metabolism. It breaks down allantoin into glyoxylate and urea and thus is involved in the use of purine as a secondary source of nitrogen. We have collected a 3 wavelength MAD SeMet dataset to 2.6A resolution but we were not able to solve the structure of this protein from these data.