EUROPEAN SYNCHROTRON RADIATION FACILITY
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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.

	Experiment title:	Experiment number:
	CNRS – LEBS-2003-2	MX-240
Beamline:	**Date of experiment**:	**Date of report**:
ID14 eh4	from: 01/12/2003 8h to: 02/12/2003 7h	
Shifts:	**Local contact(s)**:	*Received at ESRF:*
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Report: Molecular mechanisms of tubulin regulation.

The tubulin heterodimer is the microtubule building block. Microtubules are hollow cylinders made of parallel protofilaments which alternate cycles of polymerization and depolymerization in a process known as dynamic instability. GTP bound to the tubulin subunit is hydrolysed in GDP when tubulin is incorporated in microtubules. This gives rise to the paradox that the microtubule is mainly composed of GDP-tubulin, which is unable to polymerize. Our work is aimed at understanding this.

We have solved by MIRAS the crystal structure of a soluble form of tubulin, complexed with the stathmin-like domain of the protein RB3 (RB3-SLD), a member of the stathmin family of proteins, which are cellular regulators of tubulin. The complex comprises 2 tubulin molecules and 1 RB3-SLD. The structure allowed us to determine at the molecular level the regulatory mechanism of stathmin-like proteins, and to demonstrate and visualize the conformational switch between GDP-tubulin in solution and in protofilaments. In addition to proteins, tubulin and microtubules are regulated by small molecule ligands, some of them being of clinical use in particular for cancer therapy. The structure of the tubulin-colchicine:RB3-SLD complex defines the colchicine site (see Ravelli et al, Nature, in press. PDB id 1SA0 and 1AS1 – see abstract pasted at the end of this report).

The 3 shifts were devoted to 4 different topics. We tried to get data at better resolution, mainly from crystals grown in slightly different conditions. One crystal, obtained with trimethylamine as an additive, looked promising and this will be pursued.

We have already shown that the crystals of tubulin:RB3-SLD complex can be used as a template to define the binding site of tubulin ligands such as colchicine (see above) or vinblastine (manuscript in preparation). We are currently limited by the water solubility of the ligands. To circumvent this, we are developing methods to study low solubility compounds. We have chosen nocodazole as a model. Nocodazole is a small molecule supposed to bind at the colchicine site. It is widely used by cellular biologists to disrupt the microtubule network. We have obtained crystals of tubulin:RB3-SLD complex co-

crystallized with nocodazole and collected 3 dataset. The resolution is moderate (at most 4.8 Å) and there is no specific signal for nocodazole in the colchicine site. However we have found some specific signal away from this site. This unexpected result should be confirmed with higher resolution data.

Taxol and related compounds are another class of tubulin ligands which interfere with microtubule dynamic. We are developing a structure-based design study of taxoids in collaboration with Dr. F. Gueritte and colleagues (ICSN - Gif-sur-Yvette). In connection with this, we collected two datasets, one from a soaked crystal and one from a co-crystal, obtained with a soluble taxotere derivative. These are at low resolution (below 6Å). While the taxoid was not localized in these structures, the two tubulins in the complex are arranged slightly differently from the complexes we have studied so far, with a more open angle between them. We are currently working on the crystallization of tubulin with these taxoids in order to improve diffraction.

Finally, we are interested in the conformation of GTP-tubulin, the assembly-competent form of this protein. In a first approach, we crystallized the tubulin:SLD complex where tubulin is complexed with GTP or with a slowly hydrolysable analog (GMP-CPP). We collected 4 dataset, the best resolution being 5.5Å. While the GTP was hydrolyzed in the time course of crystalization, the crystals with GMP-CPP seem promising. The data allow us to determine that one tubulin molecule bears a hydrolyzed GMP-CPP, while the other shows a signal for a third phosphate on its nucleotide. We now have obtained larger crystals of this complex and will test them for higher resolution data. Hopefuly, this will put a structural basis for activation of tubulin by GTP compared to GDP-tubulin.

Abstract of manuscript in press :

Insight into tubulin regulation from a complex with colchicine and a stathmin-like domain

Microtubules are cytoskeletal polymers of tubulin involved in many cellular functions. Their dynamic instability is controlled by numerous compounds and proteins, including colchicine and stathmin family proteins. The way microtubule instability is regulated at the molecular level has remained elusive, mainly because of the lack of appropriate structural data. Here, we present the structure, at 3.5 Å resolution, of tubulin in complex with colchicine and with the stathmin-like domain (SLD) of RB3. It shows the interaction of RB3–SLD with two tubulin heterodimers in a curved complex capped by the SLD amino-terminal domain, which prevents the incorporation of the complexed tubulin into microtubules. The comparison with the structure of tubulin in protofilaments shows changes in the subunits of tubulin as it switches from its straight conformation to a curved one. These changes correlate with the loss of lateral contacts and provide a rationale for the rapid microtubule depolymerization characteristic of dynamic instability. Moreover, the tubulin–colchicine complex sheds light on the mechanism of colchicine's activity: we show that colchicine binds at a location where it prevents curved tubulin from adopting a straight structure, which inhibits assembly.