	Experiment title: The structure of the Stathmin-tubulin complex BAG - CNRS Gif sur Yvette	**Experiment number**: LS 1798
Beamline: ID14-1	**Date of experiment**: from: 7/2/01 to: 8/2/01	**Date of report**: 27/2/01
Shifts: 3	**Local contact(s)**: E. Mitchell	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Marcel KNOSSOW *, Martin GAUDIER *, Benoit GIGANT *

LEBS CNRS Gif sur Yvette

Report: The Tubulin - Stathmin complex.

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 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 (see reference and abstract below).

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. This could stem from higher resolution data or from more precise phase information, resulting in particular from the identification of an isomorphous heavy atom derivative. During this last period our work has been devoted to that purpose. We collected data from crystals soaked with heavy atom solutions and we also tried to covalently link heavy atoms to RB3 or to tubulin ligands before crystallization. Statistics of some dataset (space group P65, cell 328 * 328 * 55 ^{3}) are summarized in the table below:

Heavy atoms	TAMM	W18 cluster	W17 cluster	W30 cluster	RB3 SeMet - Tubulin Complex
Resolution	5.5	5.4	4.75	5.35	3.95
Completness	98.7	99.3	98.4	98.4	99.4
Multiplicity	3	3.2	3	3.6	3.8
Rsym	6.7	7.2	5	4.8	5.6
I/ σ (I)	14.6	14.6	19	22	20.9

These procedures have not succeded so far. The next step will involve the use of other heavy atoms, other clusters, and Hg compounds (eg TAMM) covalently linked to site specific mutants of RB3 with an incorporated cysteine. We will also test crystals of tubulins and stathmin-like proteins from sources that differ from those we have used up to now.

Publication:

The 4 Å X-Ray Structure of a Tubulin:Stathmin-like Domain Complex
Cell, Vol. 102, 809–816, September 15, 2000.
Benoît Gigant, Patrick A. Curmi, Carole Martin-Barbey, Elodie Charbaut, Sylvie Lachkar, Luc Lebeau, Samila Siavoshian, André Sobel, and Marcel Knossow.

Summary
Phosphoproteins of the stathmin family interact with the αβ tubulin heterodimer (tubulin) and hence interfere with microtubule dynamics. The structure of the complex of GDP-tubulin with the stathmin-like domain of the neural protein RB3 reveals a head-to-tail assembly of two tubulins with a 91-residue RB3 α helix in which each copy of an internal duplicated sequence interacts with a different tubulin. As a result of the relative orientations adopted by tubulins and by their α and β subunits, the tubulin:RB3 complex forms a curved structure. The RB3 helix thus most likely prevents incorporation of tubulin into microtubules holding it in an assembly with a curvature very similar to that of the depolymerization products of microtubules.