ESRF	**Experiment title:** BAG - CNRS gif sur Yvette The stathmin-tubulin complex	**Experiment number**: LS 1928
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Report: The Tubulin - Stathmin complex

Stathmin is a 17 kDa ubiquitous phosphoprotein that has been proposed to be a relay integrating diverse intracellular signalling pathways. It has recently been identified as a microtubule destabilising factor likely to be implicated in various microtubule dependant cellular functions in interphase or mitosis. Stathmin influences microtubules dynamics *in vivo* and *in vitro* either by preventing assembly or promoting disassembly of microtubules in a concentration-dependant manner. This effect is reduced upon stathmin phosphorylation by kinases.

Stathmin interacts with the αβ tubulin heterodimer to form a ternary complex, one stathmin for two tubulins. This complex has proven to be stable enough to be crystallised, whereas no good-diffracting crystal of tubulins has been reported up to now. The only near-atomic structural informations of tubulin come 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 and this work is now published (Cell, Vol. 102, 809–816, September 15, 2000).

While this structure is a milestone in the crystallography of tubulin, more precise data are required for a molecular description of the biological action of stathmin family molecules as well as of the interaction of small ligands of tubulin which interfere with microtubule dynamics. The last period was devoted to this aim.

We also made in solution (before complexation and crystallisation) mercury derivatives of cysteine mutants of RB3. We collected data from crystals of these RB3 complexed with tubulin but were not able to locate a signal for mercury near RB3. A possible explanation is that a more reactive cysteine of the tubulin displaces the Hg atom. Indeed, there is a signal near a cysteine of the α subunits of tubulin. This hypothesis has now been confirmed by Mass Spectrometry.

The two kinds of stathmin-tubulin crystals we have (with Se-met and Hg RB3) were also tested for higher resolution diffraction. They both gave an improvement and data up to 3.6 Å were collected (overall Rsym 5%, 40% in the last shell, 92% completeness).

In the very last visit of the period (July 15th), we identified a promising heavy atom derivative. We collected data from crystals soaked with YbCl3 and a preliminary processing indicated a single peak located between the two tubulins of the complex, near the GDP molecule of the β subunit.

We now plan to pursue the quest of experimental phases of the tubulin - stathmin-like domain of RB3 complex and to explore other tubulin sources, in particular those known to be homogeneous.