	Experiment title: BAG-LEBS-2004-2	Experiment number: MX-350
Beamline: ID14 eh4	**Date of experiment**: from: 16/02/2005 8h to: 17/02/2005 8h	**Date of report**: 22/2/05
Shifts: 3	**Local contact(s)**: Dr R. RAVELLI	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Benoît GIGANT* **LEBS, 1, Av. de la Terrasse, 91190 Gif-Sur-Yvette, France**

Marcel KNOSSOW* LEBS

Diep LE* LEBS

Matthieu MARCHAND* LEBS

Report: Molecular mechanisms of tubulin and actin regulation.

The tubulin heterodimer is the microtubule building block. Microtubules are hollow cylinders made of parallel protofilaments, they alternate cycles of polymerization and depolymerization in a process known as dynamic instability. In the elongation phase, the GTP bound to the tubulin subunit is hydrolysed to GDP. This gives rise to the paradox that microtubules are mainly constituted of GDP-tubulin, which does not polymerize.

The tubulin Vinca domain

We have determined the crystal structure of a soluble form of tubulin complexed with two of its regulators, namely the stathmin-like domain of the protein RB3 (RB3-SLD) and with colchicine, a small molecule ligand (see Ravelli et al, Nature. 2004 vol. 428:198-202). PDB codes 1SA0 and 1SA1. More recently, we have identified the binding site of vinblastine, an anticancer drug and the prototype of another class of tubulin ligands (manuscript in revision). The 3 shifts we had on ID14eh4 were in part devoted to characterize better the vinca domain from crystals soaked with a dolastatin 10 analog; this drug is supposed to share its binding site with vinblastine while the two structures are remarquably different. We collected a 4 Å full dataset and its analysis is under way.

Tubulin-GMPCPP

We have also pursued our quest for a structure of GTP-tubulin, the assembly competent form of this protein. We collected 2 datasets from a crystal soaked with GMP-CPP, a slowly hydrolysable analog of GTP, and from a crystal crystallized with the same compound. Unfortunately, the resolution is limited (4.6Å and 5.5Å) and will probably only allow us to determine the overall shape of the complex. Better diffracting crystals will

be needed to provide a structural basis for the activation of tubulin by GTP as compared to GDP-tubulin.

Arp2/3

Actin is the building block of microfilaments, the other major component of the eucaryotic cytoskeleton. Our purpose is to decipher the molecular mechanism of actin regulation by cellular proteins. We have already unravelled the mechanism for the actin regulation by WH2 domain proteins (Hertzog et al, Cell. 2004 May 28;117(5):611-23 PDB ID 1SQK). In this session, we tested several crystals of actin complexed with the branching protein Arp2/3 obtained in various crystallisation conditions. Unfortunately, the crystals we had either did not diffract or were salt crystals.