	Experiment title: BAG-LEBS-2005-2	Experiment number: MX-441
dth6804 **Beamline**: ID14 eh4	**Date of experiment**: from: 09/12/2005 8h to: 10/02/2005 8h	**Date of report**: 15/2/06
Shifts: 3	**Local contact(s)**: Dr R. RAVELLI	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Marcel KNOSSOW*** LEBS,** Bat. 34, CNRS FRE2930, 1 Avenue de la Terrasse, 91190 Gif-sur-Yvette, FRANCE

Report:

Marcel KNOSSOW* (3 shifts): Molecular mechanisms of tubulin regulation.

The αβ 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 β subunit is hydrolysed to GDP. This gives rise to the paradox that microtubules are mainly constituted of GDP-tubulin, which does not polymerize.

We have previously determined the crystal structure of a soluble form of tubulin in a complex with the stathmin-like domain of the protein RB3 (the T2R complex), further complexed with small molecules compounds like colchicine and vinblastine. The 3 shifts we had on ID14eh4 were devoted to characterize better these two ligand binding sites.

The tubulin vinca domain.

There are several antimitotic drugs, whose structures are remarquably different, reported to compete with vinblastine for binding to tubulin. We want to define better this so-called vinca domain on tubulin. In a previous experiment, we collected a 4 Å dataset from a crystal soaked with a dolastatin 10 analog, one of the drugs binding to this site. Dolastatin 10 is a linear penta-pseudopeptide molecule and its orientation in the T2R complex was ambiguous. In this session, we collected data from crystals soaked with shorter, three

pseudopeptide long dolastatin analogs, which retain a significant affinity for tubulin. The structures are now refined and permit to unambiguously orient the dolastatin 10 analog.

We also collected data from T2R crystals soaked with another vinca site ligand named cryptophycin. Several dataset from crystals soaked in different conditions were collected but cryptophycin was never detected in the electron density maps.

The tubulin colchicine-site ligands.

As for the vinca domain, numerous small molecules are known to compete with colchicine for tubulin binding. The project is to extend our view on this site. During this session we collected data from T2R co-crystallized with the compound called TN-16. Whereas data to only 4.2 Å resolution could be collected, a signal for the ligand was detected in the electron density maps. We will try to collect better data from these crystals in a next synchrotron experiment but it already appears that TN-16 is more buried in tubulin than colchicine, and their binding sites overlap only partially.