	Experiment title: BAG-LEBS-2005-2	Experiment number: MX-441
Beamline: ID14 eh4	**Date of experiment**: from: 20/02/2006 8h to: 21/02/2006 8h	**Date of report**: 22/2/06
Shifts: 3	**Local contact(s)**: Dr R. RAVELLI	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

The scientists in charge of the beamline used half of the first shift to fix a problem on the line.

Jenny Keller, Marc Graille (0.12 shift): yeast *Saccharomyces cerevisiae* Structural Genomics project

The systematic names of the genes are used. More details on every orf can be found on http://genomics.eu.org:targets.html

1) PH0168
Spacegroup P212121 a= 85 Å, b=95 Å and c=115Å.
Resolution 2.5 Å.
Completion= 100%
Rsym=9%

In 2002, bioinformatic analysis identified a new DNA repair system specific for thermophilic Archae and bacteria. This pseudo-operon, composed of 25 proteins, contains four nucleases, a new type of DNA-polymerase and proteins for which sequence analysis didn't provide any clues about the function. In collaboration with Dr H. Myllykallio (IGM, Université Paris XI- Orsay), we have initiated a project aimed at solving the 3D-structure of proteins encoded by *Pyrococcus horikoshii* belonging to this group. PH0168 is one of the proteins with unknown function. During this beamtime allocation, we have collected a 360 degrees dataset at the Se edge. Phasing trials are in progress.

Audrey DORLEANS* (Ph. D., CNRS), Benoît GIGANT* (2.38 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 beamtime on ID14eh4 were devoted to characterize better these two ligand binding sites. We have also made new attempts to get the structure of GTP-tubulin.

The tubulin colchicine-site ligands.

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 tested crystals of T2R co-crystallized with six different compounds and collected 5 dataset from 4 different complexes. A first processing on site indicated that the resolution is 3.6 – 4 Å resolution, which is close to the best resolution we have had with these kind of cystals. Based on previous experiments we are confident to locate and orient theses ligands in their tubulin binding site. One of these compounds is TN-16, from which we collected a 4.2 Å dataset in december. The resolution is thus significantly improved.

The crystals for the two last complexes were significantly smaller and they diffracted poorly. Bigger crystals will be needed.

The tubulin vinca domain.

We tried crystals soaked with 3 different ligands. This part of the project was less successful. The crystals diffracted poorly, and albeit we tested a lot of them no useful data could be collected. Bigger crystals and/or other soaking conditions have to be tried.

GTP-Tubulin.

As a new attempt to get the structure of soluble GTP-tubulin (or GTP-like), we collected data from crystals of GDP-tubulin in complex with RB3 and co-crystallized or soaked with Beryllium or Aluminium fluorides. These salts mimick either the ground state or a transition state of the third phosphate of di-nucleotides. We collected three dataset at 3.8 – 4 Å resolution.