	Experiment title: BAG-LEBS-2006-2	Experiment number: MX-554
Beamline: ID14-4	**Date of experiment**: from: Friday 10/11/2006 at 8:30 to: Saturday 11/11/2006 at 8:00	**Date of report**: 28/2/06
Shifts: 3	**Local contact(s)**: Dr. R. Ravelli	*Received at ESRF:*

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Report:

Marcel Knossow*, Audrey Dorleans*, Benoit Gigant* (2 shifts): Molecular mechanisms of tubulin regulation.

The αβ tubulin heterodimer is the microtubule (MT) building block. The tubulin/MT cycle is regulated by intracellular proteins. It is also perturbed by exogenous coumpounds, some of which are useful anticancer drugs.

In this session, we have continued the structural definition of the binding mode of small molecule ligands on tubulin using crystals of the tubulin- stathmin-like domain complex (T2R). We collected data for three new ligands. In particular, we have succeeded in growing crystals with nocodazole, a MT depolymerizer widely used in cellular biology. Because nocodazole is poorly water soluble, we had to adapt the crystallisation protocol. Data to 4.4 Å were collected which permit to determine the nocodazole binding site whereas its orientation could not be accurately defined.

We also collected data from crystals of T2R further complexed with fragments of different sizes of a neuronal regulatory protein. Crystals were obtained from complexes with three different fragments and in two different crystallization conditions. They diffracted to ~4Å (4 datasets collected) or 6 Å (one dataset). Whereas the protein fragments were necessary for crystals to grow in these conditions and despite the fact that these fragments are present in the crystals as judged by SDS-PAGE analysis, the space group and cell dimensions are the same as for T2R crystals. Moreover we have so far no signal for the neuronal protein fragment in electron density maps. We presently try to grow crystals with a Seleno-

methionine version of the fragments to settle the issue of whether they are ordered in these crystals.

Nicolas Leulliot* & Mark Brooks* (1.25 shifts) : yeast multi-protein complexes involved in DNA replication, ribosome biogenesis, mRNA quality control pathway and cell signalling and archeophage structural genomics project

1) Pab1020. SAD
Space group $P2_12_12_1$
Resolution 3.2 Å.
Completion= 98.4%; Multiplicity = 7.6
Rsym=13.8%

Bioinformatic analyses of the *Pyrococcus abyssi* genome have identified two proteins which are predicted to be ligases. While one was probably a homolog of human DNA ligase 1 (LIG1), the role of the second, Pab1020 was less clear. Sequence analysis has shown that despite having an ATP-dependent DNA ligase catalytic domain (prokaryotic ligases are NAD-dependent), the remainder of the protein was of unknown fold. Therefore, the gene was cloned and recombinant protein expressed for biochemical characterization, which have shown that it is capable of ligating nicked RNA, but not nicked DNA *in-vitro,* indicating that it is an ATP-dependent RNA ligase. Native datasets have been collected on ID21-EH1 to approximately 3 Å resolution, but the structure was solved by SAD using crystals containing protein labeled with selenium during this run. The sites of these heavy atoms were located by SHELX, followed by refinement and phasing with SHARP. Density modification with DM has allowed an interpretable map to be produced, the crystallographic model of which is in the process of being constructed.

2) RNA modifying complex.
Space group $P2_1$
Resolution 2.4 Å.
Completion= 98.2%; Multiplicity = 3.7
Rsym=8.3%

This RNA modification enzyme is a heterodimer. One protein (A) is responsible for the catalytic activity while the second (B) is necessary for in vivo stabilisation of the enzyme and the in vitro activity. To understand how the B protein influences the activity of A, the A protein is also studied alone a 2.4 Å dataset has been recorded previously at ESRF). During this session, we have collected a 2.4 Å resolution dataset from a crystal of native protein. SeMet labelling of both proteins is in progress so as to solve the structure of this heterodimer.

3) Virar 29 SeM
Space group $P4_22_12$
Resolution: 6 Å.
Completion= 100%; Anomalous multiplicity = 9.4
Rsym= 9.1%

This protein from *Acidianus Filamentus* Virus 3 (AFV3) is a 20.1kD protein. Bioinformatics analysis reveals homologies with TATA binding proteins (TBP). Although this homology is low (14% sequence identity with TBP from *Sulfolobus acidocaldarius*), the residues involved in DNA binding among TBP from archaea are conserved. SeMet labelled protein crystallized and a SAD data set has been collected on ID14-EH4 beamline. Unfortunately, the crystal diffracted at to low resolution (6Å) to solve the structure.