	Experiment title: BAG-LEBS-2006-2	Experiment number: MX-554
Beamline: ID23-1	**Date of experiment**: from: Friday 06/10/2006 at 8:30 to: Saturday 07/10/2006 at 8:00	**Date of report**: 28/2/06
Shifts: 3	**Local contact(s)**: Dr D. FLOT	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

M. Knossow, Audrey Dorleans*, Benoit Gigant***,** Philippe Meyer*, Emeline Leproult*, Christophe Caillat*, LEBS, Bat 34, CNRS UPR9063, 1 av. de la Terrasse, Gif-Sur-Yvette, France

Marc Graille* (CNRS researcher) & Mark Brooks* (Post-doc): Institut de Biochimie et de Biophysique Molé Cellulaire (IBBMC), Equipe Génomique Structurale, CNRS UMR8619, Université Paris-Sud, Orsay, France

Report:

Audrey Dorleans, Benoit Gigant (0.5 shift): Molecular mechanisms of tubulin regulation.

The αβ tubulin heterodimer is the microtubule building block. It is one of the few validated targets for cancer therapy. Our project deals with the definition of the binding mode of small molecule ligands interfering with microtubule dynamic. Some of these ligands are evaluated for, or used in, cancer therapy.

Having determined the crystal structure of tubulin in complex with the stathmin-like domain of the protein RB3 (the T2R complex), we use this complex as a template for tubulin-ligand interaction studies. The three known tubulin binding sites for exogenous compounds have the intriguing peculiarity to accept molecules of apparently unrelated structure. The beamtime on ID23-1 was mainly devoted to characterize two ligands of one of these sites, the colchicine site.

Previous datasets collected at ESRF have permitted to confirm that the coumpound ABT751 is indeed a colchicine site binder. But because of the low resolution diffracting crystals (3.8 Å) and of the symmetry of the coumpound, its orientation in tubulin has remained ambiguous. We thus synthetized a bromo-derivative of ABT751 to take advantage of the radiation sensivity of the C-Br bond, as we did in another case (Gigant et al, Nature 2005). We collected two low resolution dataset (5.6 Å), with minimal exposure. Inbetween them, the crystal was fully exposed to X-ray during a “high” resolution (3.9 Å) data collection. The peaks in the calculated Fobs “before exposure” - Fobs “after” correspond to

chemical groups sensitive to X-ray. Peaks in the vicinity of the ligand have permitted us to orient it.

We also collected a complete 3.8 Å dataset from a T2R crystal complexed with a ligand of the "Locus" serie for which we had no useful data so far. The refinement is under way and already confirms the presence of the ligand in the crystal and its binding in the colchicine site.

Finally, during this half shift, we preliminarily tested new crystals of T2R complexed with a microtubule regulatory protein.

Philippe Meyer, Emeline Leproult & Christophe Caillat (LEBS-CNRS): (1.25 shift): projects : complexes involving ATPases (Hps90), kinases substrates or their partners, nitroreductase and glycosyltransferase (AGT).

-Datasets collected to 3.2 and 3.1 Å (unit cell a=b=106, c=290, $\alpha=\beta=\gamma=90$) on an Hps90 deletion mutant (Middle & C-terminal domain) co-crystallised with ATP, novobiocin and analogues. The structures were solved by MR but no ligand was visible after TLS refinement.

-Data collected to 3.2 Å on AGT in complex with target DNA duplex containing a Hydroxy Methyl Cytosine. The structure was solved by MR but the limited resolution combined with important conformational changes precluded DNA reconstruction.

-Attempt to collect datasets of NRNfrA after infiltration with substrates or inhibitors (Tyrosine, NitroUracyl and NADH).

Marc Graille* & 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) TopoVI.
Spacegroup $P2_12_12_1$ a= 114.9Å; b=200.5 Å; c=329.1 Å
Resolution 3.5 Å.
Completion= 90.4%; Anomalous multiplicity = 2.75
Rsym=10.8%

DNA topoisomerases catalyse changes in the topological state of DNA molecules during biological processes as important as replication, transcription and DNA repair. We are interested in DNA topoisomerase VI produced by an archae. Strikingly, this bacterial protein is the target of different drugs currently used in cancer treatment. We have obtained crystals for this protein five years ago. Most of these crystals diffracted to 6-10 Å resolution but a few of them diffracted to 4.2 Å resolution (native data collected at the ESRF in 2004-2005). We have screened plenty of crystals of SeMet labelled protein until we have got one diffracting to 3.5A resolution during this session. This allowed us to collect a 3.5 Å resolution SAD dataset at the Se-

edge from a crystal grown in the presence of an inhibitor. From these data, we could position 38 out of the 48 Se sites expected and phase the structure. The electron density maps obtained after solvent flattening (80% solvent content) and NCS-averaging (four copies in the asymmetric unit) allowed us to rebuild the model. The structure has been refined to 3.55 Å resolution and the paper describing this first structure of an intact type II DNA topoisomerase is in preparation.

2) Virar 79.
Space group $P6_522$
Resolution 1.9 Å.
Completion= 99.5%; Multiplicity = 35
Rsym=10.7%

ORF79 from Acidianus Filamentus Virus 1 (AFV1) is a 11.6 kD protein which has no homologue identified from sequence. Although no functional information is available, sequence analysis reveals a putative Zn binding site (HCA profile). A native data set at 1.9Å resolution has been recorded. Purification of the SeMet labelled protein is currently performed.

3) Pab1020.
Space group $P2_12_12_1$
Resolution 3.0 Å.
Completion= 97.2%; Multiplicity = 7.2
Rsym=10.1%

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. During this session, we have collected a native dataset to 3 Å resolution. SeMet labeling of the protein is in progress in order to solve this structure.

4) Pab0263.
Space group $P4_32_12$
Resolution 3.4 Å.
Completion= 98%; Multiplicity = 7.5
Rsym=8.1 %

Pab0263 is a protein from *Pyrococcus abyssi* which was identified as a potential partner of Pab1020, and is predicted to possess a Pin-domain type fold. This fold is characteristic of proteins which have been shown to be exonucleases, and indeed preliminary studies show that is capable of degrading RNA *in-vitro*. The structure was solved using single-wavelength anomalous diffraction at ID23-EH1. Programs from the SHELX suite were used for phasing, in order to identify the 5 selenium atoms which had been incorporated into the protein. Despite the mediocre resolution, the electron density maps after density modification (solvent content of 80%) with RESOLVE are of traceable quality. The partially built model confirms that it is indeed a Pin domain-containing protein. Further experiments are now required to optimize crystals of Pab0263 to obtain higher resolution data.