

	Experiment title: BAG-LEBS-2007-2	Experiment number: MX-669
Beamline: ID23-2	Date of experiment: from: 31/08/2007 8h30 to: 1/09/2007 8h	Date of report: 21/2/08
Shifts: 3	Local contact(s): Dr D. NURIZZO	<i>Received at ESRF:</i>
<p>Names and affiliations of applicants (* indicates experimentalists):</p> <p>Philippe Meyer*, Nouredine Atmane*, Audrey DORLEANS*, Armelle VIGOUROUX*, Marcel KNOSSOW*: LEBS, UPR3082 CNRS, Bat 34, CNRS, 1 av. de la Terrasse, Gif-Sur-Yvette, France</p> <p>Mark Graille*, Nicolas Leulliot*: Herman Van Tilbeurgh's Group, Institut de Biochimie et de Biophysique Moléculaire et Cellulaire (IBBMC), Equipe Génomique Structurale, CNRS UMR8619, Université Paris-Sud, Orsay, France</p>		

Report:

Philippe Meyer*, Nouredine Atmane*: Structural and functional analysis of protein kinases and of the Hsp90 machinery with specific inhibitors for therapeutic purposes (1 shift).

Project ProTK :

-Dataset collected to 2.8 Å of resolution (unit cell a=b=163, c=57 ; space group I4) on the K55M inactive mutant of the bacterial tyrosine kinase chimera CapA1/CapB2. The structure was solved by MR and refined to 2.8 Å. A publication is in revision.

-Dataset collected to 1.2 Å of resolution (unit cell a=36, b=88, c=39 ; space group P21) on the bacterial tyrosine kinase chimera CapA2/CapB2. The structure was solved by MR and is currently refined to 1.3 Å of resolution with a Rcryst=19.7 % and Rfree=22.1 %.

Project HspNovo :

Many tiny needle crystals tested. A lot of time used for proper crystal centring. Partial dataset on a crystal diffracting to 3.5 Å due to rapid degradation in the microfocus beam.

We lost about 0.3 shift because of miss-settings leftover by previous users.

Mark Graille*, Nicolas Leulliot*: : yeast multi-protein complexes involved in DNA replication, ribosome biogenesis, mRNA quality control pathway and cell signalling and archeophage structural genomics project (1 shift)

1) Tpa1

Spacegroup: P2₁2₁2₁ with a= 104 Å, b=161 Å and c=213Å.

Resolution: 3.8Å.

Completion= 97%

Rsym=19%

The deletion of the gene encoding for yeast Tpa1 protein strongly affects translation termination, deadenylation and mRNA stability, suggesting a role in the control of gene expression at the level of translation. The Tpa1 protein is a component of a ribonucleotidic complex bound to the 3'-end of mRNAs. The knowledge of its 3D structure might help to decipher the precise function of Tpa1. During this run, we have been able to collect 5 angular sections of 30° from the same crystal. The data were merged to a complete set of 3.8Å resolution. The crystal suffers from serious anisotropy and its diffraction decreased during data collection.

Moreover, during this session, diffraction tests have been performed on other new proteins studied in our group. Some of the crystals were salts, others diffract only weakly.

A. Dorleans, A. Viouroux, M. Knossow: Molecular mechanisms of tubulin regulation. (1 shift)

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

In this session, we have attempted to explore new crystal forms of tubulin in complex with the stathmin-like domain of the RB3 protein (T2R). Crystals of T2R obtained in conditions different from those we normally use were tested and turned out to be salt. In addition, we used the low resolution setup of the beamline to collect data on a new crystal form obtained with the RB3 K25->A mutant. The crystals were known to diffract to low resolution so that we requested the helium cone to be installed. This was done by the local contact previous to our visit, which spared the time for elimination of air in the cone. The data go to 9-10 Å resolution and the unit cell is very large (estimated a = b = 300 Å, c = 600 Å, all angles 90°). The very intense background has prevented meaningful integration of these data (see picture). Obviously the helium setup needs to be adjusted to become usable as a routine tool.


