	Experiment title: BAG-LEBS-2007-2	Experiment number: MX-669
Beamline: ID14-3	**Date of experiment**: from: 03/11/2007 at 8:30 to: 05/11/2007 at 8:00	**Date of report**: 28/2/06
Shifts: 6	**Local contact(s)**: **Dr. A.M. Goncalves**	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Louis Renault*, LEBS, Bat 34, CNRS UPR9063, 1 av. de la Terrasse, Gif-Sur-Yvette, France

Mark Brooks*: 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

Report:

Part of the beamtime allocation was lost because of sereral problems on the beamline with the sample changer. It was blocked at some time, or showed problem in the communication with the cryo-system, and most of the crystals had to be mounted manually. Dr. A.M. Goncalves arranged a partial use of ID14-2 because of the sample changer problem on ID14-3.

Mark Brooks *: : yeast multi-protein complexes involved in DNA replication, ribosome biogenesis, mRNA quality control pathway and cell signalling and archeophage structural genomics project (2.5 shifts)

1) Decapping complex (Dcs1-Dcs2)

The heterodimeric Dcs1-Dcs2 complex is involved in mRNA decapping. We have obtained crystals of it in complex with a ligand, and are in the process of optimizing crystals and refining crystallization conditions. Crystal diffraction gave reflections up to ~3.2 Å resolution, but the data were deemed not useful for structural analyses finally. During collection from the best crystal, the cryostream broke down, resulting in the experiment being moved to ID14 EH2.

2) Acetyltransferase complex (MAK)

Small crystals have been obtained of the catalytic subunit of a yeast complex involved in acetylation of the N-termini of certain proteins. A previous dataset had been collected up to a maximum resolution of 3.1 Å, and a credible molecular replacement solution has been obtained. This experiment was therefore in an attempt to obtain higher resolution dataset than had previously been obtained. However, no crystal diffracting to higher resolution was found.

3) *P. abyssi* RNA ligase.

Space group $P2_12_12_1$

This ATP-dependent RNA ligase had been solved recently with crystals labelled with selenium by means of a SAD experiment on ID14EH4. A higher resolution (3 Å) dataset had been also obtained but higher resolution diffraction would aid the refinement of the model. Screening of crystals did not yield suitable diffraction, and no datasets were collected for this protein.

L. Renault (<2 shifts): structural studies of complexes between the methyltransferase $Rlma^{II}$ and RNA and of GTP-dependent oligomerization of GBP1.

structural studies of complexes between the methyltransferase $Rlma^{II}$ and RNA

We used less than 2 shifts on the beam line ID14-3 to collect data sets on complex crystals of the protein $RlmA^{II}$ (32kDa) in complex with a RNA substrate of different length.

$RlmA^{I}$ and $RlmA^{II}$ are bacterial methyltransferases that modify the N-1 position of 23S ribosomal RNA nucleotides G745 and G748, respectively (Gustafsson *et al.*, (1998), *J Bacteriol*; Douthwaite *et al.*, (2004), *J Mol Biol*). Methylation of G748 is associated with resistance to tylosin and related 16-membered ring macrolide antibiotics. Our specific aim is to understand, at a molecular level, the structural basis for resistance to macrolide drugs and in particular how resistance enzymes recognize specifically their rRNA target by obtaining a high-resolution structure of $RlmA^{II}$ complexed with its RNA substrate. The structure of $RlmA^{I}$ was solved (Das *et al.*, (2004), *PNAS*) but no structure of an antibiotic resistance enzyme that targets the ribosomal RNA in complex with its substrate is available yet.

A native and SAD data sets were previously collected but the analysis of the data revealed the presence of pseudo-merohedral twinning with a twinning fraction close to 50%, which emulates orthorhombic symmetry. Phasing has been initiated by combining molecular replacement and MIRAS but electron density is poor with a large part of the protein and RNA not visible. We have crystallized Rlma2 in several new crystal form with the selenomethionylated protein in order to escape the high merohedral twinning and/or to improve the resolution. During this shift, we tested 3 new different crystal forms but none of them diffracted below 8.5 Å.

structural studies of the GTP-dependent oligomerization of GBP1.

Gamma-induced Guanylate-binding proteins are characterized by nucleotide-dependent oligomerizations associated with high-turnover GTPase activities. Their mechanisms of regulation as molecular switch or mechano-chemical enzymes in key cellular pathways remain elusive. As a first model we target the poorly understood function of human GBP1 of 68 kDa which relays antiviral and anti-angiogenic effects in cells with mechanisms not understood at the molecular level (Guenzi E et al. (2003) The guanylate binding protein-1 GTPase controls the invasive and angiogenic capability of endothelial cells through inhibition of MMP-1 expression. EMBO J. 22, 3772-82.). Using ESRF beamlines, we have recently solved the first structural basis for the catalytic machinery of the Nterminal GTPase domain of GBPs which has both a GTPase and GDPase activity (Ghosh A et al., (2006) How

Guanylate Binding Proteins achieve assembly stimulated processive cleavage of GTP to GMP. Nature, 440, 101). We are focusing now on the possible cross-talk between GBP1 different domains upon nucleotide-dependent oligomerization by analysing conformational changes between the different states of full-length GB1-GTP dimers, full length GBP1-GDP/AlF tetramers and full length GBP1-GDP monomers.

To understand the link between oligomerization, membrane-anchoring, and GTPase/GDPase activity we have produced myristoylated-GBP1 and obtained crystals without nucleotide or with a GTP-analog corresponding putatively to two different oligomeric form. Several crystals forms were available. We have collected several data sets at midle resolution (at the most 3.5 Å) on these crystals. Analysis showed the presence of pseudo-merohedral twinning with a twinning fraction close to 50%, which emulates orthorhombic symmetry. We have solved the phasing by molecular replacement but electron density maps did not show the membrane-anchoring region. The GTP-induced dimer seems to be prevented by the crystallization condition as crystals with a GTP-analog do not show dimers involving the nucleotide-binding regions. New crystalization conditions in the apo-form of GBP1 and with a GTP-analog are in process to improve the resolution and to trap dimers and tetramers with the full-length protein. It is expected that the structures of the full-length GBP1 protein intercepted in different oligomeric intermediates of the GTPase/GDPase reaction will clarify with in vitro studies if the protein may act as a mechanochemical enzyme.