	Experiment title: BAG-LEBS-2007-1	Experiment number: 30-01/796
Beamline: BM30A	**Date of experiment**: from: **17-MAR-07** at 8:30 to: **19-MAR-07** at 8:00	**Date of report**: 20/2/08
Shifts: 6	**Local contact(s)**: **Franck BOREL** (borel@ibs.fr)	*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

Clotilde Husson*, ICSN , CNRS , 1 av. de la Terrasse, Gif-Sur-Yvette, France

Pierre Briozzo*, Yann Gohon* : **LEBS**, Bat 34, CNRS UPR9063, 1 av. de la Terrasse, Gif-Sur-Yvette, France , and ***Unité de Chimie Biologique*, UMR 206 INRA, Institut National Agronomique Paris-Grignon, 78850 Thiverval-Grignon, France**

Report:

C. Husson, L. Renault (3 shifts): structural studies of complexes between the methyltransferase Rlma^{II} and RNA.

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

We used 3 shift on the beam line BM30A to collect heavy-atom 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 data set was previously collected but the analysis of the data revealed the presence of pseudo-merohedral twinning, which emulates orthorhombic symmetry. Phasing was initiated by molecular replacement with Rlma1 structure as search model but electron density was poor with part of the

protein and RNA not visible. First SAD data sets were thus collected on ID14-1 with the selenomethioned protein but experimental phasing was poor too because the presence of pseudo-merohedral twinning with a twinning fraction close to 50% for all data sets. During this shift, we collected several MAD data sets on crystals of $RlmA^{II}$- RNA complexes at Se and Zn absorption edges. All crystals and data sets were pseudo-merohedrally twinned with a twinning fraction close to 50% for all data sets. The analysis of the anomalous signal for good experimental phasing is still hindered by the presence of pseudo-merohedral twinning and by the fragility of crystals which are very sensitive to radiation damage. We have combined all heavy atom data sets using MAD as SAD data sets in SHARP but electron density remains ambiguous after density modification techniques. We need therefore to recollect data from heavy-atom derivatives.

Pierre Briozzo, Yann Gohon (3 shift) project: Mutants and complex of Cytokinin oxidase with inhibitor and AMADH with NAD cofactor

- We used the beamtime on 17 March from 8h am to 12h pm (2 shifts).

- We tried to collect data on 16 crystals of cytokinin oxidase from *Zea mays*, on 7 crystals of AminoAldehyde DeHydrogenase from *Pisum sativum*, and on 8 crystals of bacterial UMP kinases. Unfortunately, none of the crystals diffracted to a satisfying resolution, and no data set could be collected.