	Experiment title: BAG-LEBS-2007-2	Experiment number: MX-669
Beamline: BM30A	**Date of experiment**: from: 07/09/2007 at 8:30 to: 08/09/2007 at 8:00	**Date of report**: 28/2/06
Shifts: 3	**Local contact(s)**: **Dr. Lilian Jacquamet**	*Received at ESRF:*

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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 1.5 shift on the beam line ID23-2 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 high twinning fraction, 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 complex with 5-Bromo-Uridine derivatized RNA. During this shift, we collected MAD and SAD data sets at Br absorption edges at the most 3.5 Å. Analysis showed that all tested crystals were pseudo-merohedrally twinned with a twinning fraction close to 50% and that the Brome on derivatized RNA is cleaved along the collect of a full MAD data set. Data sets used as SAD in SHARP while combining Molecular Replacement and MIRAS phasing with density modification techniques such averaging have improved slightly the maps. Further building and refinement in process is slow down by the pseudo-merohedral twinning with a twinning fraction close to 50% which makes the assignement of the monoclinic space group ambiguous.