	Experiment title: BAG-LEBS	Experiment number: MX-1536
Beamline: ID23-2	**Date of experiment**: from: 11/07/2014 at 9:30 to: 12/07/2014 at 8:00	**Date of report**: 01/10/2014
Shifts: 3	**Local contact(s)**: Dr. David FLOT	*Received at ESRF:*

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Report:

Structural Study of bacterial RNA-methyltransferases promoting macrolide antibiotic resistances.

Background:

Several posttranscriptional modifications of bacterial rRNAs are important in determining antibiotic resistance or sensitivity. $RlmA^{I}$ and $RlmA^{II}$ are bacterial rRNA-methyltransferases from gram-positive and negative bacteria, respectively, that have different target specificities. They modify the N-1 position of 23S ribosomal RNA (rRNA) nucleotides G745 and G748, respectively. Methylation of the bacterial rRNA by RlmA enzymes confers or increase resistance to different macrolide antibiotics such as tylosin (RlmaII), telithromycin (RlmaII) or Mycinamicins (RlmaI). We study the structural basis of how the two different Rlma bacterial enzymes with their cofactor SAM recognize and methylate specifically their rRNA target, which may represent interesting targets for the developpment of new therapeutic drugs against macrolide antibiotic resistances of gram-positive and negative bacteria.

Results:

On ID23-2, we have tested crystals of RlmaII bound to a long rRNA substrate, which usually display perfect pseudohemohedral twinning when flash-cooled with standard methods, and new crystal forms of RlmaI bound to a short rRNA substrate. All crystals were grown *in situ* 1-3 weeks earlier at ESRF on ID23 because we wanted to flash-cool the crystals at ESRF without any cryoprotectant addition by using the High-Pressure (HP) cooling apparatus developped at ESRF by P. Carpentier, P. van der Linden and colleagues (P. van der Linden et al. (2014) *J. Appl. Cryst.* 47) in order to limit physical stress on flash-cooled crystals. We previously noticed that traveling with crystals in their mother liquors in crystallization trays damaged significantly their diffraction quality.

None of the crystals diffracted beyond 6-7 Å, so no suitable data sets for solving these structures could be collected. We have obtained previously higher diffraction at ESRF up to 3.5 Å with Rmeas~38% for RlmaII bound to a long rRNA substrate from perfectly twinned crystals, and up to 5.2 Å at SOLEIL synchrotron with Rmeas~37% for RlmaI bound to a short rRNA substrate. We will therefore set up new crystals at ESRF to obtain better resolution using both standard flash cooling procedures and the HP-cooling apparatus that allowed us to reduce our crystal mosaicity and avoid more frequently perfect twinning on crystals of RlmaII bound to a short RNA substrate. Since RlmaI and RlmaII display different substrate specificities and provide different antibiotic resistance, solving each of their complexes with their rRNA sustrate is important to understand how the two different Rlma bacterial enzymes with their cofactor SAM recognize and methylate specifically their rRNA target to induce bacterial antibiotic resistance and to design potential mechanisms of inhibition of these interactions.