	Experiment title: BAG-LEBS	Experiment number: MX-1536
Beamline: ID23-eh1	**Date of experiment**: from: 19/04/2014 at 9:30 to: 20/04/2014 at 8:00	**Date of report**: 12/05/2014
Shifts: 3	**Local contact(s)**: Dr. CARPENTIER Philippe	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

Structural Study of bacterial RNA-methyltransferases.

Background:

RlmA^{I} and RlmA^{II} are bacterial methyltransferases from gram-positive and negative bacteria, respectively, that modify the N-1 position of 23S ribosomal RNA (rRNA) nucleotides G745 and G748, respectively. Methylation of the rRNA by RlmA(II) confers resistance to mycinosylated 16-membered ring macrolide antibiotics such as tylosin. We study the structural basis of how the two Rlma bacterial enzymes with their cofactor SAM recognize and methylate specifically their rRNA target, which leads for Rlma(II)-expressing bacteria to bacterial resistance to macrolide antibiotics.

Results:

On ID23-1, we have tested different crystal forms of Rlma enzymes obtained with different minimal RNA substrates and their cofactor. Our aim was to obtain first high resolution data sets using new small crystal forms of RNA:Rlma enzymes (crystals with a smallest dimension of 10-15µm) or to reduce a strong crystal defect that affect all previous best diffracting crystal forms with Rlma(II). This defect, known as perfect pseudomerohedral twinning, prevents us to solve the first crystal structure of an RNA:Rlma enzyme complex after combining both MIRAS, molecular replacement and density modifications techniques from our previous twinned crystals diffracting to 3.5 (first crystal form) or 2.8 Å (second form). On 18-19/04/2014 we have used 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**) to limit crystal damages during their cooling at cryogenic temperatures. This system allows crystal freezing without any addition of exogenous cryoprotectants. All our new small crystal forms did not diffract better than 7 Å but we have optimized the conditions for cooling twinned crystal forms at 100K and high pressure (200MPa) without cryoprotectants. By optimizing crystal High-Pressure-cooling and data collection procedures, we have

succeeded to obtain first data sets at middle resolution (~3.5 Å) where the the two previously overlayed crystal lattices of our twinned crystals can be better separated. We need now to collect new HPdata sets on these crystal forms to improve the resolution. These next data sets will allow to solve the interactions of Rlma(II) with its RNA susbstrate at high resolution. We have also started to improve the size and shapes of our new crystal forms. The latters will reveal complementary intermediate steps of the methyltransferase activities of Rlma enzymes, and will allow to decipher the full catalytic machinery of this family of bacterial methyltransferases.