	Experiment title: BAG-LEBS	Experiment number: MX-1536
Beamline: ID29	**Date of experiment**: from: 22/06/2014 at 9:30 to: 23/06/2014 at 8:00	**Date of report**: 01/10/2014
Shifts: 3	**Local contact(s)**: Dr. CARPENTIER Philippe	*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 contain a Zinc-finger domain and methyltransferase catalytic domain, and 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 ID29 or ID23-1, we have tested different crystal forms of RlmaII bound to a short rRNA substrate suffereing from epitaxial twinning and perfect pseudomerohedral twinning. Our aim was to reduce or circumvent the perfect pseudomerohedral twinning present in all crystal forms of Rlma(II):rRNA complexes and to obtain useful data sets with higher resolution. Crystals were flash-cooled in 3 different manners (to see below). An important screening was required with each flash-cooling method because most of the crystals diffracted only to low resolution (6-7 Å) or were showing too much split reflections in diffraction patterns due to the presence of strong epitaxial twinning.

We have first collected at 100K with cryoprotectant (standard protocol) an useful data set with fine-slicing giving a Rmeas~37% up to 2.95 Å (Cc1/2=50% at 2.41 Å).

To limit physical stress and potential damages of crystals during their cooling at cryogenic temperatures, we have also collected data sets using either (i) the humidity control device or (ii) High-Pressure (HP) cooling apparatus developped at ESRF by J. Sanchez-Weatherby, M. W. Bowler, and colleague (J. Sanchez-Weatherby et al. (2009) *Acta Cryst.* D65, 1237-1246), or P. Carpentier, P. van der Linden and colleagues (P. van der Linden et al. (2014) *J. Appl. Cryst.* 47), respectively. These systems allow data collection at room temperature without uncontrolled dehydratation, or crystal freezing without any addition of exogenous cryoprotectants. All successful crystals were grown *in situ* at ESRF on ID23 1-3 weeks earlier because we previously noticed that traveling with crystals in their crystallization trays damaged significantly their diffraction quality.

With the humidity control device (J. Sanchez-Weatherby et al. (2009) *Acta Cryst.* D65, 1237-1246), the progress of dehydration of our protein crystals were analyzed but crystals were too fragile to collect more than few images at room temperature or 4°C. We could collect at Zn K absorption edge a useful data set with fine-slicing. The data set displays a weak anomalous signal and a Rmeas~31% up to 3.01 Å (Cc1/2=50% at 2.54 Å) after initiating a slight slow dehydratation at room temperature up to a relative humidity of 90% and a flash-cooling in nitrogen liquid without cryptotectant addition.

With the High-Pressure (HP) cooling apparatus (P. van der Linden et al. (2014) *J. Appl. Cryst.* 47), we have brought crystals to 200 MPa prior to a flash-cooling in nitrogen liquid without cryptotectant addition. Our best data set with fine-slicing has a Rmeas~39% up to 2.93 Å (Cc1/2=50% at 2.56 Å). Using HP-cooling without any cryoprotectant improves the averaged diffraction quality of screened crystals and reduces up to 40% the crystal mosaicity compared to twinned data sets. The best data set with the the humidity control device reduces as well to 40% the crystal mosaicity compared to twinned data sets. This improvement is important to separate the epitaxial twinning present in all crystals.

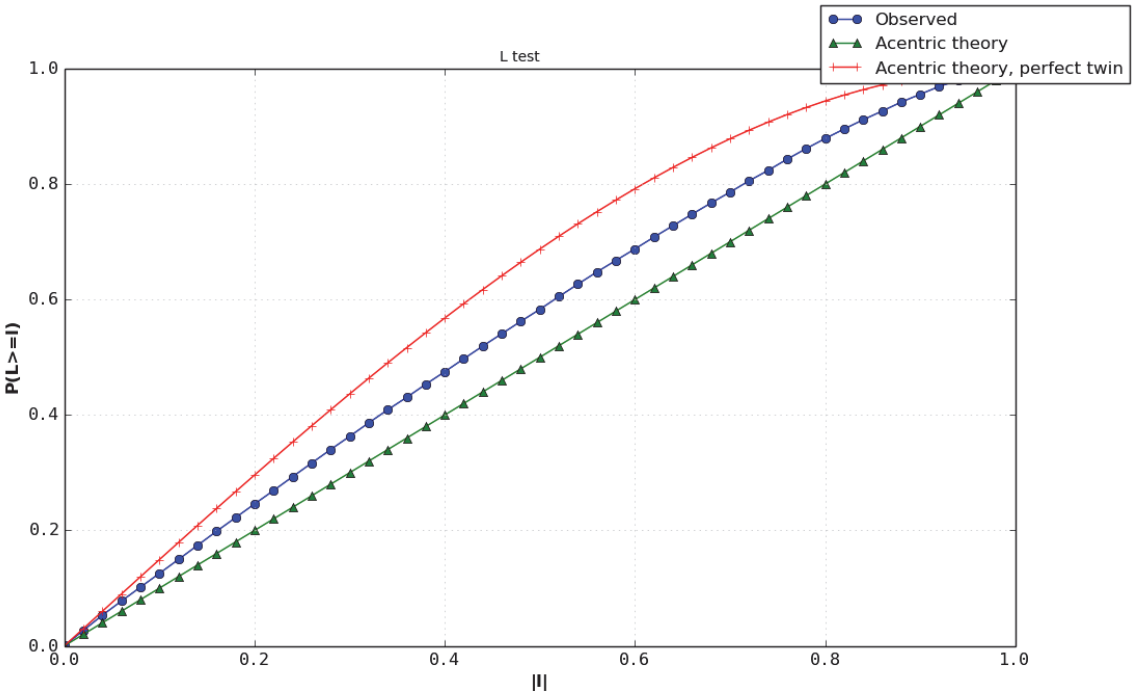
The new data sets appear to be more suitable now for solving the structure. The best crystals have changed of crystal system from monoclinic to orthorhombic and are not damaged anymore by the perfect pseudomerohedral twinning that was present in all previous crystal forms of Rlma(II):RNA complexes (Figure 1). Model building and refinement are in process with the new data sets. We are combining molecular replacement, SAD (presence of weak Zn anomalous signal), and density modification techniques (2-fold NCS symmetry) to improve our electron density maps which are of poor quality especially for the rRNA substrate with most atoms displaying very high B-factors.

Figure 1: L-test for acentric data using phenix showing in :

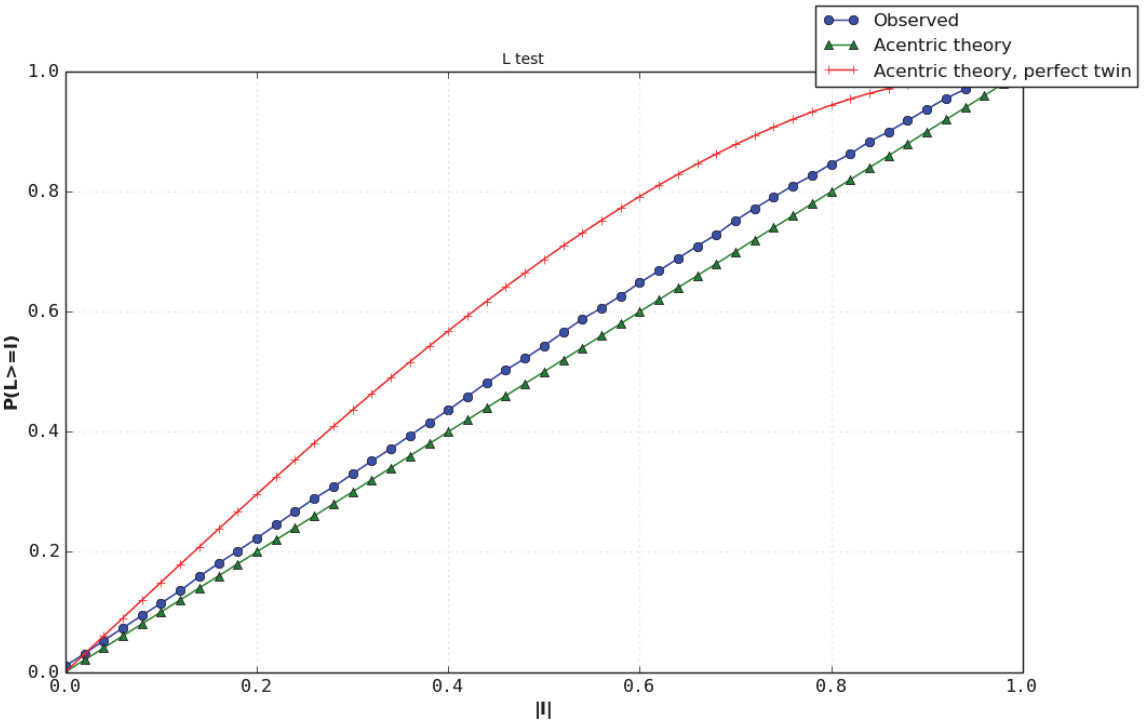
a) our previous best native data set (2.3 Å) which was 50% pseudomerohedral twinned with a fraction of 50% after a refinement blocked at 35% Rfree,

b) our new best data set (3 Å) obtained from HP-cooling for which no twinning is detected.

a)


L test
Observed
Acentric theory
Acentric theory, perfect twin
P(L>=l)
|l|
0.0
0.2
0.4
0.6
0.8
1.0

b)


L test
Observed
Acentric theory
Acentric theory, perfect twin
P(L>=l)
|l|
0.0
0.2
0.4
0.6
0.8
1.0