	Experiment title: BAG-LEBS-2006-2	Experiment number: MX-554
Beamline: ID14-4	**Date of experiment:** from: Friday 25/08/2006 at 8:30 to: Saturday 25/08/2006 at 8:00	**Date of report:** 28/2/06
Shifts: 3	**Local contact(s):** Dr. P. PERNOT	*Received at ESRF:*

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Report:

C. Husson, L. Renault: structural studies of complexes between the methyltransferase Rlma^{II} and RNA and complexes of actin with chimeric proteins of thymosin β4 and ciboulot.

As the chiller of the ID14-4 detector was broken when we arrive at ESRF, we were kindly proposed to use part of our shifts between the beam line ID14-2 and the beamline ID23-1.

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

We used our shift on the beam line ID14-2 and on the beamline ID23-1 to collect data sets on complex crystals of the methyltransferase 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 RNA substrate is available yet.

A native data set was previously collected to a 2.8Å resolution and the molecular replacement didn't lead to an appropriate solution for phasing. The protein was expressed with selenomethionine. During this shift, we collected several SAD data sets from SeMet crystals and SeMet crystals soaked with heavy atoms. The crystals were too fragile to collect a MAD data set. For all crystals, the analysis of the data revealed the presence of pseudo-merohedral twinning, which emulates orthorhombic symmetry. The presence of the pseudo-merohedral twinning may be sometimes detectable at some orientation with the presence of splitted spots but is mainly detectable during the analysis of the data, so we have to collect data set on a lot of crystals to find one which is not twinned or less twinned as possible. The true space group seems to be P21.

crystals of RlmA^{II}- RNA substrate 1 complex :

Beam line	Crystal	HA	Resolution range.	Completeness	I/sI	Rmeas %
ID14-2	1	SeMet	18-2.98 (3.10-2.98)	98.6	16.21 (4.41)	8.2 (33.1)
ID14-2	2	SeMet+Hg	No-isomorphous	93%		43% at 2.4Å
ID23-1	3	SeMet	20-2.92 (3.09-2.92)	98.1	10.69 (5.02)	11.5 (34.5)
ID23-1	4	SeMet+soak HA	18-2.96 (3.08-2.96)	97.2	10.04 (4.55)	12.0 (36.3)
ID23-1	5	SeMet+soak HA	21-3.17 (3.3-3.17	98.9	11.37 (3.97)	13.2 (41.4)
ID23-1	6	SeMet+soak HA	20-3.80 (3.90-3.80)	95.2	5.94 (3.23)	13.5 (34.4)
ID23-1	7	SeMet+Hg	21-3.7 (3.80-3.70)	93.8	13.04 (6.29)	7.6 (18.0)
ID23-1	8	SeMet+Hg	20-3.7 (3.73-3.70)	95.4	13.81 (7.62)	6.6 (13.4)

crystals of RlmA^{II}- RNA substrate 2 complex :

Beam line	Crystal	HA	Resolution range.	Completeness	I/sI	Rmeas %
ID14-2	1	SeMet	20-3.7 (3.9-3.7)	98	13 (5)	14 (49)
ID14-2	2	SeMet	20-3.5 (3.7-3.5)	96	10 (3)	11 (53)
ID23-1	3	SeMet	20-3.5 (3.7-3.5)	96	9 (3)	12 (52)
ID23-1	4	SeMet	20-3.65 (3.88-3.65)	94	8 (3)	12 (43)
ID23-1	5	SeMet	20-3.92 (4.15-3.92)	97	7 (3)	16 (52)

As we get better resolution for crystals of RlmA^{II}- RNA substrate 1 complex, we mainly focus on the structure determination of this complex. From a weak molecular replacement solution, we could locate by anomalous fourrier difference peaks of the Se atoms in 2 of the crystal data sets obtained with seleno-methionine substituted protein. Nevertheless heavy-atom phasing statistics remain of very weak quality probably because of the pseudo-merohedral twinning and experimental maps are not yet interpretable even after phase combination and density modification. We are looking for additional heavy-atom derivatives.

structural studies of complexes of actin with chimeric proteins of thymosin β4 and ciboulot.

The conserved WH2 actin-binding module, originally represented by β-thymosins, has been identified in a large number of proteins that all interact with actin and play diverse functions in cell

motility. The versatile functions of the WH2 module may be linked to changes in the actin-WH2 interface that would affect actin conformation in a way that would favor or inhibit specific actin-actin interactions, thus accounting for promotion or inhibition of actin self-assembly. It is therefore important to characterize the nature of this change and the evolutionary trends of WH2 domains. The functional evolution of the WH2 domain has thus been approached by engineering a series of chimeras of Ciboulot and Thymosin Ã_4, in order to evaluate the contributions of the different regions of these two proteins containing WH2 domains with opposite functions: promoting actin barbed-end self-assembly and sequestering actin, respectively. Biochemical and dynamic analysis by RMN are combined with structural studies to characterize the molecular determinants controlling the function of promotion or inhibition of actin self-assembly by WH2 domains.

Diffracting crystals have been obtained with different chimeric proteins having different actin-sequestering activities. No good crystals could be found upon screening on ID14-2 as crystals were either diffracting to low resolution or twinned showing splitted diffraction spots at some orientation.