	Experiment title: BAG-LEBS-2006-2	Experiment number: MX-554
Beamline: ID23-2	**Date of experiment**: from: Thursday 14/09/2006 at 8:30 to: Friday 15/09/2006 at 8:00	**Date of report**: 28/2/06
Shifts: 3	**Local contact(s)**: **Dr. E. MITCHELL**	*Received at ESRF:*

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Report:

C. Husson, L. Renault (1.5 shifts): structural studies of complexes between the methyltransferase $Rlma^{II}$ and RNA and complexes of actin with chimeric proteins of thymosin 4 and ciboulot.

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 (hereafter refered as RNA substrate 1 and RNA substrate 2).

$RlmA^{I}$ 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 set were previously collected but the analysis of the data revealed a presence of pseudo-merohedral twinning, which emulates orthorhombic symmetry. During this shift, we collected 4 data sets on each 2 crystals of $RlmA^{II}$- RNA substrate 1 complex and 3 data sets on 1 crystal of $RlmA^{II}$- RNA substrate 1 complex. Both type of complex belong to the space group P21. For crystals of $RlmA^{II}$- RNA substrate 2 complex, the merge of 4 sets give for a resolution range from 20 to 3.75Å (4-3.75 Å), Rmeas=15% (51%), completeness=88%. For the crystal of $RlmA^{II}$- RNA substrate 1 complex, the merge of 2 sets give for a resolution range from 20 to 2.75Å (2.8-2.75 Å), Rmeas=12.% (35%), completeness=90%. Unfortunately, the analysis of the data showed again a presence of pseudo-merohedral twinning and probably too much radiation damage, which could not allow to obtain complete data sets.

structural studies of complexes of actin with chimeric proteins of thymosin 4 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. A previous data set has been collected at ESRF on july 2006 with the drug Latrunculin inhibiting actin polymerization. We succeeded to obtain very small crystals of a more “physiological” chimeric protein/ actin complex without the drug in different conditions. These small rod-like crystals were tested on ID23-2 and diffracted to 2 Å at the best. Despite the presence of splitted spots on many crystals, several uncomplete data sets were collected from 2 weakly splitted crystals. The crystals have a unit cell different than those obtain with the drug but were too sensitive to X-ray damage and no complete merging data set could be obtained to make molecular replacement successful.

Carine Tisné, Pierre Barraud, Philippe Benas (1.5 shift) project: methyltransferase from *T. thermophilus*

On beam-line **ID23-2**, 8 crystals of a putative complex between *E. coli* $tRNAi^{Met}$ and m1A58 tRNA methyltransferase from *T. thermophilus* (tTrmI) have been tested and 2 partial datasets were recorded (space group P6422, a=93.8 c=219.4), at 3.5 Å and 3.8 Å resolution.
Since the crystals died quickly in the beamline, we used ID14-1 available beam time. 32 crystals have been tested and 4 complete datasets have been recorded at 4.5 Å, 4.0 Å, 3.7 Å and 4.2 Å. The structure solved by molecular replacement on crystals recorded later shows that only *E. coli* $tRNAi^{Met}$ is present in the crystal.
A 1.7 Å full data set of tTrmI complexed with S-adenosylhomocysteine (space group C2221, a=90.440 b=96.795 c=140.584) was recorded. The completion is 100 % (100 % in the last shell), Rsym=10.2 % (63.2 % in the last shell). The structure has been solved by molecular replacement and contains the ligand. The structure has been refined to R=17.9%, Rfree=20.9%.