	Experiment title: BAG-LEBS-2006-2	Experiment number: MX-554
Beamline: ID14-3	**Date of experiment**: from: Saturday 25/11/2006 at 8:30 Monday 27/11/2006 at 8:00	**Date of report**: 28/2/06
Shifts: 3	**Local contact(s)**: Dr. R. Ravelli	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

Due to a problem on all ID14 beamlines independent of our experiments, we could use ID14-EH3 for only 1.5 shift over the 6 shifts allocated. The local contact and ESRF kindly moved a small part of our 6 shift allocation on ID14-2 (for 0.25 shifts) and on BM14. We lost around 3-4 shifts.

Beatrice Golinelli, Carine Tisné, Pierre Barraud (1.5 shift) project: structural study of tRNA methyltransferases.

24 crystals of a putative complex between *E. coli* $tRNAi^{Met}$ and tTrmI have been tested and 4 complete datasets at 3.2 Å and 3.3 Å resolution have been recorded (space group P6422, a=93.8 c=219.4). The completion is 97.4 % (94.2 % in the last shell) and Rsym=10.1 % (69.2% in the last shell) for the best crystal. The structure has been solved by molecular replacement and is currently being refined (to date R = 25.8 % Rfree=28.5%).

Two full data sets of tTrmI grown in the presence of S-adenosylhomocystéine and AMP (space group P212121, a=76.3 b=79.6 c=184.5) were recorded at 2.0 Å resolution. The completion is 98.4 % (90.4 % in the last shell), Rsym=8.2 % (55.2 % in the last shell). The structure has been solved by molecular replacement but does not contain the AMP molecule.

Several crystals of the tRNA binding domain (THUMP) of tRNA m1G10 tRNA methyltransferase have been unsucessfully tested for getting a resolution better than 2.8 Å that was previously recorded. Indeed, the crystals are pseudomerohedrally twinned (space group P21, a=100, b=66.7, c=102.8) and a resolution above

2.3 Å is requested to refine the structure with SHELXL.

Because of a prblem on all ID14 ESRF beam lines independent of our experiment, we could not use all of our beam time allocation on ID14-3.

Sonia Fieulaine (0.8 shift) - Projects: structural study of peptide deformylases (PDF)

NME pathway is responsible of the first methionine cleavage of each newly synthesized protein. NME is ensured by methionine aminopeptidases (MAP) and peptide deformylases (PDF). PDF enzymes are found in bacterial cytoplasm, and eukaryotic organelles. We have started a comparative structural study of the NME pathway, in bacteria, plants and animals. During this beam-time, we have studied eukaryotic mitochondrial PDFs from plants (*Arabidopsis thaliana*) and animals (human).

Because of a prblem on all ID14 ESRF beam lines independent of our experiment, we could not use ID14-3. 2 hours (0.25 shifts) could be kindly provided by ESRF on ID14-2 instead of ID14-3, but 0.55 shifts were lost during our beam time allocation.

Crystals of free PDF1B from *Arabidopsis thaliana* were tested. One data set was collected at 1.9 Å resolution, but it was incomplete due to the beam-line failure.
Crystals of human PDF1A could not be tested due to the beam-line failure.

Marc Graille (CNRS researcher) & Lionel Trésaugues (Post-doc) (3.5 shift): yeast multi-protein complexes involved in DNA replication, ribosome biogenesis, mRNA quality control pathway and cell signalling and archeophage structural genomics project

Due to a problem on all ID14 beamlines, we could not collect on ID14-EH3 but we were kindly moved to BM14 for a part of our beam time allocation.

1) Virar 79 Zn edge
Space group $P6_522$
Resolution 3 Å.
Completion= 100%; Anomalous multiplicity = 19.1
Rsym= 12.5%

ORF79 from Acidianus Filamentus Virus 1 (AFV1) is a 11.6 kD protein which has no homologue identified from sequence. Although no functional information is available, sequence analysis reveals a putative Zn binding site (HCA profile). We have collected a 3Å resolution dataset at the Zn edge from a crystal grown in the absence of zinc. Unfortunately the low occupancy of the Zn sites did not allowed us to solve the structure. Purification of the protein in the presence of zinc is currently performed.

2) Fibronectin fragment in complex with a peptide.

Space group $P3_121$ a=b=127 Å; c=61 Å
Resolution 3.2 Å.
Completion= 100%; Multiplicity = 2
Rsym= 8.3%

Human fibronectin is a dimeric glycoprotein found in extra-cellular matrixes and blood. This is a modular extracellular matrix protein involved in cell adhesion, cell motility, wound healing and maintenance of cell morphology. It is schematically depicted as a “string of beads” composed of multiple repeats of three distinct modules: F_I, F_{II} and F_{III}. Association of these modules creates fragments able to interact with different constituents of the extracellular matrix.
During a previous ESRF session (beamline ID23-EH1), we have used the Zn anomalous signal to solve the 2.4 Å resolution structure of its 45kDa gelatin binding domain ($6F_I$-$1F_{II}$-$2F_{II}$-$7F_I$-$8F_I$-$9F_I$), which also corresponds to the C-terminal half of the migration stimulating factor, a protein over-expressed in human breast cancers. We are now trying to determine the structure of this protein in complex with a peptide derived from collagen. We have collected a 3A resolution dataset from crystals grown in the presence of a 15-mer. We could not observe the peptide in the electron density maps obtained from this dataset. Further co-crystallization tests are currently performed.

In addition, we have tested several crystals from other projects but we have not been able to collect good resolution datasets on these projects because of the limited intensity of the beam on BM14 compared to ID14.