	Experiment title: BAG - LEBS - 2011-2	**Experiment number**: MX-1292
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Shifts: 3	**Local contact(s)**: Dr A. PALENCIA	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

1) Project 1: The interaction of kinesin with tubulin. (4 hours)

During this session we have collected several datasets from 4 crystals of the head domain of a kinesin in a complex with tubulin stabilized by an anti-tubulin DARPin. The diffraction is quite variable according to the crystals, and moreover highly anisotropic (e.g. 3.5 Ang in two directions, 4.5 Ang in the third direction). Merging several crystals corrects to some extend the anisotropy problem. Statistics for the 5 dataset that were merged and corrected for anisotropy (according to Strong et al., 2006. See http://services.mbi.ucla.edu/anisoscale/) are summarized below. The structure has been solved by molecular replacement and refinement is underway.

	Rsym	I/sig(I)	Completeness (%)	Multiplicity
Crystal1 (3.4 Ang)	0.106 (1.4)	11.7 (1.3)	98.9 (94.6)	5.8 (5.5)
Crystal 1 (2^{nd}) (3.4 Ang)	0.10 (1.0)	12.7 (1.8)	99.4 (95.1)	6.2 (6.0)
Crystal 2 (3.35 Ang)	0.13 (1.4)	8.8 (1.4)	98.6 (95.7)	4.7 (4.5)
Crystal3 (3.4 Ang)	0.08 (1.37)	11.6 (1.3)	98 (88.6)	5.4 (4.7)
Crystal 3 (3.2 Ang) (helical data collection)	0.08 (1.4)	10.5 (1.5)	90.2 (87)	4.6 (4.1)
Merged 5 datasets (3.25 Ang)	Rmerge			
Before and	0.141 (1.4)	16.7 (2.1)	99.9 (99.9)	25.7 (10.2)
after anisotropy correction	0.125 (0.36)	19.6 (5.3)	81.7 (22.2)	21.5 (1.5)

2) Project 2: Structural study of PlcR, a quorum sensing effector of Bacillus cereus. (4 hours)

I have collected six datasets from crystals of the ternary complex PlcR/peptide/DNA. The best dataset, at a resolution of 2.4 Å was selected for structure determination by Molecular Replacement, using a previous published structure (2QFC). The structure was solved in spacegroup P21 with cell parameters a=88.8Å b=71.1Å c=88.8Å α=90 β=115 γ=90. The refinement has already been completed and the structure validated and deposited at the Protein Data Bank. This structure helps us to understand the DNA binding mode of the transcription factor PlcR. Paper writing is underway.

3) Projet 3 : Structural study of NprR, a quorum sensing effector of Bacillus cereus. (4 hours)

I tested 16 crystals of the apo form of NprR truncated from its DNA-binding domain (NprRΔHTH) labelled with selenium. I collected one data set at 6.3Å (anomalous signal at 7.4Å), unfortunatly, the search of selenium sites failed using shelx.

I tested 9 crystals of the ternary complex NprR/peptide/DNA. I collected 3 data sets. The best resolution is 4.3Å. The complex crystallysed in space group P321 with cell parameters a=166Å b=166Å c=104Å α=β=90° γ=120°. Molecular replacement using the NprRΔHTH –peptide complex as initial model was found by the program Phaser. However, the low resolution and the poor quality of the data impaired proper refinement of this structure. So we are still trying to optimize these crystals in order to obtain a better data set.

Project 4 : Structural study of SlrP, a T3SS effector of Salmonella typhimurium.

I tested 16 crystals of the binary complex SlrP/human thioredoxin, using SlrP labelled with selenium. I collected 2 data set at 5.2Å (anomalous signal at 7.3Å) in space group $P2_12_12_1$ with cell parameters a=107Å b=135Å c=154Å α=β=γ=90°. These data did not allow us to solve the structure of the complex. We are still working on the optimization of the crystals.

4) Project 5 : Structural study of a periplasmic binding protein-opine complex

We tested 5 crystals of this complex and collected a dataset at 2.5 Å resolution. The structure was solved by molecular replacement using the liganded-free structure previously solved. The asymmetric unit contains 4 molecules of which two are liganded. The structure is now refined and paper writing is underway.

Project 6 : Structural study of a DNA repair enzyme

We collected 5 datasets from crystals of a DNA repair enzyme co-crystallized with different modified DNA fragments from 2.2 to 3 Å resolution. 3 complexed structures were solved by molecular replacement and are now under refinement.

5) Project 7: Structure of the Hsp90 molecular chaperone in complex with the cochaperone Hop/Sti1 (4 hours)

During this session we have tested several crystals of the Hsp90 molecular chaperone in complex with the Hop/Sti1 cochapeone. We have collected datasets on native as well as Sm derivative crystals. The observed diffraction limit was between 7 and 3.4 Å of resolution. The Sm derivatives were not exploitable and the structure was solved by molecular replacement using the 3.4 Å native dataset (data statistics are shown in the table below). Model rebuilding and refinement are underway.

	Rsym	I/sig(I)	Completeness (%)	Multiplicity
3.4 Å dataset	0.089 (0.76)	11.9 (1.8)	99.2 (95.8)	6.6 (6.5)