ESRF	**Experiment title: Macromolecular Crystallography at South-East Andalusia**	**Experiment number**: MX-1739
Beamline: ID23-1	**Date of experiment**: From: 05 March 2016 to: 06 March 2016	**Date of report**: 16/03/16
Shifts: 2	**Local contact(s)**: SANTONI Gianluca	*Received at ESRF:*

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Partial Report of Mx/1739 ID21-2 (05-03-2016 / 06-03-2016):

This up-date report corresponds to the data collected at ID23-1 and ID23-2 during the third round of Mx1739. We brought 100 samples from the two team grouped as CSIC-UGR. All the samples were tested and the main results are listed below. Since one of the users at ID23-2 need to tune wavelength to collect MAD data we switch to this beam-line and let them used ID23-1 after we were done with the long wavelength data collection.

Crystals from CSIC-UGR:

i) LBD-McpU bound to several ligands. McpU is a chemoreceptor that contributed to the formation of biofilm in *Pseudomonas putida*. We are trying crystal improvement using different purification strategies and screening looking for new crystallization conditions. Even when all the crystals were of low quality we were able to collect a full data set at 3.4 Å at the sulphur edge to attempt to phase those data.

Future perpectives: Se-Met derivative has been produced and crystallization is undergoing.

ii) LBD-McpH bound to several ligands. McpH is a chemoreceptor from *P. putida* that specifically recognizes purine and its derivatives, adenine, guanine, xanthine, hypoxanthine and uric acid. The latter five compounds form part of the purine degradation pathway, permitting their use as sole nitrogen sources. We have cloned purified and crystallized the ligand-binding region (LBD) of McpH and attempted the crystallization in the presence of several of its natural ligands. So far we have been able to reproducibly produce good shape crystals but either the treatment, soaking with different ligands, or the cryo-protection have degrade the quality. The best data set was collected at 3.5 Å but at the sulphur edge. Phasing by SAD will be to attempt.

Future perpectives: Better crystal handling is a requirement and will be implemented.

iii) Structural determination of *Pseudomonas* chemotactic transducer A, B and C (PctA, B, C).

As pointed it our in previos reports we already have several model of PctA and even PctB at low resolution. We already have solved the structure of PctC bound to GABA but unfortunately the ligand shows only partial occupancy. Therefore we have persistent searching for new polymorphs that may show a better definition of the ligand pocket. We have succeed and got crystals of the PctC-GABA complex that belong to the P62 space group with seven monomers in the ASU and diffracting X-ray to 2.3 Å. The model is under refinement with current R/Rfree of 19/24. Although not all the monomers have a fully occupied ligand site, at least two monomers have excellent density to fit a molecule of GABA. Crystals from the PctB complex were all of them of very poor quality and too small.

Future perpectives: Crystal improvement of PctB from new constructs is being attempted.

iv) Ancestral Proteins. Several data sets were collected from approx. 30 crystals of different extant and ancestral lactamase variants, some of them showing polymorphism, and resolution up to 1.0 Å. Models are being obtained by MR for each variant (e.g., GncaHT belonging to P1211 space group (different to that solved previously; PDB 4B88, 2.05 Å resolution), diffracting up to 1.1 Å resolution; the model is under refinement with current R/Rfree of 16/18.

Future perpectives: Others studies, implyings ancestral proteins, are been carryed out in the frame of the three derived lines of research listed in the proposal. Therefore other ancestral lactamases and mutants will be crystallized and caracterized in future runs.

Table 1. Data collected by the CSIC-UGR.

Protein	Samples	Conditions	Cryo	Resolution
PctC-GABA	9	C7/C18	0-15% GOL	2 Full data sets 2.4 Å.
PctB-/Arg/Gln	15	PPP7 / C10	-	Poor diffraction.
McpU-/Put/Cad/Sulf	29	MixPEGs pH 5.0 & 7.0	0-15% GOL	1 Full data sets at S edge (best 3.4 Å).
McpH-/Ad/Gua/Xan/Uric	16	AS pH 6.0 & 6.5 Xantine/Guanidine/Adenine in DMSO or HCl	0-15% GOL	3 Full data sets at S edge (best 3.5 Å).
Lactamase GncaHT	11	C9/C10//C13/C18/Na-Phosphate pH 4.0	15% GOL	Several Full data sets (best 1.1 Å).
Lactamase Cons2	4	C6/C11	15% GOL	1 Full data set at 2.0 Å.
Lactamase 05cHT	1	C18	15% GOL	1 Full data set at 1.4 Å.
Lactamase TEM-HT	3	Na-Phosphate pH 4.0/MixPEGs pH 8.0	15% GOL	1 Full data set at 1.2 Å.
Lactamase 02cHT	9	C9/C11/C13	15% GOL	Several Full data sets (best 2.2 Å).
Lactamase LactB	1	C9	20% GOL	Mosaic.