ESRF	**Experiment title: Macromolecular Crystallography at South-East Andalusia**	**Experiment number**: MX-1739
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Partial Report of Mx/1739 ID23-2 (05-12-2015 / 06-12-2015):

This up-date report corresponds to the data collected at ID29 during the second 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.

Crystals from CSIC-UGR:

i) D-acylase (M7) /Succiniyl amino acid racemase (Nsaar): This bi-enzymatic system is industrially used for the dynamic kinetic resolution of D-amino acids. We are studying it application as Cross-Linked Enzyme Crystals (CLECs), and as part of this study, we want to obtain the crystal structures of the enzymes, to use this information for structural-based improvement. Initial test for diffraction from crystals grown by vapour diffusion did not produced any results (see previous report for MX1629). After crystals improvement by fine screening of pH a new set of 6 crystals were analyzed during the first run (2.7 Å) of Mx1739. Similar crystals were tested in this experiment that showed no improvement respect to the previous one. (Table 1).

Future perpectives: Crytals improvements is on going.

ii) L-*N-carbamoylase from B. stearothermophillus.* We also measured this enzyme during our first run and obtained crystals that diffracted to 1.8 Å. The current model is under refinement (R/Rfree: 0.19/0.22) showing regions that were highly disorder in our previous deposited 3D model [1]. In this sense we attempt to have sufficient data sets in the presence or not of cobalt in the crystallization media. Several data sets were collected with the best one reaching a resolution limit of 2.4 Å.

Future perpectives: After analyzing the impreved models, soaking with several sustrate will be the next step.

1. S. Martínez-Rodríguez, A. García-Pino, F. J. Las Heras-Vázquez, J. M. Clemente-Jiménez, F. Rodríguez-Vico, J. M. García-Ruiz, R. Loris and J. A. Gavira, Journal of bacteriology, 2012, 194, 5759-5768.

iii) Ancestral Proteins. Several data sets were collected from 10 crystals of the GNCA02 variant with and without HisTAG and also from the GNCA-B W22DF290W double mutant variant. From each specimen we got at least a full data set at resolution better than 1.9 Å. Models are being obtained by MR for each variant.

Future perpectives: Others studies, which implies 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.

iv) NQO1-H80R.

NQO1 is a human stress protein involved in the antioxidant defence and associated to cancer. Particularly, this mutant is designed to act as a second site suppressor for polymorphisms in NQO1 strongly associated with cancer. We have already obtained the 3D model of this mutant from the previous run in the presence of FAD and dicumarol. Both are bound at the active centre. Following our previous results, crystals of NQO1-H80R were treated in a search to have the apo protein, free of FAD.

Future perpectives: No further actions are planed with this protein. The manuscript is in preparation.

v) LBD-McpU bounded to several ligands. McpU is a chemoreceptor that contributed to the formation of biofilm in *Pseudomonas putida*. We are trying to improve the crystal using agarose gel and screening for new conditions. Crystals produced for the previos run were prepared and tested in this experiment. All of them were of low quality showing evident ageing effect.

Future perpectives: Further crystals improvements is require.

vi) LBD-McpH bounded 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 obtain crystalline material using both vapour diffusion and counter diffusion in the presence of uric acid (Figure 1). In the latest configuration, two cubic shape crystals of good size were obtained. Fifteen crystals were tested but only those above commented two crystals diffracted X-ray to a resolution of 2.5 Å. MR attempt in undergoing with those data sets.

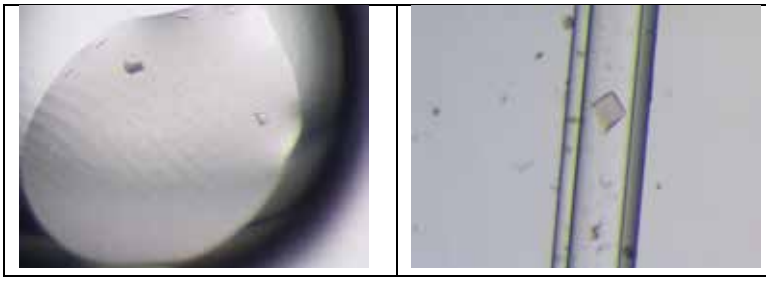

Figure 1. Crystal of the LBD-McpH grown in presence of its natural ligand, uric acid, by vapour diffusion and in capillary by counter-diffusion.

Future perpectives: Further crystals improvements is require ongoing. We have already obtained good shape crystals using the counterdiffusion technique. Those will be tested during the next experimental run.

Table 1. Data collected by the CSIC-UGR.

Protein	Samples	Conditions	Cryo	Resolution
NSAAR	14	0.2M NaMalonate/PEG3350 (pH)	0-15% GOL	Null.
L-Carbamoylase	15	15% 2-propanol, Na-Citrate pH6.5 +/- $CoCl_2$	0-15% GOL	Three data sets from two crystals. Best resolution limit is 2.5 Å.
GNCA variants (02 & B W22DF290W)	11	20% PEG 400, 15% PEG 4K, 10% PEG 8K, NaAc 0.1M pH 5.0 and 6.0	15% GOL	Six full data sets at high resolution.
NQO1-H80R	20	30% PEG 3350, pH 7.0 to 9.0.	0 to 15% GOL 0 to 15% MPD	Best data set at 2.0 Å. P212121
McpU-Put	30	C-3 30%PEG 4K, 0.2M NH4 Acetate, 0.1M Na-Acetate pH 4.60 PPP: 20% PEG 400, 15% PEG 4K, 10% PEG 8K, pH 5.0 to pH 9.0	0-15% GOL	Poor diffraction in all the cases. Crystals are too old.
McpH-Uric	20	PEG Ion/AS pH 6 and 7	15-20% GOL	Several data sets from two crystals. The best set to a limit of resolution of 2.5 Å