ESRF	**Experiment title: Macromolecular Crystallography at South-East Andalusia**	**Experiment number**: MX-1739
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Shifts: 3	**Local contact(s)**: Gordon LEONARD (leonard@esrf.fr)	*Received at ESRF:*

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Partial Report of Mx/1739 ID29 (26-09-2015 / 27-09-2015):

This up-date report corresponds to the data collected at ID29 during the first 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 this experiments. We got diffracting crystals with a maximun resolution limit of 2.7 Å (Table 1). Initial search for MR solution is ongoing.

Future perpectives: Crytals improvements is been carried out in parallel with the screening for new conditions.

ii) L-*N-carbamoylase from B. stearothermophillus.* Although we have already determined the structure of this enzyme at 2.75 Å resolution and found the role of dimerization on enzyme activity, it is our intention to firstly improve the model quality and secondly to study further the ligand binding mode by using different substrates and intermediates [1-2]. In this sense we attempt to improve crystal quality controlling several parameters such as the presence of cobalt in the crystallization media. Several data sets were collected with the best one reaching a resolution limit of 1.8 Å. Models are been determined by MR.

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

1. S. Martinez-Rodriguez, A. Garcia-Pino, F. J. Las Heras-Vazquez, J. Maria Clemente-Jimenez, F. Rodriguez-Vico, R. Loris, J. Ma. Garcia-Ruiz and J. Antonio Gavira, Acta Crystallographica Section F-Structural Biology and Crystallization Communications, 2008, 64, 1135-1138.
2. 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 30 crystals of different variant of ancestral lactamase (Table 1) in the presence or not of the reaction analogue. In table 2 are summarized the data collection, final statistic of the model and PDB ID. These two structures together with three others solved from data collected at the ESRF and one from data collected at ALBA are part of a manuscript submitted to PNAS [3].

3. Valeria A. Risso, Sergio Martinez-Rodriguez, Adela M. Candel, David Pantoja-Uceda, Mariano Ortega-Muñoz, Francisco Santoyo-Gonzalez, Eric A. Gaucher, Marta Bruix, Jose A. Gavira, and Jose M. Sanchez-Ruiz. Back and to the future: facile generation of efficient de novo catalysis in laboratory resurrections of Precambrian proteins. Submitted to PNAS (2015).

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 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 crystals of the H80R variant with its natural ligand (FAD) and in the presence of dicumarol. Conditions were refined using the counter-diffusion in capillaries applying innovative techniques to either co-crystallized or soak the crystals with dicumarol. The best results were obtained from elongated rod shaped crystals belonging to the P212121 space group and diffracted X-ray to 2.0 Å (Table 1). The structure is being refined and current R/Rfree factors are 18/23 %.

Future perpectives: No further actions are required for this project but we will attemp to obtain the protein in its apo form.

v) LBD-McpU bounded to several ligands. McpU is a chemoreceptor that contributed to the formation of biofilm in *Pseudomonas putida*. We have crystallised the ligand-binding domain of this receptor in complex with several of its natural ligands (different amines present in the natural habitat of this bacterium) but so far data are of low quality and has not yet been used to solve the structure. We are trying to improve the crystal using agarose gel and screening for new conditions. In this run crystal grown in the presence of a mix of PEGs at 6 different pH and crystals obtained in a new condition (C3), with or without agarose and with or without putrescine were tested. Any of them diffracted X-ray to a resolution better than 3.0 Å (Table 1).

Future perpectives: It is clear that further crystals improvements is require and therfore this will be the main effort with this particular protein.

Table 1. Data collected by the CSIC-UGR.

Protein	Samples	Conditions	Cryo	Resolution
NSAAR	4	0.2M NaMalonate/PEG3350 (pH)	0-15% GOL	Best data set at 2.75 Å belonging to P222.
	2	P/I 2 (11)	0-15% GOL	Best data set at 3.9 Å belonging to P222.
N-Carbamoylase	14	15% 2-propanol, Na-Citrate pH6.5 +/- $CoCl_2$ Soaking with Trp or Met	0-15% GOL 20% PEG 200	10 full data sets with the best one diffracting to 1.9 Å.
GNCA variants	30	20% PEG 400, 15% PEG 4K, 10% PEG 8K, NaAc 0.1M pH 5.0	15% GOL	Several data sets at high resolution. See Table 2.
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. Data set at 2.7 Å

Table 2. Data collection and refinement statistics of GNCA4 & mutants.

Protein	GNCA4 W229D/F290W	GNCA4
PDB ID.	5FQI	5FQQ
Beam-line	ID29 (ESRF)	ID29 (ESRF)
Resolution range	37.36 - 1.4 (1.45 - 1.4)	42.73 - 2.12 (2.196 - 2.12)
Space group	P 61	P 61
Unit cell	46.962 46.962 189.129	49.338 49.338 199.114
Unique reflections	46046 (4575)	15497 (1556)
Multiplicity	3.3 (3.2)	5.5 (5.7)
Completeness (%)	1.00 (0.99)	1.00 (1.00)
Mean I/sigma(I)	10.49 (1.75)	15.91 (1.61)
Wilson B-factor	16.33	58.80
R-merge	0.05408 (0.5408)	0.04995 (0.9418)
CC*	0.999 (0.911)	1 (0.899)
R-work	0.1544 (0.2574)	0.1883 (0.3067)
R-free	0.1665 (0.2744)	0.2251 (0.3048)
Number of non-H atoms	2422	2074
macromolecules	2138	2031
ligands	96	15
Protein residues	263	262
RMS(bonds)	0.024	0.008
RMS(angles)	0.92	1.55
Ramachandran favored (%)	99	96
Ramachandran allowed (%)	1.5	3.5
Ramachandran outliers (%)	0	0.38
Average B-factor	22.79	74.65