ESRF	**Experiment title: Macromolecular Crystallography at South-East Andalusia**	**Experiment number**: MX-1830
Beamline: ID30B	**Date of experiment**: From: 4 March 2017 at 09:30 to 6 March 2017 at 08:00	**Date of report**: 09/05/17
Shifts: 3+3	**Local contact(s)**: Andrew McCarthy	*Received at ESRF:*

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Partial Report of Mx1830 ID30B (04-03-2017 / 05-03-2017 + 05-03-2017 / 06-03-2017):

This up-date report corresponds to the data collected at ID30B during the fourth round of Mx1830 and 3 shifts to evaluate our recently produced counterdiffusion crystallization microchips. We brought 60 samples from the team grouped as CSIC-UGR. All the samples were tested and the main results are listed below, summarized in Table 1.

Crystals from CSIC:

i) LBD-PcaY bound to histamine. PcaY of *P. putida* F1, is a chemotactic sensor that responds to a number of C6-ring containing carboxylic acids. PcaY chemoeffectors include for example the non-aromatic quinate and shikimate as well as various aromatics like benzoate, 4-hydroxybenzoate, protocatechuate, vanillate and vanillin. In a previos run we got crystals, in complex with protecatechuic acid, diffracting to 2.1 Å. In this run we have measured crystals of the apo form and in complex with protecatechuic acid and quinate. Since we do have yet an initial model, we will use the 1.5 Å data sets obtained in this trip for MR.

Future perpectives: Decision to be taken after MR attempts.

ii) LBD-McpU bound to several ligands. McpU is a chemoreceptor that contributes to the formation of biofilm in *Pseudomonas putida*. Its structure has been solved from data obtained in the previous run and refinement is on-going. Still, crystals tested in this occasion were of very poor quality.

Future perpectives: The structure has been determined and refinement is on going to 2.4 Å resolution. This project may be finalized.

iii) LBD-TlpQ bound to histamine. TlpQ, a cluster I LBD, is the chemoreceptor responsible for positive chemotaxis to ethylene in some organism. As for McpU, the structure has been determined from data obtained in the previous run of MX1830 and refinement is on-going. Only four crystals were tested in this run and three data sets were collected being the best diffracting to approximately 2.0 Å. This data set could be used for refinement if the resolution is better than 2.45 Å after in-house data processing.

Future perpectives: The structure has been determined and refinement is on going to 2.45 Å resolution. This project may be finalized.

iv) Dihydropyrimidinase from *Sinorizobium meliloti*: We have recently shown that peptide-base hydrogels may help to improve crystal quality or to induce the formation of new polymorphs from data collected at ESRF and Alba. Although we already solved the structure of the unliganded form of this industrially-used enzyme (PDB 3DC8, 1.85 Å), we search to improve crystal quality or to obtain new complexes with several substrates/ligands. In this run we got more than 20 data sets from crystals grown in presence of different divalent metals (Co+2, Mn+2 and Ni+2) and with different substrates/inhibitors such as DIPH or DIBH. The best diffracting crystal got to 1.5 Å, improving our previously deposited 3D model. Extra density can be ascertained at the catalytic center, but its quality does not unambiguously allow fitting the expected ligand.

v) Ancestral lactamase GNCA02-S70A. Following our (unsuccessful) data collection of ancestral GNCA lactamase bound to different substrate/inhibitors we have soaked the variant GNCA02-S70A with different lactamase substrates, since we were not able yet to obtain a lactam-bound structure. We were able to identify Penicillin G bound to our ancestral lactamase in at least one of the crystals measured (Figure 1, P61, 2.5 Å, a=b=49.57; c=196.73). Due to the success with this variant, future work is centred in co-crystallizing the GNCA02-S70A variant with different substrate/inhibitors to shed some light on the promiscuity found with ancestral lactamases.

Table 1. Data collected by the CSIC-UGR.

Protein	Samples	Conditions	Cryo	Notes (max. resolution)
PcaY-LBD	10	C20	0 - 15% GOL	More than 10 data sets of the apo and ligated forms.
McpU-LBD	10	C2/ PPP5 / C11	0- 15% GOL	No collection.
TlpQ-LBD	4	C14	15% GOL	Three data sets, the best at approx. 2.0 Å.
Ser38	20	PPP8 / Na-Formate pH 4.0	15% GOL	More than 20 data sets from crystals grown in presence of Co+2, Mn+2 and Ni+2 with different ligands. Best dataset at approx. 1.5 Å.
GNCA02-S70A	16	PPP4, 5, 7, 8 and 9	15% GOL	Best dataset at approx. 2.5 Å. Penicillin G-bound structure obtained

vi) Microfluidic chips for counterdiffusion crystallization. One of our main research line at LEC is the development of new methodologies to screening and growth protein crystals of high quality using media to ensure that mass transport is driven mainly by diffusion. We have designed and fabricated two microfluidic chip prototypes with walls made out of millar or kapton (Fig. 1). Three model proteins, lysozyme, thaumatin and glucose isomerase, were grown within individual microchips and data collected at room temperature. Many data set were collected from the three model proteins reaching resolution close to 1.0 Å. These results will be used to write an article summarizing the main results and conclusions.

Future perpectives: A continous colaboration with the team of ID30, lead by Dr. Christoph Mueller-Dieckmann, will allow us to further test new desings of CD microchips.

Figure 1. GNCA02-S70A ancestral lactamase variant bound to Penicillin G (Left). Set-up for RT data collection at ID30B from CD microchips (Right).