ESRF	**Experiment title: Macromolecular Crystallography at South-East Andalusia**	**Experiment number**: MX-1830
Beamline: ID30B	**Date of experiment**: From: 24 September 2016 at 09:30 to 25 September 2016 at 08:00	**Date of report**: 26/01/17
Shifts: 2	**Local contact(s)**: GOTTHARD G.	*Received at ESRF:*

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Partial Report of Mx11830 ID30B (24-09-2016 / 25-09-2016):

This up-date report corresponds to the data collected at ID30B during the first round of Mx1830. We brought 50 samples from the team grouped as CSIC-UGR. All the samples were tested and the main results are listed below.

Crystals from CSIC-UGR:

i) LBD-TlpQ bound to histamine. TlpQ, a cluster I LBD, is the chemoreceptor responsible for positive chemotaxis to ethylene in some organism. The amino acid sequences of TlpQ and McpA are 73% and 70% identical, respectively, to the highly conserved domain of the *E. coli* chemotaxis transducer Tsr. Other *P. aeruginosa* strains including PA7, PA14, and 2192 possess TlpQ homologs. The sequence of the TlpQ-LBD is 29–57% identical to the N-terminal regions of MCPs from other pseudomonads, like *P. putida* strains KT2440 and F1, or *P. fluorescens* Pf-5 and PfO-1. We have obtained crystals in complex with histamine, a high affinity ligand, and tested for diffraction at Alba synchrotron getting data sets at 3.45 Å resolution. In this round, we tested 7 crystals and collected several data sets, the best at 2.12 Å, belonging to the P 2_12_12 space group.

Future perpectives: Desition to be taken after MR attempts and improving crystals quality is on-going.

ii) Lipases. Following our research studies on the improvement of protein crystal grown in gel media and the production of RCLECs (reinforced cross-linked enzyme crystals), we have crystallized the commercial lipase from *T. maritima* in different gel media. A total of 10 crystals were tested and several full data sets were collected. The best integrated data set diffracted X-ray beyond 1.9 Å belonging to the $P6_3$ space group.

Future perpectives: Other batch of crystals will be tested in the future. Crystallization condition will be applied to other comercial lipases. Crystals of other lipases will be tested in future rounds.

iii) LBD-McpU bound to several ligands. McpU is a chemoreceptor that contributes to the formation of biofilm in *Pseudomonas putida*. We keep trying to improve crystal-quality. We have produced and crystallized the Se-Met derivative bound to spermidine, but so far we do not have a single data set good enough for phasing. In this round, we tested only three crystals diffracting X-ray worse than 5 Å.

Future perpectives: Improving crystals quality.

iv) Ancestral Proteins. Besides the enhanced physicochemical properties of ancestral properties, we have previously shown that ancestral backgrounds allow to engineer *de novo* active sites more efficiently than actual

enzymes, designing an ancestral lactamase with dual activity, i.e., lactamase and kemp eliminase. Unexpectedly, the substrate promiscuity obtained with this variant goes beyond these two activities, showing also esterase activity at appreciable levels. We have crystallized and collected data of the GNCA02 W229D/F290W/S70A (GNCA02-3X) variant, and solve its structure at 1.2 Å resolution. We also soaked crystals of GNCA02-3X with several potential esterase substrates/products, such as napthyl acetate, napthol, p-nitrophenol acetate, p-nitrophenol, MUBA,...but even though crystals diffracted at high resolution we have not found any of the tested ligands at the active sites.

We are also testing a simpler methodology to obtain pseudo-ancestral enzymes (Seclet). Seclet lactamase variants have been prepared with this new methodology showing a highly improved stability (ΔTm 26-34 ºC) and higher substrate promiscuity when compared to actual lactamases, to levels closer to ancient Precambrian β-lactamases resurrected in our laboratory. Even though we already have data of the Seclet0 variant, we have found new crystallization conditions and search for crystals of improved quality. We have collected several data sets of which the best was integrated at 1.2 Å (P61). Seclet12, a new variant, was produced and crystallized. Seclet12 crystals diffracted X-ray to maximun resolution of 1.9 Å belonging to the primitive P1 space group.

Future perpectives: Among the current variants of ancestral lactamases we plan to improve the crystal quality of Seclet12 and to test new ligands of the GNCA variants. Other variants are being produced for the next round.

Table 1. Data collected by the CSIC-UGR.

Protein	Samples	Conditions	Cryo	Resolution
Seclet0	6	C28/C30/31	15% GOL	Several data sets, the best at 1.2 Å
Seclet12	6	C15	15% GOL	Several data sets, the best at 1.9 Å
McpU-LBD	3	PPP5 / PPP6	0- 15% GOL	No data set.
TlpQ-LBD	7	C14	15% GOL	Several data sets, the best at 2.6 Å.
Lipase TL	9	C23	15% GOL	Several data sets, the best at 1.9 Å.
GN02	10+4	PPP6 / PPP7 / PPP8	15% GOL	Several data sets, the best at 1.2 Å
Nsaar	5	A2 / B2	20% GOL	Bad diffraction, no data set.