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Shifts: 2	**Local contact(s)**: GOTTHARD G.	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists): Jose A. GAVIRA-GALLARDO[1]*, Ana CAMARA-ARTIGAS[2], Sergio MARTINEZ-RODRIGUEZ*[3], Mª Teresa CONEJERO-MURIEL[1], FERNANDEZ Raquel[1]

1. Laboratorio de Estudios Cristalográficos, IACT, CSIC-UGR, Spain.
2. Dto. Química y Física, University of Almeria, Spain.
3. Dto. Química Física, University of Granada, Spain.

Partial Report of Mx11830 ID30A3 (19-11-2016 / 20-11-2016):

This up-date report corresponds to the data collected at ID30B during the second round of Mx1830. We brought 60 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-McpU bound to several ligands. McpU is a chemoreceptor that contributed to the formation of biofilm in *Pseudomonas putida*. We are trying to improve crystal-quality using different purification strategies and screening looking for new crystallization conditions. We have produce and crystallize the Se-Met derivative but so far we do not have a single data set good enough for phasing. Therefore, we keep on searching for proper crystallization/cryo conditions. In this round, we tested only three crystals each one from a different crystallization condition diffracting X-ray worse than 5 Å.

Future perpectives: Improve crystals quality.

ii) LBD-TlpQ bound to histamine. TlpQ, a cluster I LBR, 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-LBR 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 getting data seta at 3.45 Å resolution. In this round, we tested 10 crystals and collected several data sets, the best at 2.6 Å, belonging to the P 2_12_12 space group.

Future perpectives: Desition to be taken after RM attempts.

iii) 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 enzyme 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: Crystallization condition will be applied to other comercial lipases. Crystals of other lipases will be tested in future runds.

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 collect data of the GNCA02W229D/F290W/S70A (GNCA0-3X) variant and solve the structure at 1.19 Å resolution (Table 2).

Furthermore, since ancestral sequence reconstruction is a tedious work, difficult to implement in most laboratories, we are testing a new and more simple methodology to obtain pseudo-ancestral enzymes (Seclet). Seclets lactamases 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. Following this research line a new variant, Seclet12 was produced and crystallized. Seclet12 crystals diffracted X-ray to very poorly and are usefuless.

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 can be produce for the next round.

Table 1. Data collected by the CSIC-UGR.

Protein	Samples	Conditions	Cryo	Resolution
Seclet0	6	C28/C30	15% GOL	Several data sets, the best at 1.2 Å
Seclet12	6	C15	15% GOL	No data set.
McpU-LBD	3	PPP5 / PPP6 / C21	0- 15% GOL	No data set.
TlpQ-LBD	10	C14	15% GOL	Several data sets, the best at 2.6 Å.
Lipase TL	10	C23	15% GOL	Several data sets, the best at 1.9 Å.
GNCA0-3X	10	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.

Table 2. Data collection and refinement statistics of GNCA0-3X.

Resolution range (Å)	40 - 1.19 (1.22 - 1.19)
Space group	P 61
Unit cell	47.343 47.343 190.705 90 90 120
Unique reflections	73336 (4114)
Multiplicity	3.2 (2.0)
Completeness (%)	88.24 (49.81)
Rsym (%)	3.2 (36.2)
Mean I/sigma(I)	29.73 (3.43)
Wilson B-factor	13.90
Refinement	14.77
R-work	0.1618 (0.3408)
R-free	0.1880 (0.3146)

Statistics for the highest-resolution shell are shown in parentheses.