ESRF	**Experiment title: Macromolecular Crystallography at South-East Andalusia**	**Experiment number**: MX-2281
Beamline: ID23-1	**Date of experiment**: From: 24 February 2021 / 25 February 2021	**Date of report**: 02/08/21
Shifts: 3	**Local contact(s)**: DE SANCTIS Daniele	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists): Jose A. GAVIRA-GALLARDO*[1], Ana CAMARA-ARTIGAS[2], Sergio MARTINEZ-RODRIGUEZ*[3], Marina PLAZA-GARRIDO*[2], Mari Carmen SALINAS-GARCIA*[2], Carmen LOPEZ[1],

1. Laboratorio de Estudios Cristalográficos, IACT, CSIC-UGR, Spain.
2. Dpto. Química y Física, University of Almeria, Spain.
3. Dpto. de Bioquímica y Biología Molecular III e Inmunología, University of Granada, Spain.

Partial Report of MX2281 ID23-1

This partial report corresponds to the first data collection experiment of Mx2281 carried out at ID23-1. We tested 110 samples from the Granada (UGR and CSIC) (Table 1) and Almeria (Table 2) teams.

Crystals from Granada CSIC & UGR (Table 1):

i) Chemoreceptor PA4633 (PA4633-LBD). The membrane-bound chemoreceptor PA4633 mediates taxis to different chemoattractant in the opportunistic human pathogenic bacterium, *Pseudomonas aeruginosa* PAO1. This receptor contains a periplasmic ligand binding domain (LBD) that directly recognizes several identified chemoattractant. Ligand binding to PA4633-LBD triggers a molecular stimulus that ultimately modulates chemotactic responses in the strain PAO1. We got crystals of the apo and bounded forms but all of them diffracted poorly and we did not collect useful data sets.

Future perspectives: New co-crystallization experiments have been set-up.

ii) hGo (Human Glycolate oxidase). Salicylic derivatives have shown to inhibit human glycolato oxidase (hGO) and, for this reason, they are considered drug candidates for the treatment of primary hyperoxaluria type I (PH-1). PH-1 is a rare disease affecting the hepatic metabolism of glyoxilate in which patients present systemic accumulation of oxalate crystals and lacks of pharmacological treatment. Salicylates have recently proved to be good inhibitors of GO and, very importantly, they show good phenotypic activities in hyperoxaluric hepatocytes. The determination of the structure of hGO-salicylates complexes will throw light to the binding mode of these compounds and will help the determination of their mechanism of inhibition. This will allow the establishment of reliable structure-activity relationships and, therefore, the development of new hits with improved activity. We have already obtained crystals of the protein without any inhibitor and co-crystallization and soaking will be done. We hope to determine the structure by MR and therefore native data sets at high resolutions is the main goal.

Future perspectives: Binding experiments are being conducted to ascertain the best ligands to be soaked/co-crystallized. Crystal improvement/optimization is being carried out.

iii) LysR-type transcriptional regulator (AdmX) from rizobacterium plymuthica. It has been shown that AdmX control the synthesis of the antibiotic andrimid in plants associated bacterium *Serratia plymuthica* A153. The environmental signals that bind to AdmX and modulate its action have been identified and can be classified a as agonists and antagonists. AdmX has been soaked with members of both classes and subject to crystallization. We have tested a total of 11 crystals but none of them diffracted to a reasonable resolution limit.

Future perspectives: We have produced and crystallized the SeMet derivatives to be test in future experiments.

iv) Ancestral lactamases. We have already solved, determined, deposited and published the structure of several ancestral beta-lactamases. We have selected the last common ancestor of Gram Negative Bacteria Class A beta-lactamase (GNCA) to carried out some preliminary investigation of the binding mode of lactamases inhibitors. In

this sense we have soaked GNCA crystals with CTX and HUBA inhibitors. We got good data set and structure determination is being done by MR.

Future perspectives: This is an on-going parallel project and other inhibitors will be assayed.

v) Dihydropyrimidinase from Sinorhizobium meliloti (SER38). We have carried out several attempts to obtain ligand-bound structures of this industrially-relevant enzyme, which are not known to date. We brought ten new crystals, changing the soaking procedures applied previously, in order to obtain liganded structures. Unfortunately, no ligand is present in any of the structures solved from five datasets obtained (2.5-2.7 Å)

Future perspectives: Active-site mutants are being prepared, in order to co-crystallize them with different substrates

Table 1. Data collected by the CSIC-UGR.

Protein	Samples	Conditions	Cryo	Resolution
AdmX	30	PPP4, C4, C8, C10	20% PEG200	Poor diffraction
PA4633-LBD	14	C2,C10	20% PEG200, 15% GOL	Several data sets, best at 2.0Å.
hGo (L1-L5)	2	C37, C38		1 dataset (3.1 Å)
GNCA	4	Sodium Formate pH 4	20% GOL	Several data sets, best at 1.1Å.
SER38	10	Sodium Formate pH 4.6	15% GOL	5 datasets (2.5-2.7 Å)

Crystals from Almeria (Table 2):

i) Chimeric constructions of the c-Src and Fyn SH3 domain. We have cloned some chimeric constructions of the c-Src-SH3 domain where the RT- (SF-RT), n-Src (SF-Src) and both (SF-2X) loops belonging to this SH3 domain have been interchanged by those present in the homologous Fyn-SH3 domain and vice versa (FS-RT, FS-Src and FS-2X). We have measured 4 crystals of SF-2X in presence of urea, which diffracted at 1.7-2.5 Å.

ii) c-Src-SH3 mutant. We are also cloned some nucleation site mutants of these SH3 domains. We have measured 6 crystals of L100I mutant of Src in presence of NS5A (high affinity peptide). One of them diffracted at resolution of 2.5 Å.

iii) Synthetic construct of GP41 (SC-GP41). Several crystals belonging to different constructions of the SC-GP41 in complex with several high affinity peptides have been obtained. Crystals were small and did not diffract or did it at low resolution (~3.4 Å)**.**

iv) Lysozyme. We have measured 36 crystals of lysozyme soaked in different concentrations of TFE and in different dyes at acidic, neutral and basic pHs. These crystals diffracted at high and medium resolution of ~1.0-2.2 Å.

Table 2. Data collected by the Almeria team.

Protein	Samples/Diffrac.	Conditions	Diffraction (Å)	Space Group/Cell
L100I	6/1	1.8 M ammonium sulphate, 0.1 M Hepes pH 7.0/2.5 M ammonium sulphate, 5 % PEG 300, 0.1M sodium acetate pH 5, Hepes pH 7 and Tris pH 8. NS5A {1:2}	2.5	P1/174 308 407, 70 82 77
SF-2X	4/4	2.5 M ammonium sulphate, 0.1 M Hepes pH 7/Tris pH 8, 3/7 M Urea	1.7-2.5	P3221/88 88 56, 90 90 120
SC-GP41	10/1	1.6 M sodium formate, 0.1 M MES pH 6.5/Hepes pH 7-7.5. 20% PEG 4K, 5 % glycerol, 0.1 M sodium acetate pH 4/MES pH 6.0	3.4	H3/ 55 55 293, 90 90 120
Lysozyme	36/33	0.1-0.6M NaCl, 0.1M buffers (sodium acetate pH4.5-5.5, tris pH8.0-8.5, imidazole pH7.0), 50mM NaH_2PO_4	1.0-2.2	P222/ 30 56 73 P4/ 80 80 37