ESRF	**Experiment title: Macromolecular Crystallography at South-East Andalusia**	**Experiment number**: MX-2281
Beamline: ID30A-3	**Date of experiment**: From: 12 November 2020 / 13 November 2020	**Date of report**: 15/02/21
Shifts: 3	**Local contact(s)**: MCCARTHY A.	*Received at ESRF:*

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Partial Report of MX2281 ID30B

This partial report corresponds to the second data collection experiment of the Mx2281 done at ID230B. We tested 60 samples from the Granada teams (Table 1) and 50 samples from the Almeria team (Table 2).

Crystals from Granada (Table 1):

i) L-amidase from *Pseudomonas* (PseAmid, 15 crystals). L-specific amidases are industrially attractive enzymes, due to its potential for the production of optically pure L-amino acids starting from racemic mixtures of amino acid-amides, which are cheap precursors. We previously solved the structure of the unliganded PseAmid from a dataset collected at ID30B in a previous bag proposal (PDB ID. 7A6G, paper submitted with revision required). We have soaked different crystals of PseAmid with different ligands. Whereas datasets with resolution up to 2.1 A were collected, all the crystals correspond to a contamination from the purification process (TIM from *E. coli*).

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). 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. We have already obtained crystals of apo hGo without any inhibitor and co-crystallization and soaking are being assayed with no succeed so far. New strategies for soaking the target ligands keep being tested but none of them seems to work so far regard-less the sufficiently good resolution obtained.

Future perspectives: We are now able to produce the apo for in a regular manner and other soaking and co-crystallization strategies will be tested.

iii) Chemoreceptor ECA2226 (ECA2226-LBD). The membrane-bound chemoreceptor ECA2226 mediates taxis to different chemoattractant in the plant-pathogenic bacterium, *Pectobacterium* atrosepticum strain SCRI1043. This receptor contains a periplasmic ligand binding domain (LBD) that directly recognizes different ligands already identified. We have obtained crystals of the apo form and in complex with betaine. We collected several data sets and structure determination is going by MR.

Future perspectives: We will attempt to improve the resolution and obtain the rest of complexes.

iv) 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.

v) 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.

Table 1. Data collected by the CSIC-UGR.

Protein	Samples	Conditions	Cryo	Resolution
ECA2226-LBD	10	HR I: C22, C15, C10	15% GOL + Ligands	Six data sets, the best at 1.9 Å
hGo (L1-L5)	16	C37 & C38	15% GOL+Ligands	Five data sets, the best at 2.5 Å.
PseAmid	16	HR I: C28, C18, C40, C37, C39	15% GOL,	Several data sets, the best at 2.0 Å
PA4633-LBDR	7		15% GOL + Ligands	Very poor crystals.
AdmX	11	HG II: C37, C46	0-15% GOL	Low quality crystals.

Crystals from Almeria (Table 2):

i) Synthetic construct of GP41 (SC-GP41). This protein and their variants yield crystals that diffracts poorly. We have tried several approaches to improve the quality of the crystals. However, the best diffracting crystal only yield a resolution of 3.5 Å. Even the complexes with peptides that stabilize the protein did not result in better diffracting crystals. In addition, the size of the crystal is small.

Future perspectives: We continuous working to improve the crystals size and quality.

ii) Chimeric constructions of the c-Src and Fyn SH3 domain. We have cloned several 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 crystals from FS-Src in presence of TFE (2,2,2-trifluoroethanol) to study the binding of the solvent. Crystals diffracted at high resolution ~1.5 Å, but not molecules of the TFE were observed in the difference maps. This solvent also has been reported to promote the formation of intertwined dimer, but our crystals belong to the monomeric form of the domain. We also measured crystals of the SF-2X in presence of urea to complete the characterization of this chaotropic agent to the SH3 domain, but not diffraction was observed.

Future perspectives: We are working to find the condition where the chimeric protein FS-Src forms the intertwined dimer using different agents that would promote the 3D domain swapping. We are trying more conditions.

iii) Chimeric constructions of the c-Src and c-Abl SH3 domain. Same as the previous chimeras, we have cloned the chimeric constructions: SA-RT, SA-Src, SA-2X, AS-RT, AS-Src, AS-2X. We have measured 3 crystals of SA-Src, which 2 diffracted at high resolution ~1.5-1.8 Å.

iv) Lysozyme. We have measured 10 crystals of lysozyme soaked in different dyes at acidic, neutral and basic pHs. These crystals diffracted at high resolution of ~1.0-1.5 Å. However, the conditions assayed did not allow us to model the dye.

Future perspectives: We are working to improve the procedure to obtain new lysozyme-dye complexes.

v) Bovine serum albumin (BSA). We have measured 5 crystals of BSA at pH 6.5. The goal is to characterize the ATCUN motif (amino terminal Cu(II)- and Ni(II)-binding) of this protein. 3 of the crystals were obtained in presence of copper and 2 in presence of nickel of the protein. Crystals not diffracted or diffracted at low resolution ~3.0 Å.

Future perspectives: We are working to improve the diffraction of the crystals.

Table 2. Data collected by the Almeria team.

Protein	Samples	Conditions	Diffraction (Å)	Space group/cell
SC-GP41	14/6	15-22% PEG 4000, 0.15M $(NH_4)_2SO4$, 0.1M MES pH6.0/ 1.4-1.5M NaF, 0.1M NaCit pH3.5	3.0	P1. 46 75 76 107 99 90 H32. 57 57 282 90 90 120 C2. 101 58 103 90 109 90
FS-Src	12/10	2.0M AS 4, 5% PEG 300, 0.1M AcNa pH 4.0/NaCit pH3.0/ 2.0M AS, 0.1M NaAc pH 4.0, 3.0M urea	1.5	P222. 28 32 60
SF-2X	3/0	0.1M MES pH 6.0, 2.0M $(NH_4)_2SO4$, 3.0M urea	-	-
SA-Src	3/2	1.8M $(NH_4)_2SO4$, 5% PEG 300, 10% glycerol, 40mM LiCl, 0.1M NaAc pH5.5	1.6-1.8	P1211. 37 93 80 90 97 90
BSA	5/1	24-26% PEG 4000, 0.15M NH_4Cl, 0.1M NaCl, 0.1M MES pH6.5, 5mM $CuCl_2$ or $NiCl_2$	3.0	C2. 224 46 148 90 114 90
Lysozyme	10/9	0.1-0.6M NaCl, 0.1M buffers (NaAc pH4.5-5.5, tris pH8.0-8.5, imidazole pH7.0), 50mM NaH_2PO_4	1.0-1.5	P222 30 56 73 P4 80 80 37