ESRF	**Experiment title: Macromolecular Crystallography at South-East Andalusia**	**Experiment number**: MX-2281
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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 (URG and CSIC) (Table 1) and Almeria (Table 2) teams. Due to our mistake, we did not realize that only Uni-pucks were supported at 23-2 and somehow force our local contact to transfer our samples from Spine-puck to Uni-pucks. We much appreciate their effort since we were able to test all our samples.

Crystals from Granada CSIC & UGR (Table 1):

i) LysR-type transcriptional regulator (AdmX) from rizobacterium plymuthica. 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. In this project we already obtained diffracting crystals but we have not been able to determine the structure by RM. We have produced SeMet crystals, WT crystals soaked with TbXo4, etc in a search for phases. We have tested almost 30 crystals but none of them diffracted to a reasonable resolution limit.

Future perspectives: We have recently solved the structure by combining the model generated by AlphaFold to generate phasing fragments searched with Arcimboldo-Borges and grown with Shredder.

ii) Chemoreceptor PA4633 (PA4633-LBD). The membrane-bound chemoreceptor PA4633 mediates taxis to different chemoattractant in the opportunistic human pathogenic bacterium, *Pseudomonas aeruginosa* 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: On-going project to improve crystal quality.

iii) Clumping Factor (ClfA) (N2N3). Modified versions of ClfA from Streptococcus ancestors. Clfa is used by Streptococci to adhere to tissues and start colonization. We tested 10 crystals and got data to 2.8 Å with crystal belonging to the P212121 space group and unit cell dimensions 76.886 129.119 168.46. We have been able to determine the structural model by MR locating four monomers in the ASU. Refinement is on-going together with crystals improvement.

Future perspectives: To improve the resolution by improving crystal quality.

iv) 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.

v) Influence of magnetic/electric field in protein crystallization. We have used Glucose Isomerase (Glucy) to study the influence of very low magnetic field in combination with electric field on the nucleation and growth (final crystal quality). We collected 10 data set at high resolution limit.

Future perspectives: Obtained result could be enough to finished this project.

vi) Histidine ammonia-lyase from *Geobacillus kaustophilus* (HAL). This thermostable enzyme belongs to the superfamily of aromatic amino-acid ammonia lyases, with high applicability in the production of optically pure amino acids. We have embarked in the production of liganded-bound structures of this enzyme, for which no structural information is available at the PDB. We measured the first 7 crystals obtained, but unfortunately, all of them diffracted poorly or were salt crystals.

Future perspectives: new crystals have been produced.

Table 1. Data collected by the CSIC-UGR.

Protein	Samples	Conditions	Cryo	Resolution
AdmX	30	PPP4, C4, C8, C10	20% PEG200	Poor diffraction
PA4633	5	C10	15% GOL / naked	Very poor diffraction. 1 set at 3.7 Å.
N2N3	10	C1& C30	15% GOL / naked	Several data sets, the best at 2.8 Å.
Glucy	10	0.2 M MgCl2 + PEG56K	15% GOL	10 data sets, the best at 1.25 Å.
HAL	7			Poor diffraction/salt

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 2 crystals of SF-2X and 1 crystal of SF-RT in presence of proline rich peptides (APP12, VSL12 and NS5A). SF-2X crystals diffracted at resolution of 2.1-2.7 Å.

Future perspectives: We are working to find the condition where the protein is bound to the peptide.

ii) 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 8 crystals of SA-RT in presence of proline rich peptides (APP12, VSL12 and NS5A), and one crystal of SA-RT in presence of PEG 300 to improve the previous data. Seven of these crystals diffracted at resolution of 1.5-1.8 Å.

iii) c-Src-SH3 mutant. We are also cloned some nucleation site mutants of these SH3 domains. We have measured 4 crystals of L100I mutant of Src in presence of NS5A (high affinity peptide), but no diffraction was observed. Two of these crystals diffracted at resolution of 1.8-3.0 Å.

iv) SH3 domain from an oncogenic c-Src tyrosine kinase (v-Src-RT). We have measured two crystals in presence of proline rich peptides (APP12, VSL12 and NS5A). One of these crystals diffracted at ~1.9 Å.

v) PDZ2-ZO1 and PDZ2-ZO3. We have measured 2 crystals of PDZ2 domain but no diffraction was observed.

vi) Concanavalin A. We have measured 20 crystals of Concanavalin A in presence of maltose, rhamnose, glucose and pyranose. 14 of these crystals diffracted in a range of resolution of 1.6-3 Å.

Table 2. Data collected by the Almeria team.

Protein	Samples/Diffrac.	Conditions	Diffraction (Å)	Space Group/Cell
SF-2X	2/2	2.0 M ammonium sulphate, 0.1 M Hepes pH 7.0 and Tris pH 8.0. APP12	2.1-2.7	P3221/87 87 55, 90 90 120 P6/87 87 54, 90 90 120
SF-RT	1/0	2.0 M ammonium sulphate, 0.1 M Tris pH 8.0. APP12 {1:2}		
SA-RT	8/6	2.0 M ammonium sulphate, 0.1 M Hepes pH 7.0 and Tris pH 8.0. APP12	1.5-1.8	C222/40 56 52, 90 90 90 P1/42 66 127, 76 79 64
L100I	5/2	2.0 M ammonium sulphate, 0.1 M MES pH 6.0 and Tris pH 8.0. NS5A	1.8-3.0	P1211/27 52 40, 90 106 90 P1/41 54 80, 82 86 88
v-Src	2/1	1.6M ammonium sulphate, 10 % glycerol, 5 % PEG 200, 40 mM LiCl, 0.1M sodium acetate pH 5.5	1.9	C121/114 76 42, 90 95 90
PDZ2-ZO1	1/0	30 % PEG 4K, 0.2 M sodium acetate, 0.1M Tris pH 8.5		
PDZ2-ZO3	1/0	0.1M sodium acetate pH 4.6, 2 M ammonium sulphate		
ConA	20/14	5 % PEG4K, 0.2M sodium acetate, 0.1M MES pH 6, 5 mM glucose, rhamnose, pyranose, maltose	1.6-3	P222/65 115 124, 90 90 90