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 some mounting-robot issue with cryo-loop vials we could not finishes data collection at ID30A-3. The next day we move to ID23-1 where a similar issue arise and loops were manually transferred to Unipuck baskets. We have to strongly thank the effort of the local contact and beam scientists doing all the sample handling.

Crystals from Granada CSIC & UGR (Table 1):

i) 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 Å. We have been able to determine the structural model by MR locating four monomers in the ASU. We are trying to get better resolution from different crystals improvement strategies. We have collected several data sets some in a different polymorph, P21, with cell parameters 70.71 106.03 71.74 Å. Analysis is on-going.

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

ii) 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 tested 18 crystals and collected 5 full data sets the best at 2.5 Å.

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

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 being attempted by MR but with no succeed so far. Crystal improvement is on-going and SeMet derivative is being produce.

Future perspectives: We keep attempt to improve the resolution by testing the co-crystallization with the rest of complexes. In parallel we are trying to obtain the SeMet derivatives.

iv) Influence of magnetic/electric field in protein crystallization: We have selected also commercial apo-transferrin (**ApoT**) and its empty form, holo-transferrin, to improve crystal quality using different strategies. So far,

we are applying the growth under diffusion-controlled regimen in capillaries with or without agarose gel prior to use external fields. We tested 12 crystals but as expected the diffraction was very poor and we collected only one data set to check space group and cell dimensions.

Future perspectives: Improvement is on-going.

v) GAP-related domain of human Neurofobromin 1 (NF1-GRD). Neurofibromatosis 1 is a human illness which mainly occurs by mutations in neurofibromin 1 gene. Among the different human clinical variants, mutations on the GAP-related domain of this protein are notorious. We have embarked in the structural characterization of this domain in order to understand the molecular basis for his illness. We measured 5 crystals, obtaining our first dataset for this protein, at a modest resolution of 2.7 Å.

Future perspectives: new crystals have been produced, in order to improve the initial resolution obtained.

vi) β-xylosidase from *Geobacillus stearothermophilus* (XynB2). Xylans are the most abundant polysaccharides forming the plant cell wall hemicelluloses, and they are degraded, among other proteins, by β-xylosidase enzymes. This enzyme has a clear biotechnogical application in the degradation of raw materials for the production of different monosaccharides, which are useful as fermentation sources or as alimentary supplements. We have embarked in the characterization of the thermostable xylosidase XynB2, belonging to family 52, which have been scarcely described from the structural point of view. We have measured 5 crystals, obtaining one dataset at 2.7 Å, from which we have been able to obtain our first structural model.

Future perspectives: new crystals have been produced, in order to improve the initial resolution obtained.

vii) Hydantoin racemase (HR). In order to finish previous studies on a site-active mutant of this enzyme (mx…), we have also produced and crystallized the WT enzyme, to complement the paper which has been written with those previous results. Two crystals were measured, but no datasets were obtained.

Table 1. Data collected by the CSIC-UGR.

Protein	Samples	Conditions	Cryo	Resolution
N2N3	12	C1&C10(T), PPP4	15% GOL / naked	Several data sets, the best at 2.2 Å.
AdmX	18	PPP8 &9, C24, C5, C6, AS8, C11	15% GOL / naked	5 data sets
ECA2226	5	C10	15% GOL / naked	Very poor diffraction. 3 set.
NF1-GRD	5	MixPEG pH8	Naked, 30% PEG 400, 15% GOL	2x Full data aprox 2.9 Å
XynB2	5	AS5	15% GOL	2x Full data aprox 2.7 Å
HR	2	C17 HRCSI	15% GOL	No diffraction
ApoT	13	selected	15% GOL / naked	Very poor.

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. We have measured 4 crystals of SF-2X in presence of proline rich peptide (VSL12). 3 of these crystals diffracted at resolution of 2.0-2.4 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. We have measured 5 crystals of SA-2X in presence of proline rich peptide (VSL12). All of them diffracted at resolution of 1.5-2.0 Å.

iii) SH3 domain from an oncogenic c-Src tyrosine kinase (v-Src-RT and v-Src-Q128R). We have measured 5 crystals of v-Src-RT and one crystal of v-Src-Q128R in presence of proline rich peptide (VSL12). Three crystals of v-Src-RT diffracted at ~2.7 Å, and the v-Src-Q128R crystal diffracted at ~1.6 Å.

iv) PDZ2-ZO1. We have cloned some mutants of PDZ2-ZO1 to get the monomeric form of this domain. We have measured 10 crystals of this domain, but no diffraction was observed.

Future perspectives: We are working to improve the procedure to obtain new crystals.

v) 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 and soaked with different concentrations of TFE (2,2,2-trifluoroethanol). Crystals were small and did not diffract.

vi) Lysozyme. We have measured 12 crystals of lysozyme soaked in different types and concentrations of alcohols and in different dyes at acidic pH. These crystals diffracted at high and medium resolution of ~1.2-2.5 Å.

vii) Bovine serum albumin (BSA). We measured 1 crystal of BSA at pH 6.5, but it did not diffract.

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

Table 2. Data collected by the Almeria team.				
Protein	**Samples/Diffrac.**	**Conditions**	**Diffraction (Å)**	**Space Group/Cell**
SF-2X	4/3	1.5 M ammonium sulphate, 0.1M Tris pH 8, [VSL12, 1:2]	2.0-2.4	P3/ 90 90 57 90 90 120
SA-2X	5/5	2.0 M ammonium sulphate, 0.1 M MES pH 6, 40 mM LiCl, 5% PEG300, 10% glycerol, [VSL12, 1:2]	1.5-2.0	P2/ 29 60 37 90 99 90 P1211/ 29 60 37 90 98 90
v-Src-RT	5/3	2.0M ammonium sulphate, 0.1 M MES pH 6, 40 mM LiCl, 5% PEG300, 10% glycerol, [VSL12, 1:2]	2.7	C222/ 40 94 80 90 90 90
v-Src-Q128R	1/1	2.4 M ammonium sulphate, 10% glycerol, 5% PEG300, 40 mM LiCl, 0.1 M MES pH 6.0, [VSL12, 1:2]	1.6	P31/ 53 53 48 90 90 120
PDZ2-ZO1-M1	10/0	0.1 M Tris pH 8.5, 8% PEG 8000		
GP41	12/0	1.6- 1.8 M sodium formiate, 0.1 M sodium acetate pH4- 5.5 1.6- 1.8 M sodium formiate, 0.1 M sodium acetate pH4- 5.5, 5% glycerol		
Lysozyme	12/11	0.2-0.6 M NaCl, 0.1 M sodium acetate pH4.0-5.0, 10 mM NaH_2PO_4	1.2-2.5	P212121/ 30 55 66 90 90 90 P41212/ 78 78 37 90 90 90
BSA	1/0	20% PEG 8000, 0.1 M sodium cacodilate pH 6.5, 0.2 M magnesium acetate		