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Partial Report of MX2281 ID23-1

This partial report corresponds to data collection experiment of Mx2281 carried out at ID23-1. We tested 112 samples from the Granada (URG and CSIC) (Table 1) and Almeria (Table 2) teams. We send a single deward since all crystals were prepared at LEC and send using the Unipuck baskets.

Crystals from Granada CSIC & UGR (Table 1):

i) 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 9 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. Now we need to get model with the natural agonist and the antagonists.

ii) Choline sulfatase from *Sinorhizobium meliloti* (SmelCOSe, C54S). We previously obtained the structures of the free and product-bound forms of SmelCOSe (PDBs 6G5Z and 6G60). However, during preparation of the corresponding manuscript, the unliganded structure was published. We then solved a C54S active site mutant soaked with different ligands in a previous BAG (MX-1938). The new structures show the binding mode of SmelCOSe, which is completely different to the hypothesis reported in the previous paper describing the free-structure. However, during manuscript preparation, we noticed that further information could greatly improve the impact of our results. We have measured 13 additional crystals soaked with new ligands, but unfortunately, all diffracted very poorly.

Future perspectives: Manuscript describing for the first time the substrate and product-binding mode of SmelCOSe is in preparation.

iii) L-amidase from *Pseudomonas* (PseAmid). 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 first structure of this class of enzymes (PDB ID. 7A6G, collected at ID30B, manuscript submitted). We brought two new crystals soaked with different ligands, but unfortunately, they were salt crystals.

iv) 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.NF1-GRD 22 crystals

soaked with different ligands. Different datasets were obtained, the best at 2.2 A, from which we have solved its structure. Unfortunately, no ligand is present in the different structures.

Future perspectives: New crystals have been produced, in order to improve the initial resolution obtained, and to obtain different ligand-bound structures to understand the molecular basis for these enzymes. Also, the complex between NF1-GRD and EVH1-SPRED1 domain has been obtained, and crystallization experiments have been set up.

v) 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. New crystals (10) in different conditions to those obtained previously have been measured. Different datasets were obtained, the best at around 2.2 A.

Future perspectives: new crystals have been produced, in order to improve the initial resolution obtained, and to obtain different ligand-bound structures to understand the molecular basis for these enzymes.

vi) Human bisphosphoglycerate mutase (BPGM). The level of 2,3-diphosphoglycerate (DPG), the allosteric ligand of hemoglobin, is controlled by BPGM. BPGM synthesizes DPG through its synthase activity and degrades it through its phosphatase activity. We have embarked in the structural characterization of BPGM and several of its mutants, in order to gain insights into erythrocytosis and hemolytic anemia. We measured 30 crystals obtained in different conditions, obtaining different datasets, the best at around 2.0 A, including ligand-bound structures.

Future perspectives: new crystals have been produced, in order to improve the initial resolution obtained. Mutants associated to clinical variants have been produced, in order to crystallize them and to conduct a profound biophysical characterization.

Table 1. Data collected by the CSIC-UGR.

Protein	Samples	Conditions	Cryo	Resolution
A41M	3	C20 Hamp II	15% GOL	No diffraction
Primpol	2	C20 Hamp II	15% GOL	No diffraction
N2N3	3	C4	15% GOL	Poor diffraction.
AdmX	9	C4	15% GOL	Poor diffraction.
PseAmid	2	C47 Hamp I	No cryo	Salt
C54S	13	C4 HRCSI	15% GOL+ Ligands	Poor diffraction (<3 Å)
NF1-GRD	22	C44 HampII, and improvement with pH	15% GOL+ Ligands	Several datasets, the best at 2.2 Å
XynB2	10	C1, C3, PPP4	15% GOL	8 datasets, the best at 2.1 Å
BPGM	30	C9, C17, C41	15% GOL, 15% MPD, naked	8 datasets, the best at 2.1 Å

Crystals from Almeria (Table 2):

i) PDZ3-PSD95. To study the polymorphism from this domain we have measured 19 crystals of PDZ3 domain. These crystals diffracted at high and medium resolution of ~1.1-2.3 Å.

Table 2. Data collected by the Almeria team.

Protein	Samples/Diffrac.	Conditions	Diffraction (Å)	Space Group/Cell
PDZ3-PSD95	19/17	30% PEG 4000, 0.2 M ammonium sulphate, 0.1 M sodium acetate pH3.5-5.0	1.1-2.3	P3/ 62 62 228 90 90 120 $P2_12_12_1$/ 29 32 88 90 90 90 P1/ 33 44 61 90 90 90