ESRF	**Experiment title: Macromolecular Crystallography at South-East Andalusia**	**Experiment number**: MX-2353
Beamline: ID30B	**Date of experiment**: From: 07 May 2022 / 08 May 2022	**Date of report**: 12/11/22
Shifts: 3	**Local contact(s)**: **SOLER LOPEZ Montserrat**	*Received at ESRF:*

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Partial Report of MX2353 ID23-1

This report corresponds to the beamtime assigned for May the 7th 2022, from our proposal Mx2353, carried out remotely at ID30-B. We sent a Dewar wit 112 samples from the Granada (URG and CSIC) (Table 1).

i) 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 previously obtained the structures form the WT and the R280K and Y52F mutants (1.1-1.6 Å resolution; SG I222) from data obtained in proposal Mx2281 and the current one, for which no structural information is available at the PDB. We have produced and crystallized a new active-site mutant (Q274N), and soaked it with L- and D-histidine; datasets up to 1.3 Å have been collected in this beamtime.

Future perspectives: Data processing is ongoing.

ii) 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 have measured new crystals, also soaked with different glycolysis ligands.

Future perspectives: Data processing is ongoing. Mutants associated to clinical variants have been produced, and crystallization experiments have been set-up.

iii) *Sinorhizobium meliloti* hydantoin racemase (HR). Hydantoin racemase is a key enzyme in the industrially used enzymatic method known as "hydantoinase process". We have solved in the past (e.g. MX2281) the first structure for this enzyme (a truncated version of the C181A mutant, paper not yet sent), alone and in the presence of different ligands. After huge efforts, we also managed to solve the structure of the WT enzyme in the previous Mx2353 beamtime (2.1 Å, actual R and Rfree values 0.191 and 0.218, respectively). Furthermore, during *in vitro* experiments for manuscript preparation, we found an unusual cross reactivity for this enzyme, and we prepared almost 60 crystals soaked with totally different ligands in order to ascertain the cross-reactivity of this enzyme. Unfortunately, and despite collecting many good datasets up to 1.7 Å, the ligands did not diffuse into the catalytic pocket.

Future perspectives: New crystallization experiments have been set up, to conduct longer in-capillary soaking experiments. A new full-length of the C181A enzyme has also been prepared based on our previous structure of the full length WT enzyme.

iv) Ancestral glycosidase (GH1-N74, N75, N80, N83, N100). Glycosidases are phylogenetically widely distributed enzymes that are crucial for the cleavage of glycosidic bonds. We previously determined the structure of the ancestral glycosidase GH1N72 from data collected in June 2019 (PDB ID. 6Z1H) and in complex with the heme-group (PDB ID. 6Z1M), showing for the first time the heme-binding this capability of GHs pocket to accept an hemo-group. Following this line of research several relevant mutants have been produce and are being characterized. Two of them, N74 & N75, diffracted below 2.0 Å and the 3D model are being determined by molecular replacement.

Future perspectives: Crystal improvement of the other proteins N80, 83 and 100 are on-going.

Table 1. Data collected by the CSIC-UGR

Protein	Samples	Conditions	Cryo	Resolution
HAL (Halo and Q274N)	21	C3 HAMP II; C6 and C9 HAMP I	15% GLY	Several datasets, up to 1.3 Å
BPGM	8	C7 HAMPT I, C25 HAMPT I	15% GLY	Several datasets, up to 2.1 Å
HR	58	C14 TRIANA	15% GLY	Several datasets, up to 1.7 Å
GH1 (N74, N75, N80, N83, N100)	25	Different conditions	15% GLY	Several datasets, up to 1.6 Å

Table 1. Crystals measured during this beamtime.