ESRF	**Experiment title: Macromolecular Crystallography at South-East Andalusia**	**Experiment number**: MX-2353
Beamline: ID23-2	**Date of experiment**: From: 03 March 2022 / 04 March 2022	**Date of report**: 12/11/22
Shifts: 3	**Local contact(s)**: **Basu Shibom**	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists): Jose A. GAVIRA-GALLARDO*[1], Ana CAMARA-ARTIGAS[2], Sergio MARTINEZ-RODRIGUEZ*[3], Marina PLAZA-GARRIDO[2], Mari Carmen SALINAS-GARCIA[2], Carmen LOPEZ[1],

1. Laboratorio de Estudios Cristalográficos, IACT, CSIC-UGR, Spain.
2. Dpto. Química y Física, University of Almeria, Spain.
3. Dpto. de Bioquímica y Biología Molecular III e Inmunología, University of Granada, Spain.

Partial Report of MX2353 ID23-2

This report corresponds to the fourth round of proposal Mx2353 carried out remotely at ID23-2. We send a Dewar with 112 samples from the Granada group (UGR and CSIC) (Table 1) and some crystals in collaboration with the Almeria team.

i) HAL (*Geobacillus kaustophilus* Histidine ammonia-lyase). 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 have also produced different active site-mutants (Y52F, R280K), in order to understand the molecular basis for the mechanism of this industrially relevant enzyme. We brought 32 crystals (ligand-free and soaked with different ligands), from which different datasets have been collected between 1.6-2.0 Å (e.g., SG I222, 1.7 Å, 105.5 108.4 112.2 90 90 90). We found an unreported MIO modification, not described till date.

Future perspectives: new crystals of HAL variants and new mutants have been produced (Q274N), co-crystallized with different ligands, in order to improve our data.

ii) BPGM (Human bisphosphoglycerate mutase). 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 the last crystal obtained in different conditions, obtaining different datasets, the best at 1.84 Å (SG P21212, 98.8 130.6 38.6 90 90 90), including ligand-bound structures.

Future perspectives: Crystal improvement is ongoing, together with the production of mutants associated to clinical variants, which have been already produced and purified.

iii) 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 shed light to the binding mode of these compounds and may 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. We keep on assaying strategies for soaking. We have collected more than 15 data sets but unfortunately the resolution is worse than 3.0 Å.

Future perspectives: We may keep trying to improve crystal quality and ligand binding procedure.

iii) HR/SER27 (*Sinorhizobium meliloti* hydantoin racemase). Hydantoin racemase is a key enzyme in the industrially used enzymatic method known as "hydantoinase process". We have solved in the past the first structure for this enzyme (paper not yet sent). In order to finish previous studies on a site-active mutant of this enzyme (HR, e.g. MX2281), we have also produced and crystallized the WT enzyme (SER27). We have been able to solve its structure at 2.1 Å (actual R and Rfree values 0.191 and 0.218, respectively; SG P321, a=b=86.28, c=135.54 α=β=90.00, γ=120.00; Figure 1). We have also found an unexpected truncation of the variant studied till date, and a new construction has been ordered.

Future perspectives: New crystallization experiments to improve the resolution are ongoing. An unexpected substrate promiscuity has been shown by TSA, and new soaking experiments are being carried out with new crystals of the mutant HR.

iv) CoVS-HR1 chimeric proteins (L3ABC). CoVS-HR1 are short chimeric proteins of 241 residues able to fold and soluble. They are present in three variants L3A, L3B and L3C. We have collected 3 data sets of L3B and L3C.

Future perspectives: Data are being analyzed.

vi & vii) Other measured samples previously described.

Besides those crystals which diffracted properly, other crystals for which no or poor diffraction were obtained were those from *Sinorhizobium meliloti* dihdyropyrimidinase (SER38) soaked with different ligands or *Rhizobacterium plymuthica* LysR-type transcriptional regulator (AdmX).

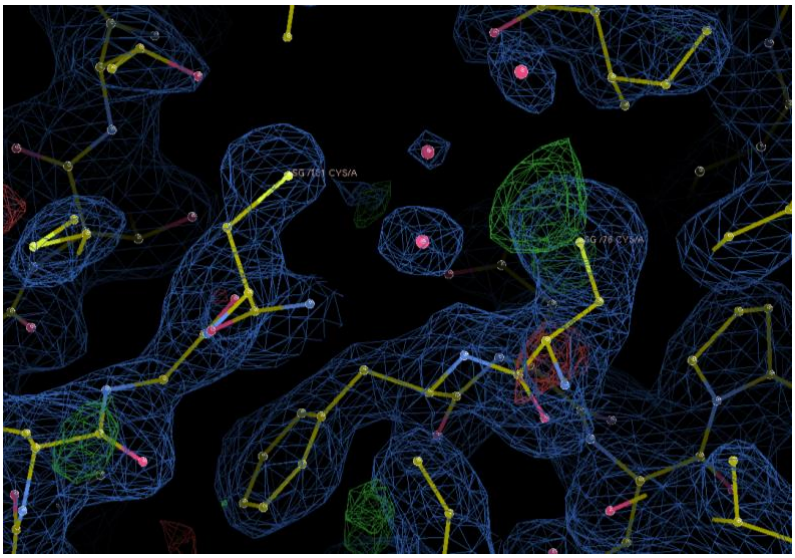

Figure 1. Catalytic environment of WT *Sinorhizobium meliloti* hydantoin racemase showing the two-base responsible for catalysis.

Table 1. Data collected by the CSIC-UGR

Protein	Samples	Conditions	Cryo	Resolution
SER38	7	NaFo 4.6	15% Glyc	No/poor diffraction
Admx	12	#22, 24, 16 and PPP8 &9	15% Glyc/naked	Poor diffraction.
HAL wt/R280K/Y52F	35	#3 hampt II	15% Glyc	Many datasets, up to 1.5 Å
BPGM	1	#41 HampI	15% Glyc	2 datasets, around 2.0 Å
GoH	30	#38 pH 7.0, #42 PH8.5	15% Glyc	15 low res data sets (> 3.0 Å)
HR/SER27	12	C17	15% Glyc	Different datasets, up to2.1 Å
L3ABC	15	MDII C1 & C2	15% Glyc/naked	Three useful data sets.

Table 1. Samples measured during this beamtime.