ESRF	**Experiment title: Macromolecular Crystallography at South-East Andalusia**	**Experiment number**: MX-2454
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Partial Report of MX2454 ID30A-3

This is the first report of our current proposal Mx2454 carried out remotely at ID30A-3. We send a Dewar with 112 samples from the Granada group (UGR and CSIC; Table 1).

i) D-amidases from microbial origin (UreDamid). Amidases are industrially attractive enzymes, due to its potential for the production of optically pure D- or L-amino acids starting from cheap racemic mixtures of amino acid-amides. We previously solved the first structure reported for an L-enantioselective amidase (PDB ID. 7A6G, collected at ID30B, MX2281). This is our second attempt to obtain usable datasets for this enzyme, for which no structural information is available at the PDB; the best dataset diffracted up to 3.5 Å.

Future perspectives: New crystallization experiments have been set-up from freshly purified UreDamid trying to obtain new crystallization conditions or better resolution. A new D-amidase (RhiDamid) has been purified, to have a second chance if this D-amidase does not behave well.

ii) D-acylase (KleDacil). D-acylases are biotechnological relevant enzymes for the production of optically pure amino acids, scarcely described from both the biotechnological and the structural point of view; only the structure from D-acylase of *A. faecalis* is known. After infructuous efforts to obtain crystals from a *Bordetella* D-acylase (MX2281and previous proposals), we produced a new recombinant D-acylase from *Klebsiellae*. We solved its structure from data obtained previously at the ALBA synchrotron; (2.27Å, R-work and R-free at the actual stage are 20.6% and 24.39%, respectively). We have produced new crystals trying to improve the resolution, and in order to carry out soaking experiments. However, the resolution obtained is below that previously obtained.

Future perspectives: new crystallization experiments will be carried out. Enzyme characterization is carried out in parallel by Master thesis students.

iii) *Sinorhizobium meliloti* hydantoin racemase (HR and HyuA). Hydantoin racemase is a key enzyme in the industrially used enzymatic method known as "hydantoinase process". After confirming an unexpected substrate promiscuity in this enzyme, we have prepared a new FL version of the C181A mutant, and have soaked it in capillary, based on previous results with a truncated form. The best crystals were obtained in condition 23 (HRCSI). We have collected different good datasets up to 1.8 Å; A different SG has been determined different to that of our previous structures. Initial analysis also reveal ligand positioning inside the catalytic pocket with ligands different to those previously obtained (Figure 1). A full data set has also been obtained for the FL WT HR (HyuA) at 2.5 Å.

Future perspectives: Data analysis is being carried out. Different crystals still stored for incoming beamtime. New crystals obtained only in condition 23 for ligand soaking. Manuscript almost prepared, waiting for new datasets confirming the broad substrate promiscuity of the enzyme

iv) Histidine ammonia-lyase from *Geobacillus kaustophilus* (HAL) and Q274N/ R280K/ Y52F mutants. This thermostable enzyme belongs to the superfamily of aromatic amino-acid ammonia lyases, with high applicability in the production of optically pure amino acids. These are the last crystals that we plan to measure for this enzyme, which arises from a collaboration with Prof. Las Heras Vázquez, at the University of Almería starting in MX2281 proposal.

Future perspectives: Analysis of all our structures is being carried out. At present, we have solved the structures of the WT and the three mutants; Y52F and Q274N mutants present ligands bound into the catalytic cleft, discerning a different mechanism to that proposed in the literature. The corresponding manuscript is being prepared.

v) *Sinorhizobium meliloti* dihydropyrimidinase (SmelDHP). This is one of our model systems to study Cross-Linked Enzyme Crystals. We have also obtained initial financial support to study different mutations on the human counterpart, and its binding to different ligands within the pyrimidine reductive pathway. In parallel to the production of the human counterpart, we have soaked SmelDHP crystals with different ligands of the previous enzyme in the route (dihydropyrimidine dehydrogenase, 5-FU, 5-IU or 5-BrU), based on previous results showing a similar scenario (PDB 6KLK). Despite obtaining a good resolution for some of our samples (the best at 1.7 Å), unfortunately, none of the crystals showed the ligand into the catalytic pocket.

Future perspectives: Human DHP clones have been purchased for expression. New crystals forms of SmelDHP have been produced, in order to try new soaking experiments.

vi) L3ABC. Novel chimeric proteins mimicking SARS-CoV-2 spike epitopes with broad inhibitory activity. They are present in three variants L3A, L3B and L3C. Several crystals of the L3C form have been tested and various data sets collected at resolutions better than 2.0 Å.

vii) BPGM. Besides the above-mentioned datasets, we brought two crystals of human BPGM grown in the presence of agarose, but they diffracted poorly.

Table 1. Data collected by the CSIC-UGR

#	Protein	Samples	Conditions	Cryo	Resolution
i	UreDamid	6	C2 triana	15% glyc	Poor resolution, the best at 3.5 Å
ii	KleDacil	3	HR I C15	15% glyc	1 full data set at 2.5 Å
iii	HRnew+Ligands HYUA	65	HR I C14, C23 & C24	15% glyc	More than 30 datasets, the best at 1.8 Å
iv	HAL (Q274N, Y52F and R280K)	12	HR II C3	15% glyc	Different data set, the best at 1.75 Å
v	SmelDHP	12	NaFormate pH 4.6	15% glyc	More than 10 data set, the best at 1.7 Å
vi	L3ABC	12	HR C3	15% glyc	Several data sets, the best below 2.0 Å
vii	BPGM + Agarose	2	HR C41	non	Poor diffraction

Table 1. Crystals measured in this beamtime.

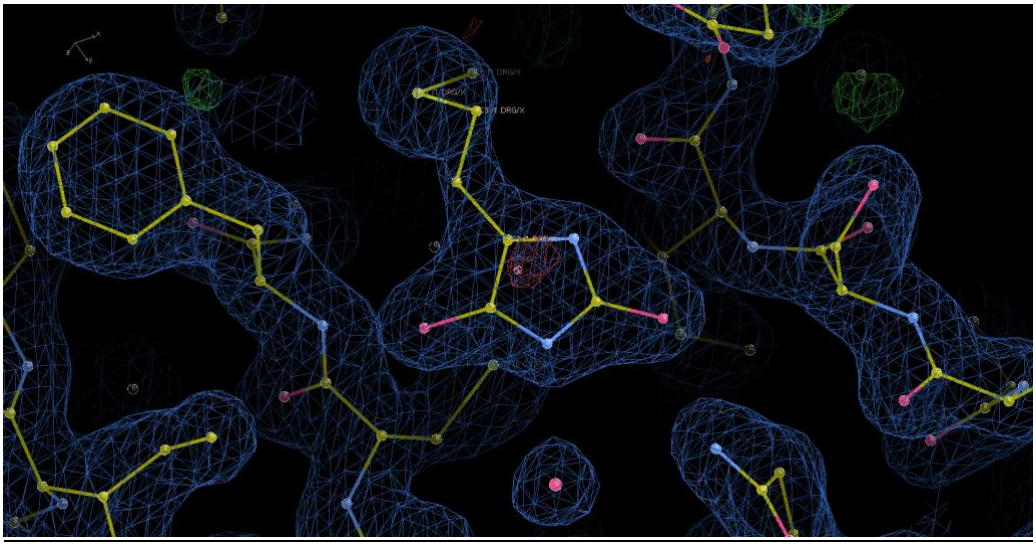

Figure 1. Hydantoin-derivative ligand into the catalytic pocket of one of the hydantoin racemase structures solved from data obtained in this beamtime.