ESRF	**Experiment title: Macromolecular Crystallography at South-East Andalusia**	**Experiment number**: MX-2353
Beamline: ID30A-3 ID23-1	**Date of experiment**: From: 08 June 2022 / 09 June 2022	**Date of report**: 13/11/22
Shifts: 3	**Local contact(s)**: **MUELLER-DIECKMANN Christoph**	*Received at ESRF:*

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Partial Report of MX2353 ID23-2

This corresponds to the sixth and last report of our proposal Mx2353. Originally programed to collect remotely at ID30A-3 some internal issue move the time to the 09/06 at beamline ID23-1. We send a Dewar with 112 samples from the Granada group (UGR and CSIC) (Table 1). Under the frame of a bilateral project (LINKC20027), we collaborate with the group of Dr. Pavlina Rezacova form the OBC Prague to apply counterdiffusion technique in an attempt to improve crystal quality for several proteins, most of them complexes systems: cGAS, NLutR and hPNP. The preliminary crystals were tested in this experiment.

Crystals from Granada CSIC & UGR (Table 1):

i) cGAS (cyclic GMP-AMP synthase). Human cyclic GMP-AMP synthase is naturally activated by dsDNA (involved in cGAS-STING immune pathway). The apo form and in complex with synthetic sDNA have been produced.

ii) NLutR (Human Glycolate oxidase). N-terminal DNA binding domain of the transcription repressor of lutABC operon for L-lactate utilization (LutR) from *Bacillus subtilis* in its apo form or bound to synthetic DNA. In general, the complex formed beautiful well-shaped crystals, but the data indicated low internal quality. Moreover, crystal growth was not reproducible in most of the cases.

iii) hPNP (Human Purine nucleoside phosphorylase). Trying to crystallize the apo-form of this enzyme is a challenge because sulphate/phosphate binds to the active site and conditions usually used for protein crystallization require the presence of one of these anions (also, there is an old structure in PDB with protein crystallized without inhibitor, but with phosphate bound to the active site).

Future perspectives: This is an open project and we may test similar proteins or new systems.

iv) Liver Catalase (Cat). This is one of our model system. We are studing the influence of self-assembled monolayers of selected compound: thiol groups, methacrylate and glycidyloxy on the nucleation and growth of protein crystals. Here we have been able to identify three different polymorphs, one of them never reported before.

Future perspectives: This is an on-going investigation. We may need to characterize other model proteins.

v) 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). Little is known about D-specific amidases, and thus, we have recently cloned and purified a putative D-amidase from microbial origin. We have brought our first crystals for this enzyme, but unfortunately, none of them diffracted.

Future perspectives: New crystallization experiments will be set-up from freshly purified UreDamid. New D-amidases have been cloned to have different targets for structural determination.

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 have measured new crystals obtained for mutant variants with pathological implications in humans (R90C, R90H and Q102K). Only the latter diffracted to a reasonable resolution (2.1 Å).

Future perspectives: Data processing is ongoing. New crystallization experiments have been set-up with the R90C and R90H variants. A new clinical R62Q variant has been purified. Q102K structure has been solved

vii) *Sinorhizobium meliloti* hydantoin racemase (HR). Hydantoin racemase is a key enzyme in the industrially used enzymatic method known as "hydantoinase process". We solved in the past the first structure for this enzyme (a truncated version of the C181A mutant, MX2281, paper not yet sent). 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). We have prepared new crystals of the C181A truncated mutant soaked for longer periods, since shorter soaking were unsuccessful in the previous beamtime. We have collected different good datasets up to 1.7 Å; this time, different ligands have been found into the catalytic pocket.

Future perspectives: a new full length C181A version has been crystallized. In-capillary long-soaking experiments with both the FL and truncated version have been carried out. Crystals have already been cryo-protected and are stored for future beamtime.

Table 1. Data collected by the CSIC-UGR

Protein	Samples	Conditions	Cryo	Resolution
cGas	26	Screening over E3	0 to 15 %gly	We collected 2 data sets, one at 2.7 Å
NLutR	15	Several conditions	0 to 15 %gly	Very poor quality not better than 4.0 Å
hPNP	24	Screening over the selected condition	0 to 15 %gly	Very poor worst than 4.7 Å
Cat	9	15% PEG4000	none	5 data sets
UreaDamid	7	HR II C24	15 %gly/no cryo	Poor/no diffraction
BPGM mutants	16	HR I C6 & C41 (3L)	15 %gly	Full datasets (Q102K) 2.1 Å
HR	15	C41 HAMP I	15 %gly	Full datasets up to 1.7 Å