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Partial Report of MX2064 ID23-1

This partial report corresponds to the first data collection experiment of the Mx2064 carried out at ID23-1. We tested 100 samples from the two team grouped as Granada.

i) Human Death-associated protein kinase 1 (DAPK1). This kinase is a positive mediator of gamma-interferon induced programmed cell death. Since it is essential for growth of p53-mutant cancers, accounting for over 80% of triple receptor–negative breast cancer (TNBC), it has been proposed as a potential therapeutic target for p53-mutant cancer. In collaboration with other groups from the Faculty of Medicine of the University of Granada, new inhibitors for this kinase have been developed. We have solved the structures from the catalytic domain of different inhibitor-soaked crystals, obtaining different datasets, the best at 1.5 Å. whereas we could ascertain clear electronic densities at the catalytic center of this domain (Figure 1), they do not correspond to the expected molecules.

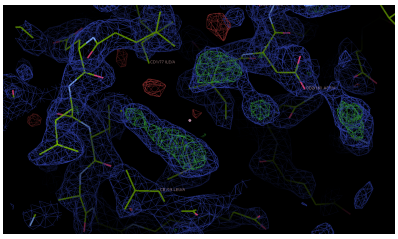

Figure 1. Structure of the catalytic domain of human DAPK1 soaked with a newly designed inhibitor, solved from one of the datasets collected during this round. Electronic densities in the catalytic center do not correspond with the expected soaked inhibitors.

Future perspectives: New crystals with other ligands will be tested in the following rounds.

ii) L-carbamoylase from *Sinorhizobium meliloti* (SmeLCar). L-specific carbamoylases are biotechnological important enzymes allowing the use of the hydantoinase process for the production of different optically pure L-amino acids. We solved in the past the first structure of an L-carbamoylase (PDB 35NF), but we have been unsuccessful in obtaining ligand-bound structures with this enzyme. We have crystallized a new L-carbamoylase of unknown structure. A dataset at modest resolution was obtained in the previous bag proposal (Mx1938 (ID30A-3)), but MR trials resulted unsuccessful. We have obtained two new dataset, the best at 2.2 Å. MR attempts are ongoing.

Future perspectives: Crystal improvement/optimization is being carried out.

iii) Hydantoin racemase from *Ensifer meliloti* (HR). Hydantoin racemase enhances the enzymatic tandem known as "hydantoinase process", utilized worldwide in the industrial production of tons of optically pure D- or L-amino acids (precursors of different commercially available antibiotics, such as ampicillin or amoxicillin). We solved the first structure of the C181A mutant of HR from a dataset collected at ID30A-3 in a previous round of

the Mx1938 proposal, and several other ligand-bound structures during this bag-proposal. In this expedition, we have diffracted 3 crystals belonging to the WT enzyme, but all diffracted at poor resolution.

Future perspectives: we have solved 8 different structures of the WT and the C181A mutant, free and bound to different ligands. The corresponding manuscript is being written, and the corresponding structures will be deposited soon.

iv) Aminomutase: aminomutases are important enzymes for the biosynthesis of Beta-amino acids. We previously crystallized this enzyme and could solve its structure from a dataset obtained at ALBA synchrotron. However, it lacked different important regions containing putative catalytic residues. We have again crystallized this protein, and have bought 7 crystals. Poor diffraction was obtained in all cases.

Future perspectives: due to the unsuccessful results obtained with this enzyme, we have decided to abandon it till we decide how to follow with it.

v) Formamidase from *Bacillus cereus* (BceAmiF). During the review process of the corresponding manuscript, the reviewers asked for new experiments to try ascertaining the modification in the catalytic cysteine of this enzyme (from structures at atomic resolution obtained previously from datasets collected at the ESRF). Different atomic resolution datasets were collected (from 15 different crystals), but we were unsuccessful to obtain a better structure to that presented in the manuscript.

The corresponding manuscript has been published this year (Martínez-Rodríguez S, Conejero-Muriel M, Gavira JA. Arch Biochem Biophys. 2019 662:151-159).

vi) 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 [1]. The environmental signals that binds 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 36 crystals but all of them diffracted poorly.

[1] M. A. Matilla, et al., Environmental microbiology, 2016, DOI: 10.1111/1462-2920.13241.

Future perspectives: We plan to improve crystal quality.

iv) Ancestral Proteins. Within this project and in collaboration with the group of Prof. Jose M. Sanchez-Ruiz (UGR) we plan to crystallize some ancestral Rubisco, if the project keeps on-going. Meanwhile are getting the crystallization conditions ready to testing using Rubisco from *Rhodospirillum rubrum*. We have bring 12 crystal with the best diffracting to approximately 2.5 Å.

Future perspectives: We may try to improve crystals quality.

Table 1. Data collected by the CSIC-UGR.

Protein	Samples	Conditions	Cryo	Resolution
DapK1	24	A.S. pH 4, 6.	15% GOL	Several data sets, the best at 1.6 Å
SmeLCar	3	C9 HR I	0-15% GOL	Several data sets, the best at 2.2 Å
HR	3	C4	0-15% GOL	No data set.
Aminomutase	7	C44, PEG-ION	20% PEG200 & 15% GOL	Several data sets, the best at 2.6 Å.
BceAmiF	15	C1, C2, PPP4	15% GOL, 15-20% PEG400	Several data sets, the best at 1.4 Å.
AdmX	36	PPP5, PPP6, PPP7, PPP8, PPP9, C10 & C11	- & PEG200	Bad diffraction, no data set.
ARub	12		20% GOL	One data set (~2.5 Å)