ESRF	**Experiment title: Macromolecular Crystallography at South-East Andalusia**	**Experiment number**: MX-2353
Beamline: ID30B	**Date of experiment**: From: 18 September 2021 / 19 September 2021	**Date of report**: 17/02/22
Shifts: 3	**Local contact(s)**: **CARPENTIER Philippe**	*Received at ESRF:*

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Partial Report of MX2353 ID30B

This corresponds to the first report of our current proposal Mx2353 carried out remotely at ID30B. We send one Dewar wit 112 samples from the Granada (URG and CSIC) (Table 1).

Crystals from Granada CSIC & UGR (Table 1):

i) Chemoreceptor ECA2226 (ECA2226-LBD). The membrane-bound chemoreceptor ECA2226 mediates taxis to different chemoattractant in the plant-pathogenic bacterium, *Pectobacterium* atrosepticum strain SCRI1043. This receptor contains a periplasmic ligand binding domain (LBD) that directly recognizes different ligands already identified. We have obtained crystals of the apo form and in complex with betaine. We collected several data sets while crystal improvement was on-going and SeMet derivative was also produce. Meanwhile and using a model generated with AlfaFold2 we have been able to find a MR solution. The structure of the complex with betaine have been determined from data collected at ID30A-3 and deposited at the PDB (ID. 7PSG) (Table 2). The manuscript, including the structure of PctD also from ESRF data, have been accepted for publication in mBIO.

Future perspectives: No further actions are planned for this project.

ii) Influence of magnetic/electric field in protein crystallization: We have selected also commercial apo-transferrin (**ApoT/HoloT**) and its empty form, holo-transferrin, to improve crystal quality using different strategies. So far, we are applying the growth under diffusion-controlled regimen in capillaries with or without agarose gel prior to use external fields. We tested 64 crystals but as expected the diffraction was very poor. Meanwhile we have solved the structure from previous data and complete the analysis form Bio-SAX data from the ESRF.

Future perspectives: No further actions are planned for this protein.

Campos-Escamilla, Camila; Siliqi, Dritan; Gonzalez-Ramirez, Luis A.; Lopez-Sanchez, Carmen; Gavira, Jose Antonio and Moreno, Abel. *X-ray Characterization of Conformational Changes of Human Apo- and Holo-Transferrin*. International Journal of Molecular Sciences. 2021, 22. Doi: 10.3390/ijms222413392.

iii) β-xylosidase from *Geobacillus stearothermophilus* (XynB2). Xylans are the most abundant polysaccharides forming the plant cell wall hemicelluloses, and they are degraded, among other proteins, by β-xylosidase enzymes. This enzyme has a clear biotechnogical application in the degradation of raw materials for the production of different monosaccharides, which are useful as fermentation sources or as alimentary supplements. New crystals (10) in different conditions to those obtained previously have been measured. Different datasets were obtained, the best at around 2.2 A.

Future perspectives: New crystals have been produced, in order to improve the initial resolution obtained, and to obtain different ligand-bound structures to understand the molecular basis for these enzymes.

Table 1. Data collected by the CSIC-UGR.				
Protein	**Samples**	**Conditions**	**Cryo**	**Resolution**
ECA2226	16	C10	15% GOL / naked	Several data sets, the best at 1.9 Å.
ApoT/HoloT	64	Optimzed conditions	15% Gol/15-25% PEG200/ naked	Many data sets but none of them better than 3.5 Å.
XynB2	32	C1	15% GOL / naked	Best at aprox. 2.2 Å..

Table 2. Data collection and refinement statistics.			
Protein / Ligand	PctD-LBD/choline	PctD-LBD/acetylcholine	**PacA-LBD/betaine**
PDB ID.	7PRQ	7PRR	7PSG
Beam Line	ID23-1	Xaloc	ID30A-3
Space group	P 21 21 21	P 21 21 21	P 1 21 1
Unit cell			
a, b, c (Å), ß (°)	56.14, 104.86, 118.60	62.29, 102.65, 104.85	80.78, 83.44, 94.91, 106.86
ASU	2	2	4
Resolution (Å) *	59.3-2.0 (2.07-2.0)	47.48-1.8 (1.86-1.8)	39.99-1.91 (1.98-1.91)
Unique reflections*	47974 (4694)	62195 (6172)	91902 (9315)
Multiplicity*	4.5 (4.5)	4.5 (4.6)	2.9 (3.1)
Completeness (%)*	99.69 (99.62)	98.62 (99.42)	98.01 (98.75)
I/σ_I *	11.25 (1.81)	12.00 (1.32)	10.95 (0.95)
Wilson B-factor	29.90	25.31	38.62
R_{merge} (%)*	0.09827 (1.033)	0.08522 (0.9069)	0.05702 (1.211)
CC(1/2) *	0.998 (0.691)	0.998 (0.626)	0.998 (0.509)
Refinement			
R_{work}/R_{free} (%)	17.0 / 21.7	16.7 / 20.7	18.5 / 22.6
No. atoms	5368	5517	8894
Protein	5005	4984	8443
Ligands	137	192	132
Solvent	312	455	395
B-factor ($Å^2$)	43.95	36.09	53.94
R.m.s deviations			
Bond lengths (Å)	0.013	0.012	0.011
Bond angles (°)	1.26	1.08	1.33
Ramachandran (%)			
Favored (%)	97.24	97.56	97.93
Outliers (%)	0.00	0.00	0.00