ESRF	**Experiment title: Macromolecular Crystallography at South-East Andalusia**	**Experiment number**: MX-2064
Beamline: ID30B	**Date of experiment**: From: 17 November 2018 / 18 November 2018	**Date of report**: 22/08/19
Shifts: 3	**Local contact(s)**: MCCARTHY A.	*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], Julio BACARIZO[4]

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.
4. GBRC, University of Glasgow, UK.

Partial Report of MX2064 ID30B

This partial report corresponds to the third data collection experiment of the Mx2064 carried out at ID230B. We tested 100 samples from the Granada (Table 1) and Almeria (Table 2) teams.

Crystals from Granada (Table 1):

i) Phycocyanin from *Spirulina platensis* (PC). PC is water-soluble phycobiliprotein highly characterized with a characteristic light blue color. We have purified this protein directly from the natural source to use it in our experiments in the design of new materials/supports for protein crystallization, since its color allows us to easily discriminate it in the interior of microcapillary devices. We have obtained different datasets, the best at better resolution to the corresponding structure deposited at the PDB (1HA7) (SG P1211, 1.92 Å; 107.6, 114.7, 182.8, 90 90 90). Whereas the structure is mostly the same, we have identified interesting features in the phycocyanobilin (PCB) groups, suggesting alternative conformations.

Future perspectives: the results obtained allow us to continue using this protein as a model in the design of new devices for protein crystallization.

ii) 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. As in a previous round, we could ascertain clear electronic densities at the catalytic center of this domain, but they do not correspond to the expected molecules.

Future perspectives: Based on new binding experiments showing the necessity of additional amino acids at the C-termini of this enzyme for inhibitor binding, a new construction with a 30-aa C-termini extension has been produced, and we have set-up new crystallization experiments.

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. We have diffracted 3 crystals belonging to the WT enzyme, but all diffracted at poor resolution.

The corresponding manuscript is being written, and the corresponding structures will be deposited soon.

iv) L-amidase from *Pseudomonas* (PseAmid). L-specific amidases are industrially attractive enzymes, due to its potential for the production of optically pure L-amino acids starting from racemic mixtures of amino acid-amides, which are cheap precursors. We have cloned, expressed purified and crystallized the enzyme from *Pseudomonas*. We have solved the structure of the unliganded PseAmid from a dataset collected previously at ID30B in a previous bag proposal (1.8 Å, $P2_12_12_1$, R/Rfree of 18.5%/23.4% respectively). We have soaked different crystals of PseAmid with the product of the reaction. Datasets at up to 2.0 Å were obtained. However, inspection at the catalytic centre of PseAmiD does not show any ligand present.

Future perspectives: Binding experiments are being conducted to ascertain the best ligands to be soaked/co-crystallized. Crystal improvement/optimization is being carried out.

v) LBD-PcaY of *P. putida* F1 (PcaY). PcaY chemoeffectors include for example the non-aromatic quinate and shikimate as well as various aromatics like benzoate, 4-hydroxybenzoate, protocatechuate, vanillate and vanillin. We have obtained our first crystal of the ligand binding domain of PcaY it is apo form to 2.1 Å. Crystals belong to P $2_12_12_1$ space group with unit cell dimension 43.58 68.56 96.43 for a, b and c implying that two monomers are accommodated in the UC. The structure of the apo form has been determined using Arcimboldo (CCP4) from those data (MX1830). Models have been deposited at the PDB IDs. 6S18, 6S1A, 6S33, 6S37, 6S38 and 6S3B.

Future perspectives: Article is in preparation.

Table 1. Data collected by the CSIC-UGR.

Protein	Samples	Conditions	Cryo	Resolution
PC	9	PPP5, PPP7, C12 & C21	0-15% GOL, 20% PEG	Several data sets, the best at 2.0 Å
DapK1	10	A.S. pH 4, 6.	15% GOL	Several data sets, the best at 1.5 Å
PseAmid	7	C27 HR I	15% GOL, 20% PEG	Several data sets, the best at 2.0 Å
HR	3	C4	0-15% GOL	No data set.
PcaY	10	C20	15% GOL+Ligands	Several data sets, the best at 1.5 Å.
GncaSub	11	PPP5, PPP6, PPP7, PPP8, PPP9, C10 & C11	0-20% PEG200	Several data sets, the best at 1.6 Å.

Crystals from Almeria (Table 2):

i) Synthetic construct of GP41 (SC-GP41). Several crystals belonging to different constructions of the SC-GP41 in complex with several high affinity peptides have been obtained. In this beamtime, we brought some crystals of the covNHR-VQ. The crystals were very small (<20 μm) and didn't diffract. Also, we try to improve the quality of the complex covNHR-VQ with CHR C34 the peptide, but the crystals diffracted at low resolution and we didn't collect data.

Future perspectives: To improve the quality of the covNHR-VQ crystals. Also, we are working with new constructions and we plan to measure these crystals soon.

ii) Chimeric constructions of the c-Src. We have cloned several chimeric constructions of the c-Src-SH3 domain where the RT-, n-Src and both loops belonging to this SH3 domain have been interchanged by those present in the homologous Fyn-SH3 domain and the non-homologous Abl-SH3 domain (SF-RT, SF-Src-SF-2X; SA-RT, SA-Src, SA-2X) and viceversa (FS-RT, FS-Src and FS-2X; AS-RT, AS-Src, AS-2X). We have measured crystals obtained at different pHs and in presence and absence of PEG of low molecular weight which has been proven to induce the dimerization of the protein by domain-swapping. Besides, we have measured crystals from SF-SRC chimeras in presence of the chemical denaturant urea at pH 8.0. Previously, we have obtained the structure of these chimeric constructions in presence of urea by growing the crystals in drops with different concentrations of urea. However, these new crystals were obtained by soaking. Although the crystals diffracted at high resolution (~1.5 Å), not molecules of urea were observed, and we concluded that the procedure to obtain the crystals affect to the binding of the denaturant. In addition, we have measured crystals of the chimeric constructions soaked with bromophenol blue in order to study the effect of the dye in the diffraction quality and radiation damage.

Crystals of the C-Src and C-Abl chimeric constructions were low quality and doesn't diffract or diffracted at low resolution.

As part of these studies we are also cloned some nucleation site mutants of these SH3 domains. The E128Q mutant of Fyn diffracted at a resolution of ~2Å. The solution of the structure and analysis is under way.

Future perspectives: These studies are part of the grant BIO2016-78020-R (Ministry of Economy and Competitiveness (MINECO) Spain, 2017-2020). We continue to crystallize new constructions in diverse conditions to study the behaviour of the loops and residues in the protein nucleation site in the propensity of these proteins to form intertwined dimers or/and amyloid fibres. Currently, we are working to improve the procedure to obtain new crystals and improve their quality.

iii) Lysozyme crystals. We have measured 30 crystals of lysozyme (crystallized between pH 5.5-7.5) soaked on presence of the dye bromophenol blue and other related compounds in order to study the binding of dyes to lysozyme and their effect in radiation damage. The analysis of these structures is under way.

Table 2. Data collected by the Almeria team.

PROTEIN	SAMPLES	CONDITIONS	Resolution (Å)	SPACE GROUP/CELL
SC-GP41 (Cov mut)	4/0	20% PEG 6000, 0.1M TRIS/HCl pH 8.0	-	No diffraction
SC-GP41 (Cov VQ free)	2/0	10% PEG 6000, 2.0M NaCl	-	No diffraction
SF-SRC	3/2	2.5 M $(NH_4)_2SO_4$, 0.1M TRIS/HCl pH8.0, 3M Urea	~1.5	P1 211: 45 40 56
FS-SRC	4/4	2.0 M $(NH_4)_2SO_4$, 0.1M sodium acetate pH 5.0	~1.0-1.5	P212121: 28 32 59
SA-SRC	4/1	0.8 M $(NH_4)_2SO_4$, 0.1M sodium acetate pH 5.0	~3	P1
F-1	3/2	3.5M HCOONa, 0.1M sodium acetate pH 4.5	~1.8	C121: 73 45 42
Lysozyme	30/30	0.2M NaCl, 0.1M sodium acetate pH 5.5-0.1M phosphate pH 7.5	~1.5	P212121: 30 56 73 P43212: 77 77 38