ESRF	**Experiment title: Macromolecular Crystallography at South-East Andalusia**	**Experiment number**: MX-1938
Beamline: ID30B	**Date of experiment**: From: 15 Abril 2018 at 09:30 to 16 Abril 2018 at 08:00	**Date of report**: 15/05/2018
Shifts: 3	**Local contact(s)**: **SANTONI Gianluca**	*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 Mx1938 (ID30B):

This up-date report corresponds to the fourth round of data collection at ID30B within the Mx1938 proposal. We brought 100 samples from the team grouped as CSIC-UGR and from the GBRC at Glasgow University. All the samples were tested and the main results summarized below.

Crystals from CSIC-UGR (Table 1):

i) Ancestral-cellulase. In collaboration with a group of the CIC nanoGune we have crystallize a reconstructed ancestral cellulase. The enzyme crystallized with two different chemical cocktails and in two different polymorhps, P 2_1 and P3 2_1 containing 6 and 2 monomers in the ASU respectively. Since the higher resolution data set was obtained in P 2_1, the 3D model has been determined from this data using MR. The PDB will be deposited shortly and the manuscript is in preparation.

Future perspectives: Even though the enzyme was crystallized in the presence of CMC (carboxymethilcellulase), the substrate is not present in the structure. Further attempts to soak/co-crystallize arabinose will be done.

ii) LysR-type transcriptional regulator (Admx) from *rizobacterium plymuthica*. Infections due to multidrug-resistant bacteria represent a major global health challenge. To combat this problem, new antibiotics are urgently needed and some plant-associated bacteria are a promising source. Andrimid (adm) is broad spectrum antibiotic produces by rizobacterium. AdmX is a transcriptional activator of the adm gene cluster that produce andrimid. In collaboration with the EEZ (CSIC) we have crystallized the ligand-binding-region bound to IAA (3-indolacetic acid) and tested in this experimental round but crystal diffracted poorly.

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

iii) L-carbamoylase from *Bacillus stearothermophillus* (BsLCar). L-specific carbamoylases are biotechnological important enzymes allowing the use of the hydantoinase process for the production of different optically pure L-amino acids. Whereas we solved in the past the first structure of an L-carbamoylase (PDB 35NF), we continue trying to obtain a complex of L-carbamoylase with a ligand. Crystals obtained in the presence of L-Met have been tested during this round, but they diffracted poorly.

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

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 during this bag proposal (1.8 Å, $P2_12_12_1$, R/Rfree of 18.5%/23.4% respectively). Substrate-soaked crystals of PseAmid diffracted poorly, so, new crystals are being produced.

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

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 clearly shown in the catalytic cysteine of this enzyme (from structures at atomic resolution obtained previously from datasets collected at the ESRF). After extensive but unsuccessful efforts to try determining its nature, we decided to soak this enzyme with different modification-agents (urea, formamide, acrylamide, hydroxylamine,…), which could support our hypothesis on the modification of this enzyme. Datasets up to 1.3 Å have been collected, thus further improving our previous structures. MR of the different datasets is ongoing to ascertain whether new ligands are bound in the catalytic centre, and some extra soaked-crystals have to be measured.

Future perpectives: Decision to be taken after solving the different datasets collected, but this project is getting to its end.

vi) Choline sulfatase from *Sinorhizobium meliloti* (SmelCOSe). We have previously obtained the structures of the free and liganded forms of SmelCOSe (PDBs 6G5Z and 6G60). However, during preparation of the corresponding manuscript, the unliganded structure has been published. We are thus attempting to solve active-site mutants of the enzyme bound to different ligands, to try to ascertain the ligand binding mode of this promiscous enzyme. We have obtained different full data sets of a C54S mutant bound to a Hepes molecule (SP C121, 2.0 Å R/Rfree of 17.5%/21.8% respectively), which inhibits SmelCOSe activity. Refinement is ongoing.

Future perspectives: New crystals are being produced for soaking experiments.

Table 1. Data collected by the CSIC-UGR team.

Protein	Samples	Conditions	Cryo	Notes
ACellulase	6	C22 & C32	15% GOL, No cryo	5 full datasets, two polymorphs.
AdmX	10	C28	20% GOL	Very poor diffraction.
Thaumatin	2	tartrate	15% GOL	No datasets.
BceAmiF	19	PPP4, PPP7, C28	15% GOL	15 full datasets.
PseAmid	3	C28	15% GOL	Very poor diffraction, no datasets.
SmelColSul	7	C16, C4	15% GOL	4 full datasets.
BsLcar	3	C5	No cryo	No datasets.

Table 2. Data collection and structural refinement statistics of a ancestral Cellulase.

Protein	Ancestral Cellulase
Data collection	
Beam line	ID30B (ESRF)
Space Group	P 1 21 1
Cell dimensions	
a, b, c (Å); ß (º)	51.436, 235.636, 74.562; 90.67
Resolution (Å)	71.08 - 1.45 (1.502 - 1.45)
R_{merge} (%)	6.78 (47.38)
I/σ_I	9.79 (2.35)
Completeness (%)	99.43 (99.29)
Unique reflections	310126 (30944)
Multiplicity	3.3 (3.3)
CC(1/2)	0.997 (0.787)
Refinement	
R_{work}/R_{free} (%)	11.82 / 15.35
CC(work/free)	0.978 / 0.971
No. atoms	17341
Protein	15105
Ligands	156
Water	2080
B-factor (Å^2)	19.71
R.m.s deviations	
Bond lengths (Å)	0.009
Bond angles (º)	1.38
Ramachandran (%)	
Favored	97.77
Allowed	2.23

Crystals from Institute of Infection, Immunity and Inflammation-University of Glasgow (Table3):

ii) Small terminase subunit (TerS) of λ phage in complex with phage inhibitor protein (ppi) from *E.Coli*

Phage small terminase subunit binds to a specific region (pac or cos site) present in the phage DNA, which is specifically cutted by the large terminase subunit (TerL). The DNA-terminase complex then interacts with an empty pro-capsid and DNA translocation begins.

For blocking the packaging of phage DNA, ppi-PICI proteins binds to the phage small terminase subunit, directly interfering with phage DNA packaging.

We have discovered several PICIs in *E.Coli* called EcCICFT073 (ppi1), EcCIppiLike (ppi2) and EcCIEC2733.1 (ppi3) where ppi binds to TerS and modify phage recognition of its cognate *cos* site, affecting its DNA binding activity and affinity.

For characterise these mechanisms we crystallized TerS λ (1-98)/ppi3, TerS λ /ppi2, ppi1 and ppi3 alone.

We obtained the PPi3 crystallographic structure in previously ESRF beamtime's and the structure of the complex TerS λ (1-98)/ ppi3 in Diamond. In this Beamtime, we improved the resolution of both targets to 2.34 Å and 2.07 Å respectively (**Figure 1**). We also got a 2.35 Å dataset of TerS λ /ppi2 in this but we were not able to get the phases to solve the structure.

We also obtained 5 crystals of ppi1 but the diffraction was around 7 Å.

Future perpectives: Improve the crystal quality of ppi1 to get better diffraction and obtain Se-Meth labelled protein crystals of TerS/ppi2 to solve the phase problem and then solve the crystallographic structure of the complex.

Table 3. Data collected by the Institute of Infection, Immunity and Inflammation-University of Glasgow				
Protein	**Samples**	**Conditions**	**Cryo**	**Resolution**
PPi3	15	0.1M Tris pH8.5; 8% Peg 8K	40% Glycerol	2.34 Å
TerS λ (1-98)/ ppi3	15	2M AmSO4;0.1M AcONa pH5	35% Glycerol	2.07 Å
TerS λ /ppi2	15	0.12 M Alcohols, 0.1 M Buffer System 3 8.5 50 % v/v Precipitant Mix 3	40% Glycerol	2.35 Å
Ppi1	5	0.1M Tris pH 8,5, 8% PEG8K	35% Glycerol	7 Å

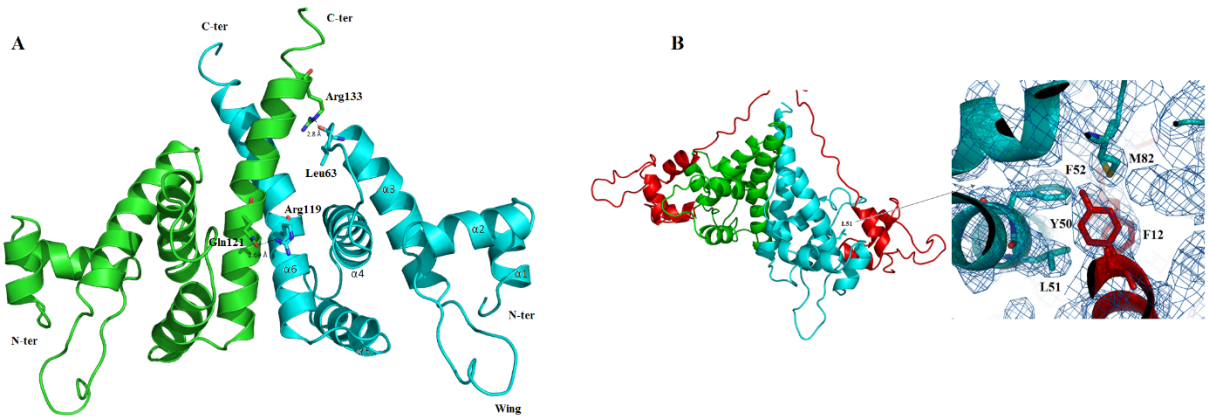

Figure1. Crystallographic structures of ppi3 and TerS λ (1-98)/ ppi3. We show the strongest interactions in the dimer stabilisation of ppi3 (A) and the binding interface of ppi3 with TerS (B).