	Experiment title: Macromolecular Crystallography at South-East Andalusia	**Experiment number**: MX-1938
Beamline: ID23-1	**Date of experiment**: From: 22 June 2018 at 09:30 to 23 June 2018 at 08:00	**Date of report**: 16/10/2018
Shifts: 3	**Local contact(s)**: Nicolas FOOS	*Received at ESRF:*

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Partial Report of Mx1938 (ID23-1 & ID30A-3):

This up-date report corresponds to the fifth round of data collection at ID23-1 & ID30A-3 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 are summarized below. We had problems at ID23-1 and we were relocated at ID30A-3.

Crystals from CSIC-UGR (Table 1):

i) LBD-TlpQ bound to different ligands. TlpQ, a cluster I LBD, is the chemoreceptor responsible for positive chemotaxis to ethylene in some organism. We have already determined the structure of TLPQ-LBD bound to histamine (PDB ID. 6FU4) from Se-met data collected at this beam-line (Table 2). The article has been accepted in mBio and will be published soon. This time we soaked LBD-TlpQ-Hist with potential ligands such as spermidine, putrescine and cadaverine. We tested 10 crystals and the structures will be determined by MR

Future perspectives: This project is finished.

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 the crystals in previous experiments with no succeed. We have optimized the crystals

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

iii) Ancestral-cellulase. We have already determined the structure of this ancestral reconstructed enzyme from data collected at ID30B (PDB UD. 6GJF and Table 2) from the P 2_1 polymorph. This structure did nor shown the ligand CMC and therefore the second polymorph, P3 2_1, was the focus in this experiment. Unfortunately, the structure derived from the two collected data set did not have the CMC in the binding pocket.

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.

iv) Choline sulfatase from *Sinorhizobium meliloti* (SmelCOSe). We have previously obtained the structures of the free and product-bound forms of SmelCOSe (PDBs 6G5Z and 6G60). However, during preparation of the corresponding manuscript, the unliganded structure has been published. We have crystallized a C54S active site mutant and have soaked different crystals with two different substrates. Whereas pNPS soaking resulted

unsuccessful, we have obtained full data sets of the Choline-O-sulphate (substrate)-bound SmelCOSe. Refinement is ongoing; the structure clearly shows the binding mode of SmelCOSe, which is completely different to the hypothesis reported in the previous paper describing the free-structure.

Future perspectives: Manuscript describing for the first time the substrate and product-binding mode of SmelCOSe is in preparation.

v) 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. We have diffracted 3 crystals belonging to the WT enzyme, with a maximum resolution of 2.2 A. These datasets have allowed us finally to obtain the WT structure for this enzyme.

Future perspectives: Final cycles of model building and refinement corresponding to different free and bound structures obtained during the Mx1938 proposal are being carried out. The corresponding structures will be deposited asap, and the corresponding paper will be prepared in the near future.

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

Protein	Samples	Conditions	Cryo	Notes
LBD-TlpQ	10	C14+P, C, E	15% GOL	8 full datasets.
AdmX	11	C18, C11, PPP8 & PPP9	20% GOL	4 full datasets.
Cellulase	4	C32 CSHRII	15% GOL	2 full datasets, the best at 1.6 Å.
C54S	23	C4, C16 & C22	15%, 20% GOL	6 full datasets, the best at 1.6 Å.
HRWT	3	Ammonium Sulphate pH 9.0	15% GOL	2 full datasets, the best at 2.2 Å.

Table 2

Protein	TlpQ-Histamine	A-Cellulase
PDB identifier	**6FU4**	**6GJF**
Data collection		
Beam line	ID23-2 (ESRF)	ID30B (ESRF)
Space Group	P 64	P 1 21 1
Cell dimensions		
a, b, c (Å)	81.62, 103.98, 147.45	51.436 235.636 74.562 90.67
Resolution (Å)	84.98 - 2.45 (2.54 - 2.45)	71.08 - 1.45 (1.502 - 1.45)
R_{merge} (%)	6.2 (36.9)	6.78 (47.38)
I/σ_I	12.5 (2.9)	9.79 (2.35)
Completeness (%)	98.12 (98.06)	99.43 (99.29)
Unique reflections	46027 (4544)	310126 (30944)
Multiplicity	3.9 (4.0)	3.3 (3.3)
CC(1/2)	99.8 (82.6)	0.997 (0.787)
Refinement		
Resolution (Å)	84.98 - 2.45	71.08 - 1.45
R_{work}/R_{free} (%)	19.3/23.7	11.82 / 15.35
B-factor (Å^2)	52.22	19.71
R.m.s deviations		
Bond lengths (Å)	0.005	0.009
Bond angles (^{0})	1.08	1.38
Ramachandran (%)		
Favored	97.86	97.77
Outliers	0.00	2.23

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

Redirecting phage packaging proteins in E.*coli*:

Phage Inducible Chromosomal Islands (PICIS) carry and disseminate bacterial superantigens and other virulence genes, as well antibiotic resistance genes. Following infection by a helper phage, they excise, replicate and are packaged (ERP) in phage-like particles composed of phage virion proteins, leading to very high frequencies of transfer. Two key features of the PICIs are their capacity to interfere with phage reproduction, and their ability to hijack the phage machinery for their own packaging and transfer. One of these elements, EcCICFT073, present

in the E. coli CFT073 strain can be mobilised by the archetypical E. coli lambda and 80 phages. We discovered a new phage interference protein present in EcCICFT073, called Redirecting Phage Packaging Protein (Rpp) and obtained several crystallographic structures. Rpp is responsible of the phage packaging intereference and interacts with the TerSλ of the phage, redirecting the DNA packaging.

In this experiment, we were able to collect several dataset of TerSλ/RppC at 3, 2.8 and 2.2Å of resolution. The TerSλ/RppC crystallographic structure has been deposited in Protein Data Bank (PDB) with the PDB code: **6HNT** and will be send with other two structures and strong *in vivo* supporting data to *Cell* in 2018/2019. We also collected 7 Å resolution data of RppA and one dataset of TerS80/RppC at 1.8 Å resolution (Table 3). Unfortunately, we were not able to solve the structure of TerS80/RppC using MR.

Future perspectives: We will improve RppA crystals to increase the resolution and also get crystals of TerS80/RppC labelled with Se-Meth to perform SAD experiments.

Table 3. Data collected by the Institute of Infection, Immunity and Inflammation-University of Glasgow

Protein	position	Conditions	Cryo	Resolution
TerSλ/RppC	B1x2	2M AmSO4 0.1M AcONa pH 5	25% Glycerol	2.2 Å
TerSλ/RppC	B1x1	2M AmSO4 0.1M AcONa pH 5	25% Glycerol	2.8 Å
TerSλ/RppC	B1x3	2M AmSO4 0.1M AcONa pH 5	25% Glycerol	3.0Å
TerS80/RppC	B1x8	0.09 M NPS 0.1 M Buffer System 3 8.5 50 % v/v Precipitant Mix 1	30% Glycerol	1.8 Å
RppA	B5x7	0.1M Tris pH8.5 8%Peg 8K	25% Glycerol	7 Å

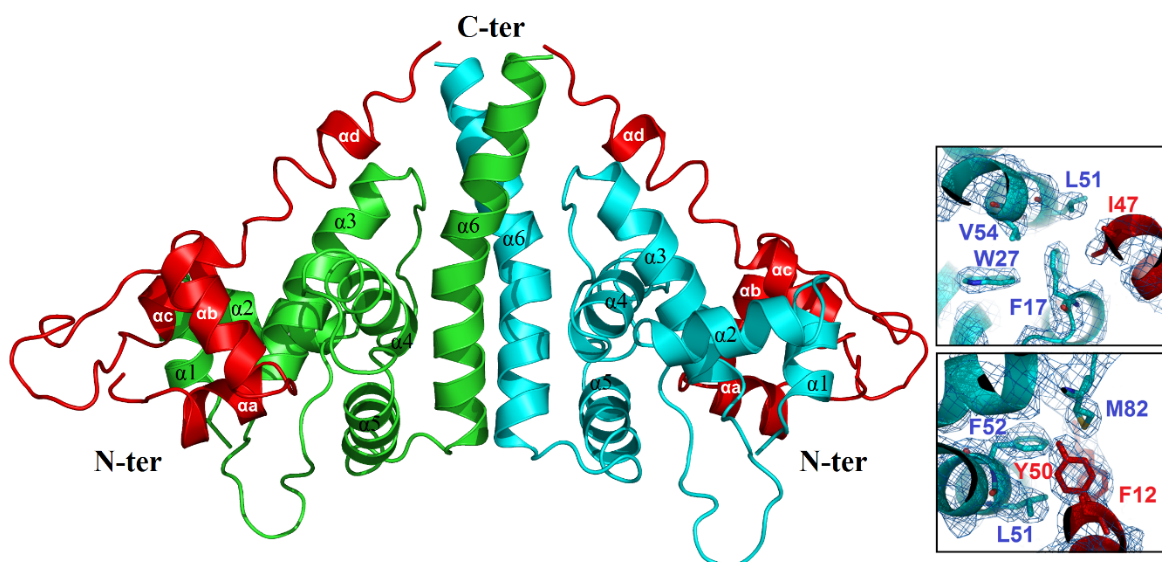

Figure 1. Crystallographic structure of TerS λ (1-98) in complex with RppC. Cartoon representation of RppC where chain A is coloured in green, B in cyan, and the TerS λ (1-98) is coloured in red (C, D). Electron Density Map (EDM) containing important residues involved in the heterodimer binding