ESRF	**Experiment title: Macromolecular Crystallography at South-East Andalusia**	**Experiment number**: MX-1830
Beamline: ID30A-3 ID29	**Date of experiment**: From: 18 June 2017 at 09:30 to 19 June 2017 at 08:00	**Date of report**: 14/08/17
Shifts: 3	**Local contact(s)**: VON STETTEN David	*Received at ESRF:*

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Partial Report of Mx1830 ID30A-3:

This up-date report corresponds to the data collected at ID30A-3 during the fifth round of Mx1830. We brought 100 samples from the team grouped as CSIC-UGR and from our recently incorporated Bag-member from the GBRC at Glasgow University. Due to some troubles at ID30A-3 our local contact found free ID29, used for this run. All the samples were tested and the main results are listed below, summarized in Table 1 and 2, respectively.

Crystals from CSIC-UGR:

i) LBD-PcaY bound to histamine. PcaY of *P. putida* F1, is chemotactic sensor that responds to a number of C6-ring containing carboxylic acids. 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 measured the first crystals of this LBD in a previous run. We have tried to get crystals of the complex with its natural ligands but once more only the apo form produced good looking crystals assayed in this run. We have tested 8 crystals and collected two data sets, the best at 2.3 Å.

Future perpectives: Co-crystallization and soaking with other ligands is on going.

ii) LBD-TlpQ bound to histamine. TlpQ, a cluster I LBD, is the chemoreceptor responsible for positive chemotaxis to ethylene in some organism. As for McpU, the structure has been determined from data obtained in the previous run of MX1830 and refinement is on-going (2.45 Å). A different approach to crystallize this LBD was tested using the single capillary 3L configuration of the counterdiffusion technique. Five crystals from the 3L configuration and 13 from standard Capillary CD were tested in this run and five data sets were collected being the best diffracting to approximately 2.5 Å.

Future perpectives: As already mentioned, the structure has been determined and refinement is on going to 2.45 Å resolution. This project may be finalized although further assays to improve crystal quality may be tested.

iii) Hydantoin racemase from Ensifer meliloti: We already crystallized and collected data of this enzyme at ESRF [1]. Although the structure was determined from those data, the lack of density at a loop containing the active site precluded us to deposit and publish this structure. This project has been

recovered and new crystals were produced corresponding to an active site-mutant, and tested during this run. Unfortunately, none of them diffracted properly and therefore we did not collect any data set.

[1] Martinez-Rodriguez et al., Acta Cryst. (2008). F64, 50–53

Future perpectives: Improvement of crystal quality is on going.

iv) Ancestral lactamase GNCA02-S70A. Following our (unsuccessful) data collection of ancestral GNCA lactamase bound to different substrate/inhibitors we have soaked the variant GNCA02-S70A with different lactamase substrates, since we were not able yet to obtain a lactam-bound structure. From the previous data-collection we were able to identified Penicillin G bound to GNCA02-S70A. This time we tested 16 crystals soaked with four different substrate/inhibitors.

Due to the success with this variant, future work is centred in co-crystallizing the GNCA02-S70A variant with different substrate/inhibitors to shed some light on the promiscuity found with ancestral lactamases.

Table 1. Data collected by the CSIC-UGR.

Protein	Samples	Conditions	Cryo	Notes (max. resolution)
PcaY-LBD	8	C4, C7 & C20	0 - 15% GOL	Two full data sets of the apo form, the best diffracting at 2.3 Å.
HR	10	C4 and Tartrate	0- 15% GOL	No collection.
TlpQ-LBD	16	C14	10-15% GOL	Five full data sets in complex with histamine, the best diffracting at 2.5 Å.
GNCA02-S70A	16	PPP pH 4, 5, 7, 8 & 9	15% GOL	Seven full data sets collected, the best dataset at approx. 1.5 Å.

Crystals from GBRC:

The phage-inducible chromosomal islands (PICIs) are a family of highly mobile genetic elements (MGE) that contribute to horizontal gene transfer, host adaptation, and virulence. They are molecular parasites that exploit phages as helpers, using a variety of elegant strategies to manipulate the phage life cycle for promote their own spread. Phages can drive evolution of bacterial pathogens by serving as vehicles for the transfer of fragments of chromosomal DNA and other mobile genetic elements, a process called transduction.

Following infection by a helper phage the PICI genome excises, replicates using its own replicon, and is efficiently packaged into infectious particles composed of phage virion proteins (Figure 1).

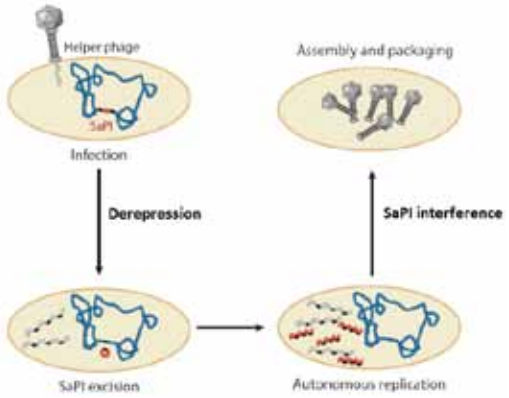

Figure 1

Staphylococcus aureus pathogenicity islands (SaPIs) are the prototype members of the family and contain four distinct modules that mediate their regulation, excision and integration, autonomous replication and phage packaging exploitation (ERP cycle). For study the ERP cycle in PICIs and SaPIS we have crystallized several protein complexes:

i) STL repressor of SaPI1 in complex with antirepressor 80α Sri.

After infection by a helper phage, a phage anti-repressor protein relieves Stl-mediated repression of the SaPI, initiating the ERP cycle. We obtained several crystals of the antirepressor Sri (80α) in complex with STL (SaPI1) to characherize the de-repression mechanism. In this round, we tested 20 crystals with different cryoprotectants and precipitants and collected one data set at 3.72 Å, belonging to the P 6_1 2 2 space group. Sri has a phage inhibitor protein homolog crystallized before (PDB code: 5HE9) and STL shares around 42% of homology with others Helix Turn Helix (HTH) domains (PDB:1YQ9) that will help to obtain a MR solution.

Future perpectives: Desition to be taken after MR attempts and improving crystals quality is on-going.

ii) Small terminase subunit (TerS) in complex with phage inhibitor protein (ppi).

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 the empty pro-capsid and DNA translocation begins.

For blocking the packaging of phage DNA, ppi-SaPI protein binds to the phage small terminase subunit, directly interfering with phage DNA packaging:

- ppi prevents interaction of phage TerS with TerL introducing its own TerS as it happens in TerS(80α)/ppi of SapIbov2.

-ppi modify phage TerS recognition of its cognate pac site, affecting its DNA binding activity and affinity as it happens in TerSλ/ppi *E.Coli*.

For characterise these mechanisms we crystallized both complexes and TerS alone. Crystals appear in different precipitant conditions and we tested different cryoprotectants resulting in an initial diffraction with maximum resolutions around 5-10 Å in two datasets (table1). We realized which precipitant agents works better and that MPD helps to increase the diffraction quality. NMR structure of TerS (λ phage) is available (PDB code:1J9I) and ppi share a 30-40% of homology with different crystallographic structures (PDB code: 3E4W, 3E4Y..) which will help to obtain a MR solution.

Future perpectives: Improving crystals quality is on-going.

Table 2. Data collected by the Institute of Infection, Immunity and Inflammation-University of Glasgow

Protein	Samples	Conditions	Cryo	Resolution
STL/Sri	20	41SC1, 6SC1, 12PP1, 33SC2	35% PEG200, 30% PEG 400	1 data set at 3.72 Å
TerSλ/ppi *E.Coli*	16	39SC1	40% PEG200, 10% MPD	Not collected data but diffraction observed to 5 Å.
TerSλ	6	24SC1, 36SC1	20-40% PEG 400 5%MPD 20%GOL	No data set.
Terϕ80α/ppi Sb2	8	45SC2, 12PP1	40% PEG400	Not collected data but diffraction observed to 9 Å.