ESRF	**Experiment title: Macromolecular Crystallography at South-East Andalusia**	**Experiment number**: MX-1938
Beamline: ID30A-3	**Date of experiment**: From: 02 December 2017 at 09:30 to 03 October 2017 at 08:00	**Date of report**: 07/02/18
Shifts: 3	**Local contact(s)**: SOLER LOPEZ Montserrat	*Received at ESRF:*

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Partial Report of Mx1938 ID30A-3:

This up-date report corresponds to the second round of data collection at ID30A-3 within the Mx1938 proposal. We brought 100 samples from the team grouped as CSIC-UGR and from our recently incorporated Bag-member from the GBRC at Glasgow University. All the samples were tested and the main results summarized below (Tables 1 and 2).

Crystals from CSIC-UGR:

i) LBD-McpH bounded to several ligands. McpH is a chemoreceptor from *P. putida* that specifically recognizes purine and its derivatives, adenine, guanine, xanthine, hypoxanthine and uric acid. The latter five compounds form part of the purine degradation pathway, permitting their use as sole nitrogen sources. We have cloned purified and crystallized the ligand-binding region (LBD) of McpH. In previous experiment we got diffraction data at 2.7 Å resolution but did not find MR solution. We have produced the Se-Met derivatives and obtained crystals in different conditions. Right now, we are improving crystal quality prior MAD/SAD data collection. We tested ten crystals grown in ammonium sulphate with different cryo-protectant solutions. Any of them diffracted X-ray at a reasonable resolution to collect data.

Future perpectives: Further crystals improvements is required.

ii) Structural determination of Pseudomonas chemotactic transducer A, B and C: We have produced crystals of PctA, PctB and PctC pre-incubated with several of their natural ligands (manuscript in preparation). Crystallization with other amino acids has fail and therefore we decide to use PctA-Ile and PctA-Tpr crystals to soak other amino acids within the capillaries. A full data set of PctA-Ile soaked with methionine was collected at 2.0 Å resolution (Mx1549) and the structure solved. Following our previous experiment with methionine, we have produce crystals of PctA bounded to Ile and Trp and soaked with a mix of different amino acids. This approach may allow us not only to get other 3D models but also to stablish a feasible protocol to soak different ligands using the benefits of working under diffusion mass transport regime. Here we bring three crystals of PctA-Ile, soaked with a mixt of three other aminoacids. We have collected four data sets and the structure determination is on-going.

Future perpectives: Different aproaches to test this methodology are planed.

iii) Hydantoin racemase from Ensifer meliloti (HR): We already crystallized and collected data of this enzyme at ESRF [1] but the lack of density at the active site precluded us to deposit and publish this model. We have obtained crystals of different mutants which are being tested. In this opportunity we bring 30 crystals from different conditions. We collected 12 full data sets from different conditions. Fortunately, we obtained a new crystal form C2, for which the active site is traceable and therefore will allow us to get a good 3D model at 2.5 Å resolution.

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

Future perspectives: To obtain different complexes at the binding site.

iv) LBD-McpU bound to several ligands. McpU is a chemoreceptor that contributes to the formation of biofilm in *Pseudomonas putida*. We have produced and crystallized the Se-Met derivative bound to putrescine and solve the structure from data collected at ID23-1 (PDB ID. 6F9G). The manuscript has been submitted to JMB. In this round, we tested seven remaining crystals searching for better resolution but any of them were good enough.

[2] Gavira, J.A., et al "Structural basis for polyamine recognition at bacterial chemoreceptor" submitted to JMB.

Future perspectives: This project is finished.

Table 1. Data collected by the CSIC-UGR.

Protein	Samples	Conditions	Cryo	Notes (max. resolution)
McpH	10	AS pH 6.0	0 - 20% GOL	0 full data sets.
McpU	7	PPP6	0 - 15% GOL	0 full data sets.
HR	30	C10, C16, AS pH 5.0.	15% GOL	12 data sets.
PctA	3	C22	15% GOL	4 Data sets.

Crystals from GBRC: Crystals from Institute of Infection, Immunity and Inflammation-University of Glasgow:

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 have obtained several crystals of the antirepressor Sri (80α) in complex with STL (SaPI1) to characherize the de-repression mechanism. In this round, we tested 40 crystals with optimized cryoprotectants and precipitants and collected several native data sets between 3.19-2.85Å of maximum resolution. After this beamtime, we have been purifying the STL/SRI complex using a Se-Meth labelling method from *Molecular Dimensions* and we got a model from Single Anomalous diffraction (SAD) phasing method. Running Molecular replacement with the native B1x6 dataset (Table 2) using the SRI/STL-SeMeth model we obtained a unique solution (Top LLG 5000, Top TFZ 74.1) belonging to the space group $P6_122$ (**Figure1**).

Future perpectives: Now that we have a good model for MR we will increase the quality of the crystals to get better resolution in next rounds.

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

Protein	position	Conditions	Cryo	Resolution
STL/Sri	B2x8	0.1M AcONa pH4.6 8%PEG4K	25%PEG200	3.02 Å
STL/Sri	B1x6	0.1M AcONa pH4.6 8%PEG4K	25%PEG200	2.85 Å
STL/Sri	B1x4	0.1M AcONa pH4.6 8%PEG4K	25%PEG200	3.02 Å
STL/Sri	B1x3	0.1M AcONa pH4.6 8%PEG4K	25%PEG200	3.19 Å

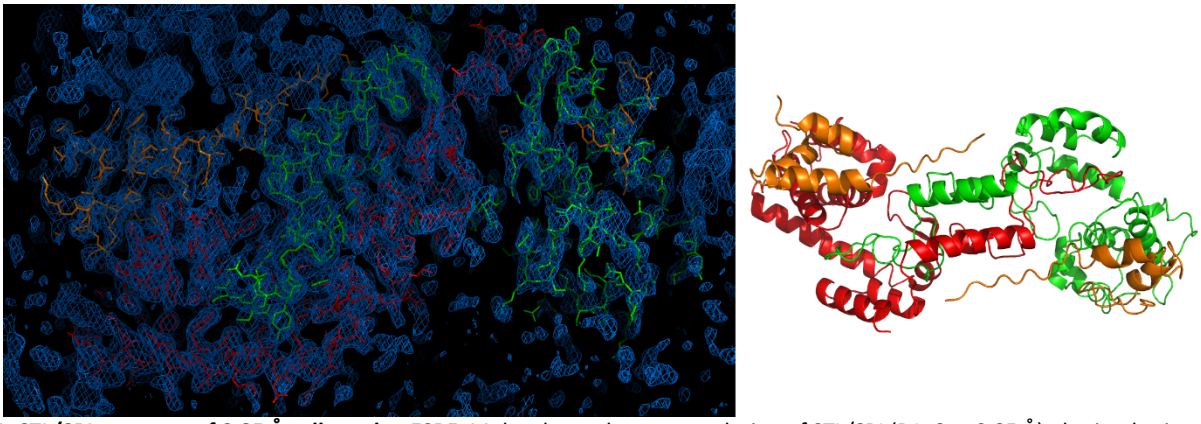

Figure1. STL/SRI structure of 2.85 Å collected at ESRF. Molecular replacement solution of STL/SRI (B1x6 at 2.85 Å) obtained using a model from SAD phasing method is shown. STL/Sri crystallographic structure consists in a dimer formed by 2 molecules of STL (Green and Red) and 2 molecules of Sri interacting in the DNA Binding domain (DBD) of STL (Orange).