	Experiment title: Macromolecular Crystallography at South-East Andalusia	Experiment number: MX-1830
Beamline: ID23-1	**Date of experiment**: From: 11 February 2017 at 09:30 to 12 February 2017 at 08:00	**Date of report**: 07/05/17
Shifts: 3	**Local contact(s)**: Max Nanao	*Received at ESRF:*

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Partial Report of Mx11830 ID23-1 (11-02-2017 / 12-02-2017):

This up-date report corresponds to the data collected at ID23-1 during the third round of Mx1830. We brought 60 samples from the CSIC. All the samples were tested and the main results are listed below.

Crystals from CSIC:

i) LBD-TlpQ bound to histamine. TlpQ, a cluster I LBD, is the chemoreceptor responsible for positive chemotaxis to ethylene in some organism. The amino acid sequences of TlpQ and McpA are 73% and 70% identical, respectively, to the highly conserved domain of the *E. coli* chemotaxis transducer Tsr. Other *P. aeruginosa* strains including PA7, PA14, and 2192 possess TlpQ homologs. The sequence of the TlpQ-LBD is 29–57% identical to the N-terminal regions of MCPs from other pseudomonads, like *P. putida* strains KT2440 and F1, or *P. fluorescens* Pf-5 and PfO-1. We have obtained crystals in complex with histamine, and collected data to 2.4 Å but all attempts to solved the structure by MR failed. Therefore, we produced the SeMet derivative protein and crystallized it. In this round, we collect both MAD and high redundant SAD data from several crystals. We were able to obtain phases by merging several high redundant data set by SAD method to 3.4 Å.

Future perpectives: The structure has been determined and refinement is on going to 2.45 Å resolution. This project may be finalized.

ii) 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 obtained our first crystal of the ligand binding domain of PcaY it is apo form and in complex with one of its identified ligand, protecatechuic acid. Only crystals of the apo form diffracted well producing several data sets, the best to 2.1 Å (Table 1.). Crystals belong to the P222 (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. Structual determination is being attempt by MR.

Future perpectives: Desition to be taken after MR attempts. Co-crystallization and soaking with other ligands will be assayed.

iii) 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 cadaverine, one of its identified natural ligands. In this round, we tested 20 crystals and collected several MAD data sets and high redundancy data sets at the peak of Se (Table 1). Phases were obtained aster merging six data sets and the refinement is in progress at 2.4 Å. Current R and Rfree values are 20 and 26% respectively (Table 2).

<u>Future perpectives</u>: The structure has been determined and refinement is on going to 2.4 Å resolution. This project may also be finalized.

Table 1. Data collected by the CSIC-UGR.				
Protein	**Samples**	**Conditions**	**Cryo**	**Resolution**
TlpQ-LBD	20	C14	15% GOL	Several data sets, the best at 2.6 Å.
PcaY-LBD	20	C6 / C7 / C20	0- 15% GOL	Seven data sets, the best at 2.1 Å.
McpU-LBD	20	C2/ PPP7 / PPP6	0- 15% GOL	Several data sets, the best at 2.4 Å.

Table 2. Data collection and refinement statistics of McpU/Cad.	
Resolution range (Å)	80.43 - 2.329 (2.412 - 2.329)
Space group	C 2 2 21
Unit cell	135.318 165.242 125.636 90 90 90
Unique reflections	60055 (5623)
Multiplicity	15.1 (13.3)
Completeness (%)	99.42 (94.50)
Rsym (%)	0.3866 (2.064)
Mean I/sigma(I)	10.22 (2.05)
Wilson B-factor	35.68
CC (1/2)	0.984 (0.651)
Refinement	
R-work	0.2061 (0.3069)
R-free	0.2671 (0.3747)
CC (work)	0.953 (0.780)

Statistics for the highest-resolution shell are shown in parentheses.