ESRF	**Experiment title: Macromolecular Crystallography at South-East Andalusia**	**Experiment number**: MX-1739
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Shifts: 3	**Local contact(s)**: SOLER LOPEZ Montserrat	*Received at ESRF:*

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Partial Report of Mx/1739 ID23-2 (11-06-2016 / 12-06-2016):

We brought 100 samples from CSIC. All the samples were tested and the main results and future actions are listed below.

Crystals from CSIC:

i) LBD-McpU bounded to several ligands. McpU is a chemoreceptor that contributed to the formation of biofilm in *Pseudomonas putida.* Besides the effort put on crystal improvement, we have also produce the SeMet derivatives and produce crystals from them. We have tested those McpU-SeMet crystals bounded to one of its natural ligand, putrescine. Unfortunately, all the crystals diffracted X-ray below 4 Å.

Future perpectives: Further crystals improvements is on going with McpU-SeMet.

ii) 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. In this experiment we tested the remaining crystals stored at home. Crystals did not diffract better than 2.8 Å.

Future perpectives: Further crystals improvements is required.

iii) 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. Preliminary results from ID14-4 have already been pubished [1] but improved diffraction quality for other protein-ligands complexes are undergoing. We have collected data to high resolution of the PctA-Ile ($P2_12_12_1$: 70.28 77.04 115.676, to 2.15 Å and P61: 132.50, 132.50, 76.97 to 2.2 Å) and PctA-Trp ($P2_12_12_1$ to 2.25 Å) and in the case of PctB-Arg ($P3_121$: 111.6, 111.6, 117.5 to 3.1 Å) and PctB-Gln (same SG to 3.5 Å) and PctC-GABA ($P3_121$: 78.20, 78.20, 69.13 to 2.1 Å) (Mx1406/Mx1541). 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 set 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 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.

Future perpectives: The follow-up of this methodology will depend on the results obtained from those data.

iv) Protein crystallisation in short peptides hydrogels. Following our previous studies on the use of short peptide supramolecular hydrogels for the crystallization of biological macromolecules [1-2] we are producing

doped gel with nanotubes to be incorporated within the protein crystals. We are already proven that nanotubes are incorporated and in this study crystal quality will be evaluated as a function of crystals composition.
Future perpectives: We are preparing similar protein crystals composites of protein with redox properties for which full characterization will be required.

Table 1. Resume of data collected by the CSIC.

Protein	Samples	Conditions	Cryo	Resolution
McpU-Put	20	C-3 30%PEG 4K, 0.2M NH4 Acetate, 0.1M Na-Acetate pH 4.60 PPP: 20% PEG 400, 15% PEG 4K, 10% PEG 8K, pH 5.0 to pH 9.0	0-15% Glycerol	Poor diffraction in all the cases. Crystals are too old.
McpH-Uric	20	PEG Ion/AS pH 6 and 7	15-20% Glycerol	Several data sets from two crystals. The best set to a limit of resolution of 2.8 Å
PctA-X	40	Mix of amino acids.	15% Glycerol	Several data sets ranging from 2.2 to 3.5 Å resolutions.
Lysozyme/Glucose Ism.	20	Short peptide hydrogels and agarose doped with single and multiple walls nanotubes.	15% Glycerol	12 Data set at fixed configuration [2].

1. Conejero-Muriel, M.; Gavira, J. A.; Pineda-Molina, E.; Belsom, A.; Bradley, M.; Moral, M.; Duran Jde, D.; Luque Gonzalez, A.; Diaz-Mochon, J. J.; Contreras-Montoya, R.; Martinez-Peragon, A.; Cuerva, J. M.; Alvarez de Cienfuegos, L., Influence of the chirality of short peptide supramolecular hydrogels in protein crystallogenesis. *Chemical communications* **2015,** *51* (18), 3862-5.
2. Conejero-Muriel, M.; Contreras-Montoya, R.; Díaz-Mochón, J. J.; Álvarez de Cienfuegos, L.; Gavira, J. A., Protein crystallization in short-peptide supramolecular hydrogels: a versatile strategy towards biotechnological composite materials. *CrystEngComm* **2015**.