ESRF	**Experiment title: Macromolecular Crystallography at South-East Andalusia**	**Experiment number**: MX-1938
Beamline: ID23-1	**Date of experiment**: From: 21 October 2017 at 09:30 to 22 October 2017 at 08:00	**Date of report**: 21/11/17
Shifts: 3	**Local contact(s)**: SOLER LOPEZ Montserrat	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists): Jose A. GAVIRA-GALLARDO*[1], Ana CAMARA-ARTIGAS[2], Sergio MARTINEZ-RODRIGUEZ*[3], Mª Teresa CONEJERO-MURIEL[1], FERNANDEZ Raquel[1], LOPEZ Carmen[1], Julio BACARIZO*[4]

1. Laboratorio de Estudios Cristalográficos, IACT, CSIC-UGR, Spain.
2. Dto. Química y Física, University of Almeria, Spain.
3. Dto. Química Física, University of Granada, Spain.
4. GBRC, University of Glasgow, UK.

Partial Report of Mx1938 ID23-1:

This up-date report corresponds to the first round of data collection at ID23-1 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-McpN bound to its natural ligand. We have identified a new chemoreceptor in *P. aeruginosa* PAO1, which specifically recognizes nitrate [1]. We have cloned, overexpressed and purified the ligand binding domain (LBD) of McpN. Crystals were obtained in the presence of its natural ligand, nitrate, using the capillary counter-diffusion technique (Figure 1). Crystals were extracted from the capillary and directly flash-cooled or cryo-preserved with 15% prior the flash-cooling. We tested 12 crystals and collected 10 datasets, the best at 1.5 Å. We attempted to solve the structure by MR but so far all attempts failed.

1. Á. Ortega, I. B. Zhulin and T. Krell, *Microbiology and Molecular Biology Reviews*, 2017, 81, 1-28. DOI. 10.1128/MMBR.00033-17.

Future perpectives: Se-met derivatives are being prepared.

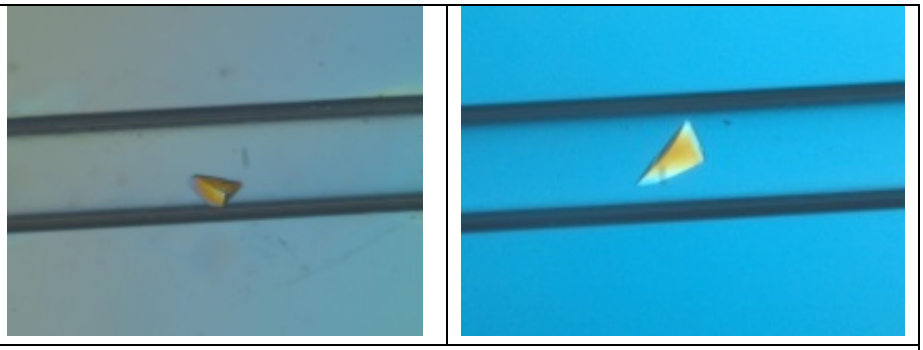

Figure 1. Crystals of McpN-LBD obtained in capillaries of 0.2 mm inner diameter using the capillary counterdiffusion technique.

ii) Formamidase from *Bacillus cereus*. This enzyme has proved very efficient for the biosynthesis of acetohydroxamic acid (lithostat), and was used as a model to study the presence of a catalytic C-E-E-K tetrad instead of the long-stablished C-E-K triad in the nitrilase superfamily. We have obtained crystals of free and liganded forms of this enzyme. We already collected data at acidic pH values at XALOC beamline, ALBA (Barcelona, Spain) to a resolution of 1.73 Å. Crystal have also been grown at a wide range of conditions and pH. The corresponding structures will be used to get insights into the enzymatic "ping-pong" mechanism. In all cases the cysteine at the active center appears modified with which looks like an acetylation. In order to descipher if this could be a radiation damage effect, we have collected many data sets with different crystals. After solving different structures, we continue observing the modified cysteine, and we have improved the resolution of the previous structure to 1.3 Å by using the counter diffusion technique. We have also solved a new structure bound to the substrate of the reaction, formaide.

Future perpectives: Together with new MS experiments, we are preparing the corresponding manuscript with the previous and the new structures.

iii) Lysozyme: Ten crystals from the model protein lysozyme were obtained as modification result of the precipitant composition following the Hofmeister series [2]. This data are part of an studend Master degree Thesis in which the influence of this serie over crystals packing, polymorhpism, and resolution limit are been studied. We collected 11 data sets, several at very high resolution.

2. Ries-Kautt MM & Ducruix AF (1989) Relative effectiveness of various ions on the solubility and crystal growth of lysozyme. *J Biol Chem* 264(2):745-748.

Future perpectives: No further experiments resquired.

Table 1. Data collected by the CSIC-UGR.

Protein	Samples	Conditions	Cryo	Notes (max. resolution)
McpN	12	C23, C27	0 - 15% GOL	10 data sets, the best diffracting at 1.5 Å from C23 without glycerol.
BceAmif	33	CCC4 + formamide /formate	15% GOL	25 data sets.
Lzm	10	CsCl, KNO_3, NaF, $MgCl_2$, Na_2HPO_4, KI & $CaCl_2$	0 - 15% GOL	11 full data sets.

Crystals from GBRC:

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. In previous ESRF beamtime, we checked the diffraction obtained of several antirepressor Sri (80α phage) in complex with STL (SaPI1) crystals to characherize the de-repression mechanism, producing 3.7 Å of maximum resolution. In this round, we tested 30 optimized crystals with different optimized cryoprotectants, collecting several data sets improving resolution up to 2.6 Å (**Table 2**), belonging to the P 6_1 2 2 space group and unit cell constats: a=99.1, b=99.1, c=297.8, α=90, β=90, γ=120. The estimation of molecules for the AU is 2 complexes with 50% of solvent in it.

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 could help to obtain a MR solution. We have tried to solve the structure with the homology models available but the resulting electron density maps (EDM) are still not very accurate (**Figure 2**).

Future perpectives: We get now better crystals of the complex that probably will give to us better resolution and the purification with Se-Met kit from Molecular Dimensions is on going to obtain phases using SAD or SAD+MR.

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

Protein	Sample	Conditions	Cryo	Resolution
STL-Sri	B1X1	0.4M Ammonium Phosphate monobasic	30% PEG 200	2.8 Å
STL-Sri	B1X3	0.4M Ammonium Phosphate monobasic	30% MPD	3.8 Å
STL-Sri	B1X4	0.4M Ammonium Phosphate monobasic	20% glycerol	4.0 Å
STL-Sri	B1X6	0.1M AcONa pH4.6 8% PEG 4K	25% PEG 200	2.6 Å

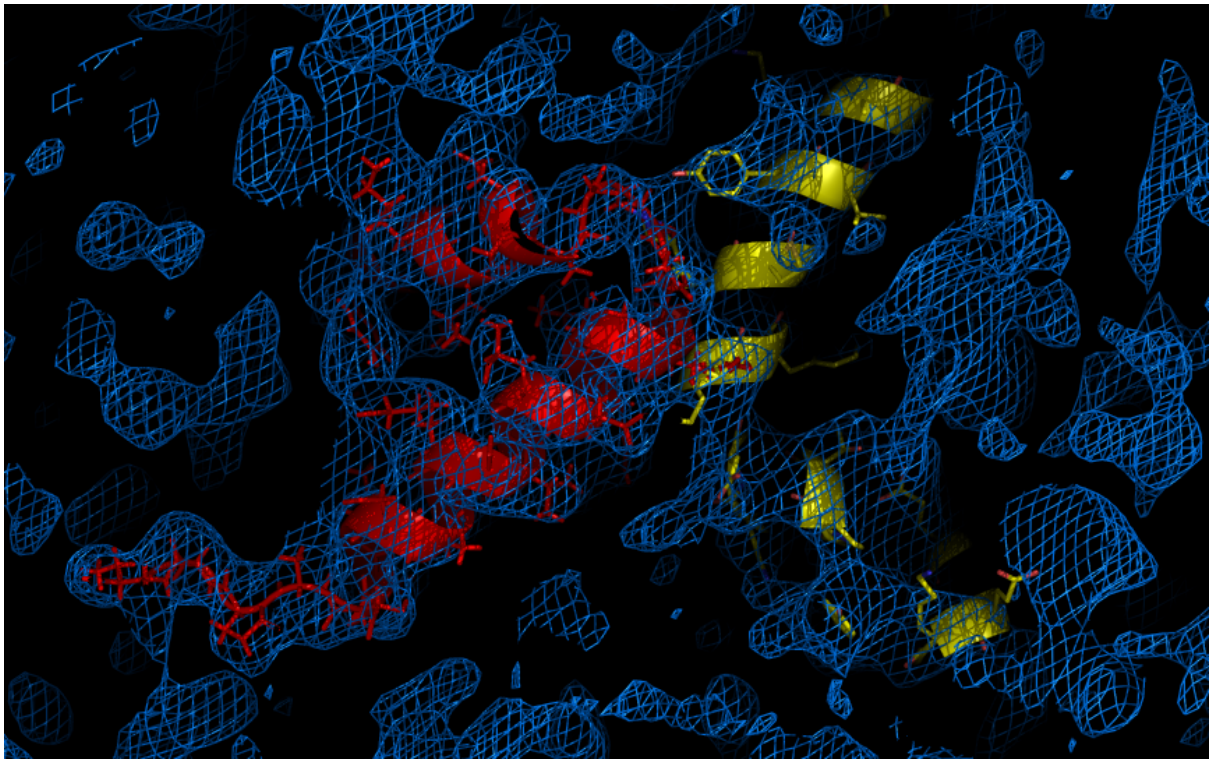

Figure 2. EDM of complex Sri(red)/STL(yellow).