ESRF	**Experiment title: Macromolecular Crystallography at South-East Andalusia**	**Experiment number**: MX-1629
Beamline: ID23-1	**Date of experiment**: From: 22 November 2014 to: 23 November 2014	**Date of report**: 22/01/15
Shifts: 3	**Local contact(s)**: POPOV Alexander	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists): Jose A. GAVIRA-GALLARDO*[1], Ana CAMARA-ARTIGAS*[2], Sergio MARTINEZ-RODRIGUEZ[3], Julio, BACARIZO-ROA*[2], Duane CHOQUESILLO-LAZARTE[1], Mayte CONEJERO-MURIEL*[1]

1. Laboratorio de Estudios Cristalograficos, IACT, CSIC-UGR, Spain.
2. Dto. Química y Fisica, University of Almeria, Spain.
3. Dto. Química Fisica, University of Granada, Spain.

Partial Report of Mx ID23-1 (22-11-2014 / 23-11-2014):

This is up-dated report of the data collected at ID23-1 (+ID23-2, +ID29) during the first round of MX-1629. We brought to the ESRF 110 samples from the teams CSIC-UGR and UAL. All the samples were tested and the main results are listed below.

The experiment at ID23-1 was satisfactory but since ID23-2 was not occupied in the morning we asked to our local contact to have access to the beamline. We collected data at this beamline until the designed users arrived. This allow us to expend some time to learn the new software implemented in the line to test the best spot to collect data from the crystals and take advantage of the new feature. In the afternoon we learned that ID29 was available and asked the Control Room permission to use it. This allowed us to perform a more effective collection of the data and to finish the full collection of the 110 crystals before 3 AM. Also to collect data at ID-29 allowed us to overlook the problem of basket-jams at ID23-1, which was solved following the protocol.

Crystals from CSIC-UGR (Granada):

i) Structural determination of Pseudomonas chemotactic transducer A, B and C (PctA, B, C).

The structure of PctA bound to Ile and Trp have already been solved and deposited at the PDB (ID 4CU3 and 3D27, respectively). However, as mentioned in previous reports, crystallization with other amino acids has fail. We were able to obtain the PctA-Met by soaking PctA-Ile crystals with methionine (data collected at ID23-1, MX-1541) and 3D model is currently under refinement (R/Rfree=0.19/0.24). Following this strategy, crystals of the PctA-Trp complex were soaked also with methionine and asparagine. We collected one data set of the PctA-Trp/Ans crystal that diffracted X-ray to a resolution ~ 4 Å but these data are useless to solve the structure.

New crystal of PctB and PctC are been produce from new constructs.

iii) 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 enzymatic "ping-pong" mechanisms. Different polymorph were obtained at extreme pH values, wich also affect crystal quality and resolution limit, i.e. at pH 4.5 and pH 9.0 crystal diffracts at 1.8 Å and 3.4 Å, respectively. At this time we try to obtain data at intermediate pH. We tested crystals grown at pH 5 and 6 with no succed.

ii) Ancestral Lactamases. We plan to use a minimalist design to introduce the novo activity in a resurrected ancestral lactamase. Therefore we have mutate ancestral GNCA lactamase achiving activity levels similar to the best rational design in the literature. Two mutants, one single (W2O4D) and one double (P203A/W204D), have been crystallized and the data collected during this sesion. In both cases two crystals forms (*P*21212 and *I*41) were

identified in each capillary (Fig. 1) and full data sets have been collected to solve the structures of the two polymorphs by MR.

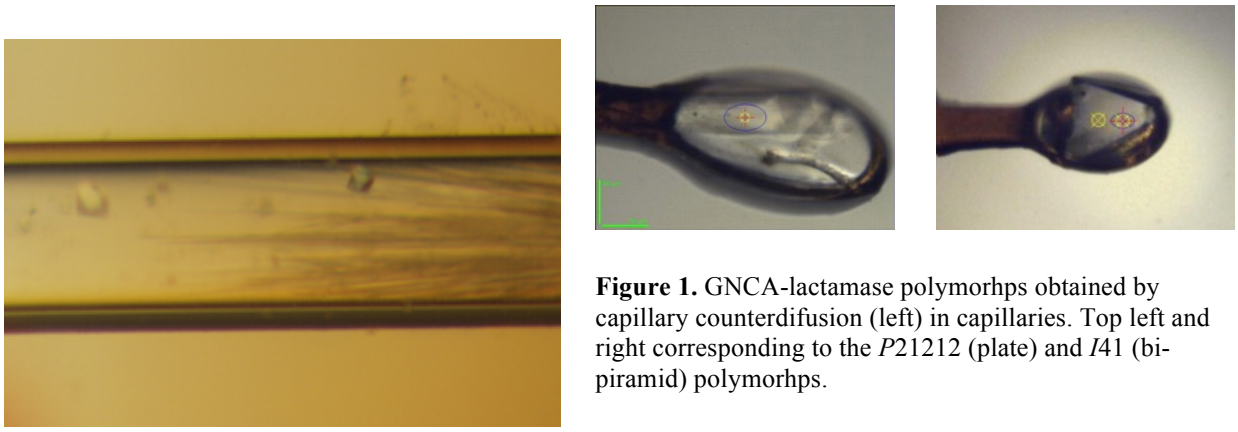


Figure 1. GNCA-lactamase polymorhps obtained by capillary counterdifusion (left) in capillaries. Top left and right corresponding to the *P*21212 (plate) and *I*41 (bi-piramid) polymorhps.

We are now producing crystal of the single mutant bound to an inhibitor analogue to gain inside into the mechanism of this the novo activity.

iv) LBD-McpU bounded to several ligands. McpU is a chemoreceptor that contributed to the formation of biofilm in *Pseudomonas putida*. We have crystallised the ligand-binding domain of this receptor in complex with several of its natural ligands (different amines present in the natural habitat of this bacterium) but so far data are of low quality and has not yet been used to solve the structure. We are trying to improve the crystal quality and toanalyse crystal behaviour using different cryo-protectant combinations.

Table 1.- Crystals samples from Granada.

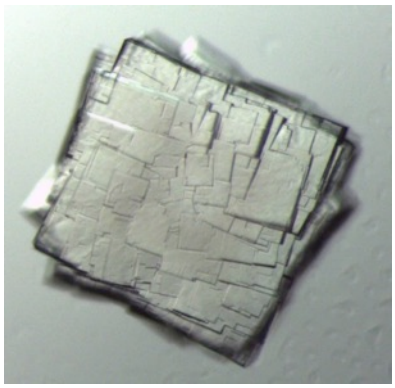
Protein	Samples	Conditions	Cryo/s	Results
PctA-Trp	3	C-4 :1.25M Na Citrate, 0.1M Na-Hepes pH 7.50	20% Glycerol	Bad diffraction. One data set at 4 Å.
McpU-Put	15	C-2 30%PEG 4K, 0.2M NH4 Acetate, 0.1M Na-Acetate pH 4.60 C-4 :1.25M Na Citrate, 0.1M Na-Hepes pH 7.50 PPP5: 20% PEG 400, 15% PEG 4K, 10% PEG 8K, NaAc 0.1M pH 5.0	15% Glycerol/30% PEG400	Poor diffraction. Data set at 2.7 Å
McpU	7	20% PEG 400, 15% PEG 4K, 10% PEG 8K, NaAc 0.1M pH 5.0	15% Glycerol	Bad diffraction. Data sets at 3.0, 3.2 and 4.0 Å.
			30% MPD	Data set at 3.5.
	2	C-20 30% PEG 8K, 0.1M Na Acetate, 0.1M Na- Cacodylate pH 6.50	15% Glycerol	Low resolution.
	3	C-2 30%PEG 4K, 0.2M NH4 Acetate, 0.1M Na-Acetate pH 4.60	15% Glycerol/ No-cryoprotectant	Poor diffraction.
McpU-Esp	4	C-20 30% PEG 8K, 0.1M Na Acetate, 0.1M Na- Cacodylate pH 6.50	20% Glycerol	Poor diffraction.
GNCA mut	12	Sodium Formate pH 4.0	15% Glycerol	Several data sets at high resolution. See table 2.
Tcm16	6	20% PEG 400, 15% PEG 4K, 10% PEG 8K, NaAc 0.1M pH 5.0	15% Glycerol/30% MPD/No cryo.	Several data sets no better than 3.3 Å.
	5	20% PEG 400, 15% PEG 4K, 10% PEG 8K, NaAc 0.1M pH 6.0	15% Glycerol/30% MPD/No cryo.	

Table 2. Data collection and refinement statistics of GNCA mutants.

	Single mutant		Double mutant	
Resolution (Å)	26.63 - 1.305 (1.351 - 1.305)	38.37 - 1.19 (1.233 - 1.19)	41.23 - 2.212 (2.291 - 2.212)	66.51 - 1.22 (1.261 - 1.217)
Space group	P 21 21 2	I 41	P 21 21 2	I 41
Unit cell	70.14 50.57 69.72	93.92 93.92 94.01	72.18 50.92 70.28	93.75 93.75 94.36
Unique reflections	60139 (5763)	121631 (12656)	13317 (1297)	121380 (12023)
Completeness (%)	98.56 (95.68)	93.61 (97.34)	98.91 (99.62)	99.88 (99.66)
Mean I/sigma(I)	16.93 (2.50)	11.16 (2.10)	7.93 (2.10)	16.70 (2.20)
Wilson B-factor	12.65	11.27	27.91	12.96
Refinement status				
R-work	0.1638 (0.3023)	0.1535 (0.2449)	0.1725 (0.2456)	0.1482 (0.2318)
R-free	0.1849 (0.3138)	0.1669 (0.2400)	0.2230 (0.3737)	0.1667 (0.2539)
Statistics for the highest-resolution shell are shown in parentheses.				

In this BAG at the beamline ID23-1(+ID29) the UAL lab collected data from crystals of several proteins:

i) Proline rich sequences (PRMs) binding domains. We have collected data from several crystals of the SH3 domain of the Fyn tyrosine kinase (Fyn-SH3) in complex with the synthetic peptide VSL12 with the purpose of determine the molecular basis of the differences in binding affinities between several members of the Src family of tyrosine kinases. Crystals were obtained from a big cluster of a square Fyn-SH3 crystal (Fig. 2A) and one of the pieces diffracted to 2.1 Å. The structure was solved by molecular replacement (Fig. 2B).



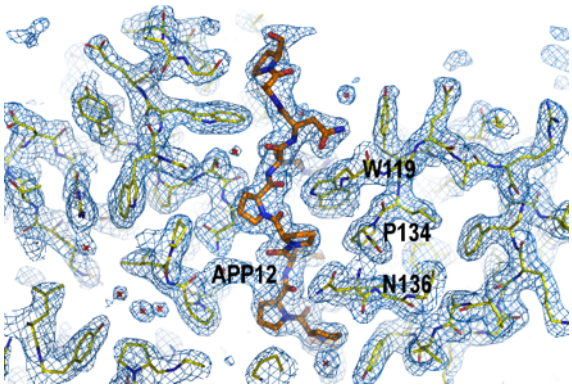


Figure 2. Crystal of the Fyn-SH3. 2Fo-Fc map showing the binding site of the APP12 peptide to the Fyn SH3 domain.

We have improved the crystal diffraction of a new crystal form of the TSG101-UEV (previous crystals diffracts at >2 Å resolution).

ii) Protein miss-folding and disease. From this subject we measure the carboxyl-truncated form of the third PDZ domain of the PSD95 (*h*-PDZ3-PSD95). The crystals diffracted poorly and no data were collected.

iii) Choline sulphatase. We collected data at ~2 Å resolution that allowed us to get a MR solution and to solve the first structure of this protein. The AU is composed by four molecules of Choline sulphatase and the refinement is in progress (actual R/Rfree values 0.20/0.21).

iv) Ring1B. Crystals of this protein show serious decay and it is difficult to obtain a full data set. At this experiment we have collected data from several crystals in order to obtain a complete data set. Crystal belongs to a monoclinic space group $P2_1/C2_1$. MR search didn't report a successful solution yet.

Table 3.- Data collected by the UAL laboratory.

ESRF Experiment Beamline: ID23-1 T[a]: 100 K BAG: MX-1629

Protein	Samples	Conditions	Cell	Resolution
Fyn-SH2/APP12	10	3.5 M NaForm, 0.1 M Hepes pH 7.5	$P2_1$; 31.7 76.7 73.2 (94.7)	2 Å
TSG101-UEV	10	0.1M AcONa pH 4.6, 25% PEG 4K, 0.2M $(NH_4)_2SO_4$ +/- 0.1 M Proline	$P3_2$ 1; 170.65 170.65 38.71	1.8 Å
N-Ring1B	10	pH5-6, 15 % PEG 4k, 5 mM $CoCl_2$	$P2_1$, 42.32 96.04 63.31 (97.85)	>3 Å
Choline sulfatase	10	1M LiSO4 0.1M Hepes pH 7.5	$C2_1$; 28.2 206.3 116.7 (110.49)	2 Å
h-PDZ3-PSD95	8	0.2 M $Mg(AcO)_2$, 20 mM AmPO4, 25% MPD, 0.1 M Hepes pH 7	No data	-