ESRF	**Experiment title: Macromolecular Crystallography at South-East Andalusia**	**Experiment number**: MX-1629
Beamline: ID23-1	**Date of experiment**: From: 19 April 2015 to: 20 April 2015	**Date of report**: 10/08/15
Shifts: 3	**Local contact(s)**: LEONARD Gordon	*Received at ESRF:*

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Partial Report of Mx ID23-1 (19-04-2015 / 20-04-2015):

This is up-dated report of the data collected at ID23-1 during the 4th round of MX-1629. We brought to the ESRF 100 samples from the teams CSIC-UGR and UAL. All the samples were tested and the main results are listed below.

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 two crystals of PctA-Ile soaked with methionine were tested with no succeed. A new construct of PctB with a single histidine tag is been produced and crystallization assays are on going.

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. We have obtained crystals of free and liganded forms of this enzyme and data have been collected data at acidic pH (4.5) to a resolution of 1.73 Å (XALOC, ALBA) and in a wider range of conditions and pH to resoluton ranging from 1.8 Å to 3.4 Å (ID23-1, ESRF). At this time we try to obtain data at intermediate pH and from crystal soaked or co-crystallized in presence of urea or formamidase. The corresponding structures will be used to get insights into enzymatic "ping-pong" mechanisms.

iii) D-acylase (M7) /Succiniyl amino acid racemase (Nsar): This bi-enzymatic system is industrially used for the dynamic kinetic resolution of D-amino acids. We are studying it application as Cross-Linked *Enzyme Crystals* (CLECs), and as part of this study, we want to obtain the crystal structures of the enzymes, to use this information for Structural-based improvement. We got two data sets from M7 crystals which are been processed for MR search.

ii) Ancestral Lactamases. Following our studies on the use of ancestral scafolds for the minimalist design of the novo activity in resurrected lactamases several crystals of the ancestral GPBCA and extant mutant F230R were tested. Data from GPBCA did not improved the resolution limit but those collected from TEM-F230R diffracted to high resolution and refinament is going from a data set scaled to 1.4 Å. These physicochemical study is still on going and new contruct are been produced that needs to be crystallized and chracterized.

iv) Protein crystallisation in short peptides hydrogels. Following our previous studies on radiation remediation from data collected at BM30 and ID29 (ESRF) and Xaloc (ALBA) [1] we have attempt the generalization of the used of short peptide supramolecular hydrogels for the crystallization of biological macromolecules. In this study crystal quality of model proteins are compared as a function of the media of growth. The produced data have

already been compared and used in our recent publication Conejero-Muriel et al., (2015) [2] in which we generalized the used of this type of gels as an alternative to improve protein crystal quality.

Table 1.- Crystals samples from Granada.

Protein	Samples	Conditions	Cryo/s	Results
Formamidase	21	20% PEG 400, 15% PEG 4K, 10% PEG 8K, NaAc 0.1M v.s pH and ligand (Urea/formamide)	20% Glycerol	Several useful data sets. Current R/Rfree factor for the structure with urea (2.0 Å) is 0.18/0.23.
PctA-Ile/Met	2	C-4 :1.25M Na Citrate, 0.1M Na-Hepes pH 7.50	20% Glycerol	Poor diffraction.
M7	7	Cond 46 JCSG2 (20º)	KI+20% Glycerol	Two data set at 1.94 A and 1.82 A, resolution. (P212121)
		Cond 20 PEG Ion 1 (20º)		
GPBCA	4	C-2 30%PEG 4K, 0.2M NH4 Acetate, 0.1M Na-Acetate pH 4.60	15% Glycerol	Several data sets at a resolution of 2.0 Å. Not to be considered as improvement.
TEM-F230R	4	C-2 30%PEG 4K, 0.2M NH4 Acetate, 0.1M Na-Acetate pH 4.60	15% Glycerol	Several data sets at high resolution. The structure is been refined at 1.38 Å.
Lysozyme/Glucose Ism.	6	Short peptide hydrogels and agarose.	15% Glycerol	12 Data set at fixed configuration [2].
	6	Short peptide hydrogels and agarose.	15% Glycerol	

Crystals from UAL (Almería):

In this BAG at the beamline ID-23-1 the UAL lab collected data from 50 crystals. Table 2 shows a resume of the data collection.

Table 2.- Data collected by the UAL laboratory.

ESRF Experiment Beamline: ID23-1 Tª: 100 K BAG: MX-1629

Protein	Samples/Data collected	Conditions	Cell	Resolution
Complex TSG-UEV/Ubiquitina	10/3	0.1M MES pH6; 0.15M AmSO4; 15% PEG 4K 0.1M Citrato Sódico pH4.5; 20% PEG 4K 0.1M MES pH6; 0.2 M LiSO4; 20% PEG 4K 0.1M Citrato Sódico pH4.5; 20% PEG 8K	P 31 2 1/ 169.92 169.92 39.57 90 90 120	Unit cell corresponding to the Tsg101-UEV domain: crystals without Ubiquitin
Src-2X mutant basic pH	10/6	2M Ammonium Phosphate, 0.1M Tris pH 8	P 63 2 2/ 95.16 95.16 66.25 90 90 120	Only two good data set at 1.9 Å
Src-2X mutant acidic pH	2/1	1.5 M Ammonium Sulphate, 20 % Glycerol	P 63 2 2/ 67.34 67.34 45.75 90 90 120	2.0 Å, structure previously solved at 1.35 Å (PDB code 4LE9: 68.239 68.239 46.495 90.00 90.00 120.00 P6322)
Nedd4-WW3 domain	8/0	0.1M Potassium Chloride; 0.1 M Hepes pH 7.0, 15% PEG 6K	No diffraction	-
Cov-NHR-ABC	10/0	0.1M Sodium Chloride, 0.1 M Sodium Acetate pH 4.6	No diffraction	-
PZD3-PSD95 D332G mutant	10/6	Solution 1: NH4-acetate 0.17M, Na-citrate (pH 5.6) 0.085M, PEG 4000 25.5%, Glycerol 15% Solution 2: NH4-sulfate 1.6M, Na-acetate (pH 4.6) 0.08M, Glycerol 20% Solution 3: 0.2 M Ammonium sulfate, 0.1 M Sodium cacodylate trihydrate pH 6.5, 30% w/v PEG8k Solution 4: 0.1 M Sodium acetate trihydrate pH 4.6, 2.0 M Ammonium sulfate Solution 5: 0.2 M	Solution 1: I 41 2 2 / 92.9 92.9 86.82 90 90 90 Solution 2: I 41 2 2 / 89.92 89.92 85.13 90 90 90 Solution 3: P4 2 2/ 42.9 42.9 47.0 90 90 90 Solution 4: P21/ 58.47 46.85 60.65 Solution 5: Solution 2: I 41 2 2 / 88.74 88.74 83.95 90 90 90	Solution 1: 2.3 Å Solution 2: 2.0 Å Solution 3: 1.2 Å Solution 4: 1.1 Å Solution 5: 2.5 Å

		Magnesium acetate tetrahydrate, 0.1 M Sodium cacodylate trihydrate pH 6.5, 20% w/v PEG8K		

Briefly, we have measured crystals from five proteins, but we have obtained data from only three of them:

i. **Tsg101-UEV domain in complex with Ubiquitin**. We brought to the ESRF crystals of this complex obtained in different conditions. Only three crystals diffracted but the unit cell correspond to the Tsg101-UEV domain alone. We are planning different strategies to obtain this complex structure.

ii. Structure of the chimeric protein Src-2X. This protein has been contructed interchanging the RT- and n-Src loop of the c-Src-SH3 domain with thos of the c-Abl-SH3. We have published the structure of the intertwined structure of this chimera, which suffer the opening through two loops: the RT- and the n-Src-loop (PDB code 4LE9)[3]. We have crystallized the protein at neutral/basic pHs to try to obtain the monomeric form of the protein. The dependence on the pH in the oligomer equilibrium has been characterized in solution and is similar to that found in the WT protein that is an intertwined dimer at acidic pHs and a monomer at neutral pH [4]. Structure is under analysis.

iii. **PZD3-PSD95 D332G mutant.** We have previously measured several crystals of this protein. Protein crystallize in different conditions in different crystals forms. Moreover, under the same crystallization conditions we have found up to three different crystals forms of this mutant. This polymorphism is a consequence of the conformational flexibility of this modular domain. Previously, we solved the structure of the WT protein that shows a succinimide residue at this position [5]. With the characterization of several mutants at this position, we try to study the role of this loop in the recognition of short carboxyl terminal sequences. Structures are under refinement.

Future & perspective: We continuous working in the different proteins collected in this table to reach the goals exposed in the proposal of this BAG.

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