ESRF	**Experiment title: Macromolecular Crystallography at South-East Andalusia**	**Experiment number**: MX-1629
Beamline: ID30A-1	**Date of experiment**: From: 25/02/2015 to: 26/02/2015	**Date of report**: 07/07/15
Shifts: 3	**Local contact(s)**: Didier NURIZZO (didier.nurizzo@esrf.fr)	*Received at ESRF:*

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Partial Report of Mx1629 ID30A-1 (25/02/2015 to 26/02/2015):

This is up-dated report of the data collected at ID30A-1 during the second round of MX-1629. We send to the ESRF 100 samples from the teams CSIC-UGR and UAL. All sample were tested and those indexed were collected.

Crystals from CSIC-UGR (Granada):

Ancestral Lactamases

From the original studies of the resurrected ancestral enzymes (thioredoxins and lactamases) this research line has derived in four approaches [1-3]: i) to study the conservation preference of selected amino acid across long evolutionary time-scale by comparing the cross-mutational effect on TEM and ancestral lactamases, ii) to use a minimalist design to introduce the novo activity in a resurrected ancestral lactamase, iii) to evaluate a new computational method for the fast reconstruction of ancestral-like sequences and iv) in order to further understand whether the information on substrate promiscuity of ancestral enzymes could be used to anticipate how extant lactamases might evolve for antibiotic resistance, we are attempting the crystallization of ancestral lactamases with different clinically used lactamase drugs and other related compounds. In the four cases crystals have been produced and data collected for several of the single and multiple mutants.

1. Risso, V. A., Manssour-Triedo, F., Delgado-Delgado, A., Arco, R., Barroso-delJesus, A., Ingles-Prieto, A., Godoy-Ruiz, R., Gavira, J. A., Gaucher, E. A., Ibarra-Molero, B. and Sanchez-Ruiz, J. M. *Mutational Studies on Resurrected Ancestral Proteins Reveal Conservation of Site-Specific Amino Acid Preferences throughout Evolutionary History*. Mol Biol Evol, **32** (2015) 440-55.

2. Valeria A. Risso, Jose A. Gavira, Diego F. Mejia-Carmona, Eric A. Gaucher, and Jose M. Sanchez-Ruiz, Hyperstability and Substrate Promiscuity in Laboratory Resurrections of Precambrian β-Lactamases. JACs **135** (2013) 2899-902

3. Zou, T., Risso, V. A., Gavira, J. A., Sanchez-Ruiz, J. M. and Ozkan, S. B. *Evolution of conformational dynamics determines the conversion of a promiscuous generalist into a specialist enzyme.* Mol Biol Evol, **32** (2015) 132-43.

Summary of results: i) To study the conservation preference of selected amino acid across long evolutionary time-scale we have produced a number of single mutants in TEM and at the corresponding *loci* in PNCA, GPBCA, GNCA and ENCA. Crystals have been produced in many of them and data are being collected (Table 1); ii) Following our study on the introduction of the novo activity in a resurrected ancestral lactamase for which we already collected data on the GNCA-W204D mutant, we have produce this mutant in PNCA and GPBCA, crystallized and solve the structures from data collected during this beam-time period (Table 2). ; iii) to evaluate a new computational method for the fast reconstruction of ancestral-like sequences, several consensus sequence of lactamase have been designed, crystals produced and the 3D structure will be determined from data sumarized in Table 1; iv) we have also atempt to obtain crystals of ancestral lactamases with different clinically used lactamase drugs and other related compounds in order to further understand whether the information on substrate promiscuity of ancestral enzymes could be used to

anticipate how extant lactamases might evolve for antibiotic resistance. We have attempted to co-crystallized several ancestral protein in the presence of relevant componds or to soaked crystals already obtained by counterdiffusion in capillaries. Collected data are also sumarized in Table 1.

Table 1.- Data collected by the CSIC-UGR.

Protein	Samples	Conditions	Cell	Resolution
PNCA-W204D	3	C-20 (20ºC)	P61: 48.30, 48.30, 197.80	1.813 - 1.75
GPBCA-W204D	3	C-2 (20ºC)	P212121: 38.17, 50.55, 119.60	46.56 - 1.6
GPBCA-204D	1	NaF4	P212121: 38.08, 50.52, 119.32	46.52 - 1.89
GNCA-W204 + Inhibitor	11	NaF4 (20ºC)	I41: 94.00, 94.00, 92.20	100 - 1.41
GNCA-W204 + soaked with Ds	3	NaF4 (20ºC)	No diffraction	-
GNCAhis + Sulbactam	4	AS6 (20ºC)	I41: 94.49, 94.49, 92.32	47.24 - 1.41
GNCAtazo01	2	AS6 (20ºC)	I41: 94.74, 94.74, 92.06	46.03 - 1.48
Consensus Lact.	2	C-2 (20ºC)	I432: 134.10, 134.10, 134.10	100 - 1.55
Consensus Lact.	2	C-5 (20ºC)	I23: 134.38, 134.38, 134.38	47.51 - 1.75
Consensus Lact.	2	C-8 (20ºC)	I432: 134.70, 134.70, 134.70	100 - 1.53
Consensus Lact.	2	C-9 (20ºC)	No diffraction	
Consensus Lact.	2	C-14 (20ºC)	P3121: 85.99, 85.99, 106.79	43.39 - 1.64
Consensus Lact.	1	C-17 (20ºC)	I432: 134.20, 134.20, 134.20	100 - 1.71
Consensus Lact.	2	C-18 (20ºC)	P321: 86.50, 86.50, 106.70	100 - 2.38
Consensus Lact.	1	C-23 (20ºC)	P3121: 87.40, 87.40, 106.80	100 - 2.67
Consensus Lact.	1	AS4 (20ºC)	P3121: 86.20, 86.20, 107.10	100 - 3.16
TEM-S285L	2	C-20	P212121: 41.60, 59.70, 97.90	100 - 1.38
TEM-L201P	2	C-20	P212121: 61.40, 92.20, 41.20	100 - 1.48
TEM-L40SP	1	C-2	No diffraction	-
TEM-C69M	2	C-2	P212121: 41.18, 57.59, 97.61	49.6 - 2
TEM-L30S	1	NaF4	P21: 99.00, 116.80, 100.70	100 - 2.15

Table 2.

Protein	**PNCAW204D**	**GPBCAW204D**
Crystallization Conditions	PEG 8K, 0.1M Na-Acetate, 0.1M Na-Cacodylate pH 6.50	30%PEG 4K, 0.2M NH4 Acetate, 0.1M Na-Acetate pH 4.60
Data collection		
Space Group	*P61*	*P212121*
Cell dimensions (Å)	48.30, 48.30, 197.80	38.17, 50.54, 119.58
ASU	1	1
Resolution (Å) *	59.79 - 1.75 (1.813 - 1.75)	41.88 - 2.471 (2.558 - 2.471)
R_{merge} *	0.06167 (0.5698)	0.07897 (0.7572)
I/σ_I *	13.79 (2.43)	17.36 (1.74)
Completeness (%)*	99.46 (98.69)	99.05 (93.08)
Unique reflections*	23963 (2346)	9283 (901)
Multiplicity*	4.0 (4.1)	8.3 (4.4)
CC(1/2)	0.998 (0.772)	0.998 (0.63)
Refinement		
R_{work}/R_{free} (%)	0.1630 (0.2465)	0.1608 (0.2716)
No. atoms	0.1927 (0.2896)	0.2260 (0.3776)
Average B-factors (Å^2)	181	21
R.m.s deviations	264	261
Bond lengths (Å)	0.007	0.007
Bond angles (^{0})	1.08	1.13
Ramachandran (%)		
Favored	99	98
Outliers	0	0

Future & perspective: Following our three main research lines with ancestral proteins, new mutants of GNCA have been produce and crystallize to characterized the detected enzymatic activity reported here; new mutants of TEM and ancestral lactamases have been produced and crystallized to study the conservation preference of selected amino acid across long evolutionary time and finally new contructus of the ancestral-like sequences, generated by new computational method, have also been cloned, purified and crystallized. Several of those structures will also be subject to co-crystallization with several substrates and/or inhibitors.

In this BAG at the beamline ID-30 the UAL lab collected data from 50 crystals of several proteins:

i. **Choline sulphatase (6 crystals)**. We brought to the ESRF 6 crystal of this protein in presence of substrate and alone. We have already solved the structure this protein which show a modified cysteine residue at the active site and that of the active-site mutant His104Ala. These new data allowed us to determine differences at the active site upon binding of the substrate. The best crystal diffracted at 1.85 Å resolution. Refinement of the structure is under way.

ii. **Proline rich sequences (PRMs) binding domains (27 crystals).** We collected data of three different SH3 domain: c-Src, α-spectrin and Fyn. Besides we collected data from several mutants (see Table 3). We have solved the structure at 1.1 Å resolution of a chimeric c-Src-SH3 where the RT loop has been changed by that present in the Abl SH3 domain. The crystals where obtained at neutral pH and show the usual fold of the SH3 domains. We have measured also 3 crystals of the TSG101-UEV domain in complex with the VP40 protein, but the crystals didn't diffract.

iii. **PDZ domains (9 crystals).** We have collected data from several mutants of the third PDZ domain of the PSD95.

iv. **Ubiquitin (3 crystals).** We have collected data from only one of the crystals but this crystal diffracted at 1.3 Å resolution and we have solved the structure.

v. **VP40 (2 crystals).** No diffraction.

vi. **Ring1B (3 crystals).** No diffraction.

Table 3.- Data collected by the UAL laboratory.

ESRF Experiment Beamline: ID30A-1 T[a]: 100 K BAG: MX-1629

Protein	Samples	Conditions	Cell	Resolution
Choline sulfatase	6	1.35 M $LiSO_4$; 0.1M Hepes pH7	C121/128.6 206.9 116.6 (90.0 110.2 90.0)	Data good at 1.84 Å/ Structure under refinement
c-Src-SH3 RT-Abl mutant	6	1.7-1.8 M $(NH_4)_2SO4$, 0.1M MES/Hepes pH 6.5-7.5	C 2 2 2/ 40.8 55.9 52.2 (90.0 90.0 90.0)	Data good at 1.20 Å/ Structure under refinement
c-Src-SH3 T114S mutant	2	1.6 M $(NH_4)_2SO4$, 0.1M Acetate pH 5.0	P 61 2 2/46.45 46.45 127.44 (90.0 90.0 120.0)	Data good at 1.6 Å/ Structure under refinement
c-Src-SH3 S94A mutant	3	2M $(NH_4)_2SO4$, 0.1M Acetate pH 5.0	P 61 2 2 / 46.61 46.61 128.01 (90 90 120)	Data good at 1.6 Å / Structure under refinement
c-Src-SH3 H122R-Q128K mutant	2	1.7 M $(NH_4)_2SO4$, 0.1M MES pH 6.5, 5 mM $NiCl_2$	35.7 35.7 81.3 (90 90 120)	Data good at 1.35 Å / Structure under refinement
c-Src-SH3 E97T mutant/APP12 complex	2	1.5M $(NH_4)_2SO4$, 0.1M Acetate pH 4.0	P1 21 1/ 30.7 31.7 66.7 (90 98.9 90)	Data good at 1.65 Å / Structure under refinement
Fyn-SH3 domain/ APP12 complex	2	3.5 M Sodium Formate, 0.1 M Hepes 7.5	P121/76.51 76.51 108.8 (90 92.1 90)	Data good at 2.0 Å/ Twin
α-Spc-SH3	2	1.5 M $(NH_4)_2SO4$, 0.1M MES pH 6.0	P222/ 31.93 42.09 49.29 (90 90 90)	Data good at 1.55 Å / Structure under refinement
PDZ3-PSD95 D332G mutant	2	20% 2-Propanol, 20 % PEG 4k, 0.1M Sodium Citrate pH 5.6	P 1 21 1/ 58.55 46.87 60.73 (90 93 90)	Data good at 1.0 Å / Structure under refinement
PDZ3-PSD95	3	30% PEG 8k, 1.5 M $(NH_4)_2SO4$	P 6 2 2 / 34.1 34.1 113.7 (90 90 120)	Data good at 1.7 Å / Structure under refinement
Ubiquitina	3	30% PEG 4K, 0.1M Tris pH 8.5	P1/29.9 30.1 41.4 (88.5 79.2 67.3)	Data good at 1.3 Å / Structure under refinement