



Experiment title: Macromolecular Crystallography at South-East Andalusia

Experiment number:
MX-1629

Beamline: BM14U	Date of experiment: From: 30 January 2015 to: 31 January 2015	Date of report: 12/02/15
Shifts: 3	Local contact(s): BELRHALI H.	<i>Received at ESRF:</i>

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Partial Report of Mx1629 BM14U (30-01-2015 / 31-01-2015).

This up-dated report corresponds to the data collected at BM14U during the second round of Mx1629. We brought 60 samples from the different team grouped as CSIC-UGR and UAL. Among the 60 samples, 15 were not tested due to beam-line operational mal-function.

Incidents: Since the beginning of data collection a problem of communication arise between MDII and mxCUBE or Spec. Initially the Omega motor did not move properly and the centering routine did not work. Fixed by reconfiguration of mxCUBE and resetting MDII we could continue. Later on this communication issue affected sequentially to the goniometer-front-light (did not stoop), the beam-stop also got suck in its upper position and finally to the mount/un-mount routine being this last one impossible to recover after a second failure. The first failure of the mount/un-mount routine fixed after restarting the hardware (MDII / SC3) and software but a second failure was impossible to recover. We give-up with 15 crystals not tested. This is not an issue since next 25th February we have allocated time at ID30.

Crystals from CSIC-UGR:

i) 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 published [1] but improved diffraction quality for other protein-ligands complexes are undergoing. We have collected data to high resolution of the PctA-Ile (P₂₁2₁2₁: 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 (P₂₁2₁2₁ to 2.25 Å) and in the case of PctB-Arg (P₃₁2₁: 111.6, 111.6, 117.5 to 3.1 Å) and PctB-Gln (same SG to 3.5 Å) and PctC-GABA (P₃₁2₁: 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-Trp 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. Finally we try to collect data from the PctB-Ans complex and to improve crystal quality of PctC-GABA (Table 1 and 6).

Table 1				
BM14U (Glutamine (Gln), Acid Gamma Amino Butirico (GABA))				
Protein	N. Crys.	Conditions	Cryos	Results
PctB+Gln	6	C5: 1.7M NH4 Sulphate, 3.5% PEG 400 0.1M Na-Hepes pH 7.50 C24: 20%PEG 8K, 0.05M K Phosphate	25% Glycerol 20% Glycerol	No diffraction 1 full data set at 3 Å
PctC+GABA	4	C3: 20%PEG 8K, 0.2M Mg Acetate, 0.1M Na-Cacodylate pH 6.50	20% Glycerol	4 full data sets, the best at 2.5 Å

Future perspectives: New constructs have been designed for PctB in an attempt to improve crystal quality.

1. M. Rico-Jiménez, F. Muñoz-Martínez, T. Krell, J. A. Gavira and E Pineda-Molina. (2013) *Acta Cryst.* F69, 1431-1435.

ii) Formamidase from *Bacillus cereus*: We have obtained crystals of the free and liganded forms of this enzyme at acidic pH values at a resolution of 1.73 Å (XALOC, Alba). Crystals have been grown also at a wide range of conditions and pH. Different polymorphs were obtained at extreme pH values affecting also crystal quality and resolution limit, i.e. from 3.4 at pH 9.0 to 1.8 Å at pH 4.5 (Mx1541) but we keep trying to fill the gap at several pHs. A resume of the data collected at pH 5.0, 6.0 and 8.0 are shown below (Table 2).

BM14U Formamidase				
Protein	N. Crys.	Conditions	Cryos	Results
Tcm16	8	0.1M AcNa pH 5.0, 20% PEG 400, 15% PEG 4000 & 10% PEG 8000	15% Glycerol	1 full data sets at 3.0 Å.
		0.1M AcNa pH 6.0, 20% PEG 400, 15% PEG 4000 & 10% PEG 8000	No cryo	1 full data sets at 3.5 Å.
		0.1M Tris-HCl pH 8.0, 20% PEG 400, 15% PEG 4000 & 10% PEG 8000	No cryo	-

Future perspectives: We plan to grow crystals at different conditions, i.e. pHs, which gives also different polymorphs and pursued the soaking or co-crystallization with different substrates.

iii) Dihydropyrimidinase from *Sinorizobium meliloti*: We have recently shown that peptide-base hydrogel may help to improve crystal quality or to induce the formation of new polymorph [2] from data collected at ESRF and Alba. Although we have already solved the structure of the unliganded form of this industrially-used enzyme at a home source [3, 4] we search to improve crystal quality or to obtain new polymorph by growing the crystal in supramolecular hydrogels. Unfortunately the enzyme did not behave as expected and obtained crystals were of low quality (Table 3).

BM14U Dihydropyrimidinase				
Protein	N. Crys.	Conditions	Cryos	Results
Ser38	2	1-4: 0.1M Na acetate pH 4.5 & 5M Na formate	20% Glycerol	No diffraction.

Future perspectives: We plan to grow better crystals and co-crystals with several ligands to complete the picture that could allow us to establish a possible mechanism for the observed enantioselectivity.

1. Conejero-Muriel, M., Gavira, J. A. et al., *Chem. Commun.* (2015) DOI: 10.1039/c4cc09024a.
2. Martínez-Rodríguez S, González-Ramírez LA, Clemente-Jiménez JM, Rodríguez-Vico F, Las Heras-Vázquez FJ, Gavira JA, García-Ruiz JM. *Acta Cryst.* F62 (2006)1223-6.
3. Martínez-Rodríguez S, Martínez-Gómez AI, Clemente-Jiménez JM, Rodríguez-Vico F, García-Ruiz JM, Las Heras-Vázquez FJ, Gavira JA. *J Struct Biol.* 2010. 169(2):200-8.

iv) D-acylase (M7) /Succinyl amino acid racemase (Nsaar): This bi-enzymatic system is industrially used for the dynamic kinetic resolution of D-amino acids. We are studying its 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. Several crystals of the Nsaar enzyme grown in capillaries by counterdiffusion were tested for diffraction. Any of them were useful for data collection (Table 4).

BM14U Nsaar				
Protein	N. Crys.	Conditions	Cryo.	Results
Nsaar	5	C4:	20% Glycerol	Very poor or not diffraction.

Future perspectives: We plan to remove the His-tag and start the screening for crystallization conditions.

v) Ancestral Lactamases/Thioredoxins: Following our data collection of ancestral GNCA bounded to different inhibitors we have co-crystallized this enzyme with sulbactam. From previous experiment in BM14U (Mx1541) we have been able to identify a sub-structure bounded to the active centre of GNCA. In order to get better density map we intend to improve crystal resolution (Table 5 and 6).

BM14U GNCA co-crystals				
Protein	N. Crys.	Conditions	Cryo.	Results
GNCA-Inh	5	NaF pH 4.0	20% Glycerol	Several data sets, the best at 1.35 Å.

Table 6. Best data sets for BM14U data collection.						
Protein	GNCA-Inh		PctB-Gln		PctC-GABA	
Space Group	I ₄		P 3 ₁ 2 1		C 1 2 1	
Unit cell	94.48 94.48 91.59		111.44 111.44 117.73		109.15 62.61 63.47 (97.87)	
	Overall	OuterShell	Overall	OuterShell	Overall	OuterShell
Low resolution limit	47.24	1.32	48.25	3.39	44.08	2.48
High resolution limit	1.28	1.28	3.27	3.27	2.39	2.39
Rmerge (within I+/I-)	0.035	0.686	0.207	1.473	0.079	1.500
Rmerge (all I+ and I-)	0.040	0.763	0.217	1.548	0.082	1.590
Rmeas (within I+/I-)	0.043	0.858	0.230	1.644	0.088	1.687
Rmeas (all I+ & I-)	0.044	0.869	0.229	1.635	0.087	1.687
Rpim (within I+/I-)	0.025	0.504	0.101	0.724	0.038	0.761
Rpim (all I+ & I-)	0.019	0.404	0.073	0.520	0.027	0.550
Rmerge	0.011	-	0.040	-	0.043	-
Number of observations	546756	32923	129845	12380	166882	11012
Total number unique	101172	8506	13437	1278	16235	1239
Mean(I)/sd(I)	24.1	1.7	11.8	1.7	14.1	1.2
Mn(I) half-set correlation CC(1/2)	1.000	0.602	0.994	0.557	0.999	0.611
Completeness	98.1	84.3	99.8	98.9	96.2	74.5
Multiplicity	5.4	3.9	9.7	9.7	10.3	8.9
Refinement						
Current R/Rfree	0.13/0.16		-		-	

In this BAG at the beamline BM14U the UAL lab was planning to collect data from crystals of several proteins:

- i) **Proline rich sequences (PRMs) binding domains.** We brought to the ESRF of two mutants of the c-Src-SH3 domain and a chimeric protein (c-Src-SH3 domain with the RT loop corresponding to the Abl-SH3 domain).

We measure only 15 crystals:

1. Only one crystal of the Gln128His mutant yields good diffraction. However once solved the structure, the density maps revealed that the mutant correspond to the lysine mutant (Gln128Lys) in its intertwined form, which is already deposited at the PDB (PDB entry 4OMP, 2 Å resolution). We didn't improve the resolution.
2. We collected two data sets of the second mutant, Glu97Thr, and we solved the structure. The structure corresponds to the intertwined dimer of the mutant. Data were collected at 2 Å. The structure is under refinement.
3. The chimeric protein yields long-thin needles. We were able to measure only 5 crystals. We collected two data sets but the quality of the data was very bad and the resolution was higher than 3 Å.

ii) **Choline sulphatase.** We brought to the ESRF 10 crystal of this protein in presence of its substrate and alone. We have already solve the structure this protein with a sulphate ion bound to a reactive cysteine present in the active site and the active-site mutant His104Ala. We didn't collect data of this protein. The robot didn't work.

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	
c-Src-SH3 (Gln128His)	5	0.1 M sodium acetate, 1.6 M ammonium sulphate, 10% PEG 300	P6 ₅ 22 (46.500 46.500 127.080)	2 Å	
c-Src-SH3 (Glu97Thr)	5	0.1 M sodium acetate, 2.2 M ammonium sulphate, 10% PEG 300	P6 ₅ 22 (46.290 46.290 126.550)	2 Å	
c-Src-SH3-Abl-RT loop	10	0.1 M MES pH 6.5, 1.8 M ammonium sulphate, 5% PEG 300, 10% Glycerol	No data	Only 5 crystals were tested. Only two were collected	
Choline sulfatase	10	1.4 lithium sulphate, 0,1 M Hepes, pH 7.0 (+/- substrate)	No data	We were not able to collect data of this protein because problems in the beamline	