



Experiment title: Macromolecular Crystallography at South-East Andalusia

Experiment number:
MX-1629

Beamline: ID30A-1	Date of experiment: From: 08/07/2015 to: 08/07/2015	Date of report: 07/01/16
Shifts: 1	Local contact(s): BOWLER M	<i>Received at ESRF:</i>
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 Física, University of Almeria, Spain. 3. Dto. Química Física, University of Granada, Spain.		

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 as a MXpressE automatic data collection under MX-1629 due to delivery-samples issues in our previous run (see previous report). 50 samples were analysed belonging to the CSIC-UGR team. All sample were tested and those indexed were collected.

Crystals from CSIC-UGR (Granada):

i) Ancestral Proteins. Several data sets were collected from 17 crystals of different variant of ancestral lactamase and thioredoxin crystals (Table 1). In table 2 are summarized the data collection, final statistic of the model and PDB ID.

ii) 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). We have not been able to get stable diffracting crystal with any of them. Focus only in the complex with spermidine, we have obtained crystal in the presence of low concentration agarose. Ten crystals were tested and some improvement was observed. Crystals diffracted to a maximum resolution of 2.6 Å belonging to the orthorhombic space group P21212 (see Table 1). We expect to be able to improve those crystals further.

iii) Structural determination of *Pseudomonas* chemotactic transducer A, B and C (PctA, B, C). We tested one crystal of the PctC-GABA complex but did not diffract.

iv) NQO1-H80R.

NQO1 is a human stress protein involved in the antioxidant defence and associated to cancer. Particularly, this mutant is designed to act as a second site suppressor for polymorphisms in NQO1 strongly associated with cancer [1]. Objectives. Molecular dynamic simulations have suggested a structural switch caused by H80R that stabilizes NQO1. We want to characterize these effects by X-ray crystallography.

We have already obtained crystals of the H80R variant with its natural ligand (FAD) and in the presence of dicumarol. We have obtained crystals of the H80R mutant, in the presence of FAD, using vapour diffusion and improved their size by the capillary counter-diffusion technique (Figure 1). Conditions were refined using the CD in capillaries. Different protocols for soaking the dicumarol have been tested and also cryo-protectant protocols and conditions. The best results were obtained from bi-pyramidal shaped crystals belonging to the P41212 space group and diffracted X-ray to 3.7 Å.

[1] Angel L. Pey, et al., *FAD binding overcomes defects in activity and stability displayed by cancer-associated variants of human NQO1*, BBA, 2014, 1842(11): p. 2163-73.

iv) Insulin. Several crystals of insulin were tested for improved diffraction limit with no succeed.

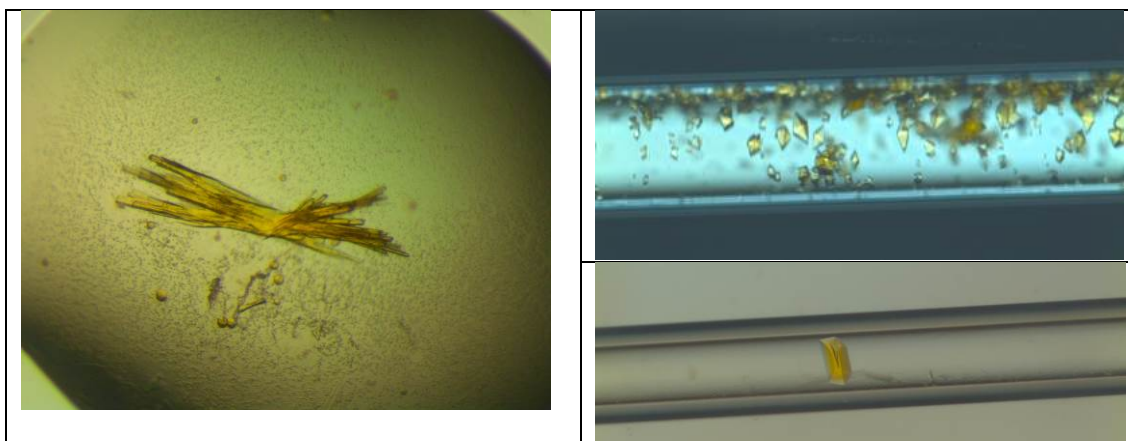


Figure 1. Crystals of NQO1-H80R grown by vapour diffusion (left) and improved crystals obtained in capillaries by counterdiffusion (right).

Table 1. Data collected by the CSIC-UGR.

Protein	Samples	Conditions	Cryo	Resolution
GNCA lactamase/NTE	4	Sodium Formate pH 4.0	15% GOL	Best data set at 2.7 Å. It has the analog. Deposited PDB P21212: 51.17, 69.79, 69.92
	6	Sodium Formate pH 4.0	15% GOL	Best data set at a resolution of 1.2 Å. Analog not bounded. I41: 93.95, 93.95, 93.11
Ancestral TRX	7	Several conditions.	15% GOL	Several data set at good resolution limit.
PctC/GABA	1	20%PEG 8K, 0.2M Mg Acetate, 0.1M Na-Cacodylate pH 6.50	20% GOL	No diffraction.
LBD-McpU	10	30%PEG 4K, 0.2M NH ₄ Acetate, 0.1M Na-Acetate pH 4.60 PPP5: 20% PEG 400, 15% PEG 4K, 10% PEG 8K, NaAc 0.1M pH 5.0	0 - 15% GOL	Best data set at 2.6 Å. P21212: 133.89, 148.15, 161.47
NQO1-H80R	20	30% PEG 3350, pH 7.0 to 9.0.	0 to 15% GOL	Best data set at 3.5 Å. P41212: 93.33, 93.33, 196.7
Insulin	2	Standard in the presence of gel.	15% GOL	Two data sets.

Table 2. GNCA4 bound to NTE.

PDB ID.	5FQJ
Beam-line	ESRF ID30A-1
Resolution range	41.3 - 2.274 (2.355 - 2.274)
Space group	P 21 21 2
Unit cell	51.172 69.793 69.955 90 90 90
Total reflections	77503 (7336)
Unique reflections	11969 (1116)
Multiplicity	6.5 (6.6)
Completeness (%)	0.99 (0.95)
Mean I/sigma(I)	9.66 (1.85)
Wilson B-factor	34.11
R-merge	0.1575 (1.113)
R-meas	0.1715 (1.208)
CC1/2	0.995 (0.76)
CC*	0.999 (0.929)
Reflections used in refinement	11962 (1115)
Reflections used for R-free	572 (61)
R-work	0.1993 (0.2763)
R-free	0.2299 (0.2831)
CC(work)	0.951 (0.860)
CC(free)	0.935 (0.818)
RMS(bonds)	0.006
RMS(angles)	0.86
Ramachandran favored (%)	96
Ramachandran allowed (%)	3.7
Ramachandran outliers (%)	0
Average B-factor	40.43