ESRF	**Experiment title: Macromolecular Crystallography at South-East Andalusia**	**Experiment number**: MX-1541
Beamline: ID29	**Date of experiment**: From: 16 February 2014 to: 17 February 2014	**Date of report**: 26/02/14
Shifts: 3	**Local contact(s)**: Christoph MUELLER DIECKMANN (christoph.mueller_dieckmann@esrf.fr)	*Received at ESRF:*

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Partial Report of Mx/1541 ID29 (16-02-2014 / 17-02-2014):

This up-dated report corresponds to the data collected at ID29 (+ID23) during the second round of Mx1541. We brought 160 samples from the different team grouped as CSIC-UGR and UAL. All the samples were tested and the main results are listed below.

Crystals from CSIC-UGR:

i) LBD-McpS bounded to several ligands. It was pointed it out on the proposal and in previous reports we kept searching for crystal of sufficient quality to undoubtedly identified the ligands (benzoate, citrate, etc.) bound to the near and distal pockets of the ligand binding domain of McpS. In previous opportunities (Mx1406) we have been able to collect data beyond 3.17 Å (McpS-Bz), the maximum resolution we have reached preciously. At ID29 we have definitively improved the resolution limit. We have collected 6 full data sets, several with resolution below 3.0 Å being the best 2.64 Å for the complex McpS-Ct and 2.3 Å for the complex McpS-Bz. We expect to get useful 3D structures in both cases.

Table 1

ID29 (*Benzoate (Bz) Malate (Ml) Acetate (Ac) Citrate (Ct) Tartrate (Tr)*)

Protein	N. Crys.	Conditions	Cryos	Results
McpS-Bz	9	1: 0.1M MES pH 4.8, 25% PEG 4000 & 0.25M AS	20% PEG 400	Only PEG 400 seems to work properly as cryo-protectant. 4 full data set
		2 & 3:0.1M MES pH 4.8, 20% PEG 4000 & 0.25M AS		
		4 & 5:0.1M MES pH 5.8, 20% PEG 4000 & 0.15M AS		
		6-9: 0.1M MES pH 5.4, 20% PEG 4000 & 0.15M AS		
McpS-Ct	10	1-3: (Protein pH 8.0) 0.1M sodium acetate pH 5.2, 20% PEG 4000 & 0.15M AS	20% PEG 400	Only PEG 400 seems to work properly as cryo-protectant. 2 full data set
			Fomblin oil	
		4-6: (Protein pH 8.0 + NaCl) 0.1M sodium acetate pH 5.2, 20% PEG 4000 & 0.15M AS	20% PEG 400	
			Fomblin oil	
		7-10: (Protein pH 8.0) 0.1 M Na Act pH 5.4, 20% PEG 4000 & 0.15 M AS	20% Glycerol	

Future perpectives: We will made only marginal efforts in the search for data improvement and to obtain new complexes.

ii) Structural determination of Pseudomonas chemotactic transducer A, B and C. The chemotaxis of *P. aeruginosa* to different amino acids is mediated by three paralogous chemoreceptors termed PctA, PctB and PctC. We have shown recently that PctA, PctB and PctC bind to 17, 5 and 2 amino acids, respectively, and mediate corresponding chemotactic responses [1]. We also showed that the neurotransmitter gamma-amino butyrate (GABA) binds to PctC with much higher affinity than the two amino acids being the first to identify a GABA chemoreceptor although its physiological relevance remains to be established. To elucidate the way of binding of these co-factors, we have produced crystals of PctA, PctB and PctC pre-incubated with several of their natural ligands. Preliminary results from ID14-4 (ESRF) have already been pubished [2] but improved diffraction quality for other protein-ligands is undergoing. We have collected data to high resolution of the PctA-Ile ($P2_12_12_1$: 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 ($P2_12_12_1$: 72.1, 116.4, 78.4 to 2.25 Å) and at lower resolution in the case of PctB-Arg ($P3_121$: 111.6, 111.6, 117.5 to 3.1 Å) complex all of them from previos data allocation slots (Mx1406 (ESRF) and Proxima I (SOLEIL)). The three structures are under refinement and will be part of an article in preparation. We have included an example of PctA binding pocket bunded to isoleucine (Figure 1). Following this line several crystal of the PctA incubated with isoleucine and tryptophane and PctC plus GABA were tested. The results are sumarized in Table 2. In the case of PctC-GABA co-crystals, several data sets were collected at resolution near 2.0 Å of crystals belonging to the P321 (77.41, 77.41, 67.41, 90, 90, 120) space group and are been used for MR solutions search using the refined structures of PctA.

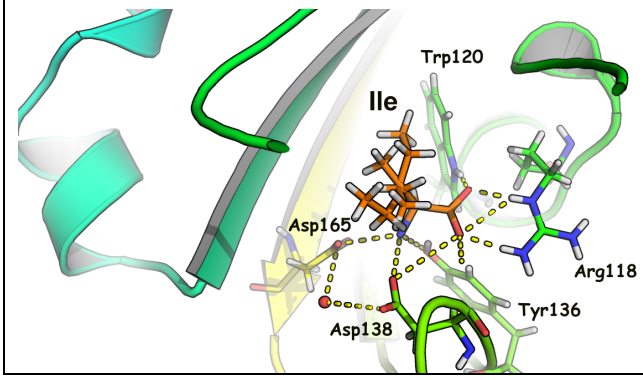


Figure 1. Current PctA model structure under refinement (R= 0.17/Rfree = 0.21) showing the Isoleucine binding pocket and residues interaction. The structure was obtained by MR from data collected at ID14-1 (Mx1406) and the manuscript is in preparation.

Table 2

ID29 (*Tryptophan (Trp), Isoleucine (Ile), Acid Gamma Amino Butirico (GABA)*)				
Protein	N. Crys.	Conditions	Cryos	Results
PctA+Trp	4	1-4: 1.25M Na Citrate & 0.1M Hepes/NaOH pH 7.5	20% Glycerol	1 full data set
PctA+Ile	2	1-2: 30% PEG 8000 & 0.2M AS	20% Glycerol	No difraction
PctC+GABA	8	1: 20% PEG 8000, 0.2M Mg Acetate & 0.1M Na cacodylate pH 6.5 2-7: 2M AS & 0.1M Tris/HCl pH 8.5	20% Glycerol	4 full data sets

Future perpectives: We will focus or efforts on getting new co-crystals of the three chemoreceptors with the remaining natural ligands.

1. Rico-Jimenez, M., Munoz-Martinez, F., Garcia-Fontana, C., Fernandez, M., Morel, B., Ortega, A., Ramos, J. L., and Krell, T. (2013) Mol. Microbiol. 88, 1230-1243.
2. M. Rico-Jiménez, F. Muñoz-Martínez, T. Krell1, J. A. Gavira and E Pineda-Molina. (2013) *Acta Cryst.* F**69**, 1431-1435.

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 [3]. 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 been grown also at a wide range of conditions and pH (Table 3). The corresponding structures will be used to get insights into enzymatic "ping-pong" mechanisms. Diferent polymorph 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.

Table 3

ID29 Formamidase				
Protein	N. Crys.	Conditions	Cryos	Results
Tcm16	36	1-3: 30% PEG 4000, 1.4M $MgCl_2$ & 0.1M Tris-HCl pH 8.5	20% Glycerol	6 full data sets of at least two polymorhps difracting x-ray to different resolution limits, from 1.8 to 3.4 Å.
		4-8: 30% PEG 4000, 0.2M AS & 0.1M Na Acetate pH 4.6	20% Glycerol	

		9-13: 20% PEG 8000, 0.2M Mg Acetate & 0.1M Na cacodylate pH 6.5	20% Glycerol	
		14-15: 30% PEG 400, 0.2M $CaCl_2$ & 0.1M Hepes/NaOH pH 7.5	No cryo	
		16-20: 25% PEG 4000, 0.2M AS & 0.1M Sodium acetate pH 4.6	15% Glycerol	
		21-26: 20% PEG 8000 & 0.1M Tris-HCl pH 8.5	15% Glycerol	
		27-32: 30% PEG 8000, 0.2M Na Acetate & 0.1M Na cacodylate pH 6.5	15% Glycerol	
		33-36: 0.1M Tris-HCl pH 9.0, 20% PEG 400, 15% PEG 4000 & 10% PEG 8000	No cryo	

Future perpectives: We plan to grow crystals at different conditions, i.e. pHs, which gives also different polymorhps and persued the soaking or co-crystallization with different subtrates.

3. Soriano-Maldonado P, Martínez-Gómez AI, Andújar-Sánchez M, Neira JL, Clemente-Jiménez JM, Las Heras-Vázquez FJ, Rodríguez-Vico F, Martínez-Rodríguez S. Appl Environ Microbiol. 2011. 77(16):5761-9.

iv) Dihydropyrimidinase from *Sinorizobium meliloti.* We have previously solved the structure of the unliganded form of this industrially-used enzyme at a home source [4, 5]. Crystals belonged to the $C222_1$ space group with unit cell dimensions of a = 124.89, b = 126.28, c = 196.10 and diffracted to a resolution of 1.85 Å. Despite there are several deposited structures of homolog enzymes, including two structures with ligands, the structural basis of the enantioselectivity of this enzyme remains unknown. We have co-crystallizing this enzyme with different ligands to unravel the mechanisms behind its enantioselectivity with the aim of enhancing its substrate promiscuity. Apo protein and protein-ligand-A crystals were diffracted at ID23 to high resolution limit, 1.35 Å in both cases in the same space group $C222_1$. Although initial autoprocessing suggested higher simetry space group (P422) it may be due to the tendency of this system to form twined crystals.

Table 4

ID23 Dihydropyrimidinase				
Protein	N. Crys.	Conditions	Cryos	Results
Ser38	4	1-4: 0.1M Na acetate pH 4.5 & 5M Na formate	20% Glycerol	2 full data set
Ser 38 + ligand A	5	1-5: 0.1M Na acetate pH 4.5 & 5M Na formate	No cryo	3 full data set

Future perpectives: We plan to grow co-crystals with other ligands to complete the picture that could allow us to stablished a possible mechanism for the observed enantioselectivity.

4. Martínez-Rodríguez S, González-Ramírez LA, Clemente-Jiménez JM, Rodríguez-Vico F, Las Heras-Vázquez FJ, Gavira JA, García-Ruíz JM. Acta Cryst. F62 (2006)1223-6.

5. Martínez-Rodríguez S, Martínez-Gómez AI, Clemente-Jiménez JM, Rodríguez-Vico F, García-Ruíz JM, Las Heras-Vázquez FJ, Gavira JA. J Struct Biol. 2010. 169(2):200-8.

v) Remadiation of radiation damage. We have shortly started a collaborative project together with scientist at XALOC beamline (ALBA, Barcelona, Spain) to investigate different strategies to remediate the radiation damage during data collection at room temperature. In this project we have selected several model systems i.e. GST from *E. Coli* and several target systems i.e. Hyal, to study the effect of treatment, the use of scavangers, etc. Initial test to get space groups and resolution limits under cryo condition were carryied out at ID23 during this slot (Table 5). Crystals of GST diffracted to a maxima resolution of 2.0 Å while Hyl crystal reached 1.5 Å. Data processing is on going and future experiments are been designed.

Table 5

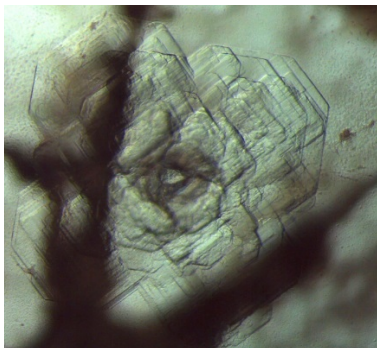
ID23 GST				
Protein	N. Crys.	Conditions	Cryo.	Results
GST	5	1: 0.1M Sodium acetate pH 5.0 & 3M Ammonium sulphate	20% Glycerol Fomblin oil	4 full data sets
ID23 Hyl				
HYL	6	1: 20% PEG 8000, 0.2M Mg acetate & 0.1M Na cacodylate pH 6.5 2-6: 30% PEG 8000 & 0.2M AS	20% Glycerol	3 full data sets.

Crystal from the UAL team:

In this BAG in the beamline ID29 the UAL collected data from crystals of several proteins:

i) Protein miss-folding and disease. From this subject we measure crystals from a PDZ domain, the third PDZ domain of the PSD95 (PDZ3-PSD95). This PDZ3 is anomalous among the PDZ domain because bear an additional α-helix at the carboxyl terminal. We have deleted this α-helix (PDZ3-PSD95-*h*) and characterized the resulting PDZ domain [6]. These crystals take four years to grow. Besides there are very thin and polycrystaline

(Fig.1). These crystals diffract at 1.5 Å resolution and we were able to obtain a molecular replacement solution in the space group P21 (unit cell, 54.12 53.97 62.61 90 93.25 90) with four molecules at the AU.



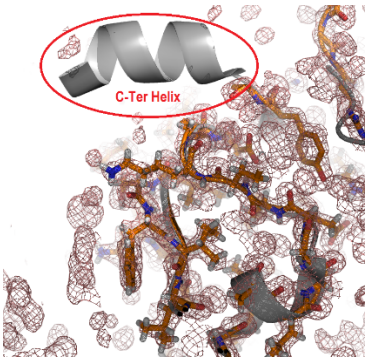


Figure 2.- Left panel. Crystal of the PDZ3-PSD95-*h* obtained in ammonium sulphate at pH 6.0. Crystal size ~200x2002x20 µm.

Right panel. Density maps of the MR solution. Red circle show the position of the α-helix, where no electron density have been found.

6. Murciano-Calles, J., Martinez, J. C., Marin-Argany, M., Villegas, S. & Cobos, E. S. (2014). *Biophysical chemistry* **185**, 1-7.

ii) Proline rich sequences (PRMs) binding domains. We manage to obtain crystals of the first WW domain of the YAP65 in abcense and presence of several high affinity proline rich motifs. In October 2013, we collected data at 1.5 Å from very tiny crystals (<20µm) of the apo form in the beamline XALOC of the ALBA synchrotron (Barcelona, Spain). The crystals diffraction was very good and we have written the manuscript that will be submitted soon. In this beamtime allocation, we tried to collect data of the complexes. Crystal does not diffract.

iii) HIV vaccines. We have obtained crystals of the covNHR antigen in two different crystallization conditions using ammonium sulphate and NaCl. These small protein constructs are derived from the gp41 HIV-1 protein and have been rationally engineered to display well known neutralizing epitopes of gp41, as well as being stable, soluble and easily producible by E. coli expression in recombinant form. Previously, we collected data at 2.5 Å from crystals grown in ammonium sulphate at the beamline XALOC of the ALBA synchrotron (Barcelona, Spain). We have a molecular replacement solution and at this beamtime allocation we wanted to improve the data's resolution. Unfortunately, in this beam time allocation we were only able to collect a single set of data from 20 different crystals and the resolution was not improved.

Additionally we brought samples of other proteins object of study in our group:

iv) Ring1B. We have recently published the physico-chemical characterization of the N-terminal segment of this protein. We have obtained crystal of this protein. Up to the date, the only structure available of this protein is in complex with its partner protein Bmi1 (B-cell-specific Moloney murine leukaemia virus integration site 1) which is critical components of the chromatin modulating PRC1 complex.

7. Martínez-Gómez AI et al. Protein Eng Des Sel. 2014 Jan;27(1):1-11. doi: 10.1093/protein/gzt056.

v) Choline sulphatase. Crystals of this protein grown in litium sulphate. We already collected data at the beamline XALOC of the ALBA synchrotron (Barcelona, Spain). Crystal diffract a medium resolution (2.5 Å) and showed fast decay. Up to the date, we were not able to obtain a MR. We have grown crystal in presence of metals to obtain the phases by MIR/SAD. At this beam time allocation we have collected several set of data, some at better resolution that the previous. Besides, we have obtained crystal in the presence of cadmium and we have collected several set of data to try to obtain the phase. Crystals brought to the beamline ID29 are detailed in Table 6 and those resulting in diffraction are indicated.

Table 6. Crystals samples from the UAL laboratory.

ESRF Experiment: ESRF Grenoble		**Beamline: ID29** **Tª: 100 K**		**BAG: MX-1541**
Xtal (Protein)	**# samples**	**Xtal Conditions**	**Diffraction**	**Data collected**
PDZ3-PSD95-h	4	0.1 M buffer pH 6.0, /1 M$(NH_4)_2SO_4$; cryo 10 % glycerol	Yes	Yes (1.5 Å)
Antigen covNHR	20	0.1M Mes pH6, 2M NaCl	Yes (only 1 crystal)	Yes (2.8 Å)
WW1 domain from YAP65	6	PEG 8k/$(NH_4)_2SO_4$ 0.1 M buffer + additives	No	No
N-Ring1B	10	pH5-6, PEG 4k; Cryo glicerol	Yes, weak	>2 Å
Choline sulphatase	4	1.2M LiSO4, 0.1M Hepes pH 7+ 0,5mM CdC12	Yes	>2 Å