ESRF	**Experiment title: Macromolecular Crystallography at South-East Andalusia**	**Experiment number**: MX-1541
Beamline: ID23-1	**Date of experiment**: From: 28 June 2014 to: 29 June 2014	**Date of report**: 25/08/14
Shifts: 3	**Local contact(s)**: Giullame Gotthard	*Received at ESRF:*

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Partial Report of Mx/1541 ID23-1 (28-06-2014 / 28-06-2014):

This up-dated report corresponds to the data collected at ID23-1 (+ID23-2) 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.

The experiment at ID23-1 was routinely ok, with some basket-jams in the sample-changer which could be easily solved by following the instructions of our local contact (Giullame Gotthard). At approximately 2:00 A.M., the Pilatus 6M detector had an error, and we could/did not know how to re-initialize it. However, we could continue and finish our experiment the next day in ID23-2 beamline, thanks to the support of both Giullame (who suggested us to ask for it) and to Christoph Mueller-Dieckmann, who gave support to us to start to measure at ID23-2.

Crystals from CSIC-UGR (Granada):

ii) Structural determination of Pseudomonas chemotactic transducer A, B and C.

Summary of results:

The structure of PctA-Ile and PctA-Tpr have already been solved and deposited at the PDB (ID. 4CU3 and 3D27). Crystallization with other amino acids has fail and therefore we decide to use PctA-Ile and PctA-Tpr crystals to soak other amino acids within the capillaries. Only crystal originally from the adduct PctA-Ile soaked with methionine survive the treatment (Table 1). A full data set was collected at 2.0 Å resolution.

Table 1. Resume of collected data at ID23-1/2 from PctA crystals.

ID23-1 (28-Jun-2014)					
Protein	N. Crys.	Conditions	Cryo	Loop / Capillary	Results
PctA-Trp/Ala	1	1.4M Na Citrate & 0.1M Hepes pH 7.5	20% Gly	crystal in capillary	No crystal
PctA-Trp/Thr	1	1.4M Na Citrate & 0.1M Hepes pH 7.5	20% Gly	1 crystal in capillary	No diffraction
PctA-Trp/Met	2	1.4M Na Citrate & 0.1M Hepes pH 7.5	20% Gly	2 crystal in loop	Salt crystal
PctA-Trp/Asn	2	1.4M Na Citrate & 0.1M Hepes pH 7.5	20% Gly	2 crystal in loop	Bad diffraction
PctA-Ile/Met	3	2.0M Na Formate & 0.1M Na Acetate pH 4.6	20% Gly	3 crystal in loop	2 Full data set.
PctA-Ile/Asn	1	2.0M Na Formate & 0.1M Na Acetate pH 4.6	20% Gly	1 crystal in capillary	No crystal

Future perspectives: New family proteins are been produced and will be subject to crystallization trials.

Table 1 (continuation). Resume of collected data at ID23-1/2 from PctB crystals.					
ID23-1 (28-Jun-2014)					
Protein	N. Crys.	Conditions	Cryo	Loop / Capillary	Results
PctB+Glu	1	1: 2.0M Ammonium sulphate & 0.1M Tris/HCl pH 8.5	20% Glycerol	1 crystal in loop	No diffraction

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.

iii-b) Allantoinase (AllBali): As a parallel project production of Reinforced Cross-Linked *Enzyme Crystals* (RCLECs) is also been intended with allantoniase from *Bacillus licheniformis* ATCC 14580 allantoinase (AllBali). Allantoinase, a member of the amidohydrolase superfamily, exists in a wide variety of organisms, including bacteria, fungi, plants, and a few animals, such as fishes and amphibians, and catalyzes the reversible hydrolysis of allantoin into allantoate by hydrolytic cleavage of the N^1-C^2 amide bond of the five-member hydantoin ring. AllBali has been cloned, overexpressed in *Escherichia coli,* and purified to homogeneity in a two-step procedure by metal affinity and size exclusion chromatography.

Summary of results:

Crystals of both proteins, M7 and Nsar, obtained in different crystallization conditions than tested previously were selected and screened for X-ray diffraction (Table 2). Only one crystal from M7 diffracted X-ray to a resolution limit of 1.7 Å. Structure solution has been attempted by MR with no succeed to date.

Crystals of AllBali were obtained by the vapour diffusion method using 0.1 M potassium thiocyanate and 20% w/v polyethylene glycol 3,350 as crystallization solution. X-ray diffraction data (Table 2) were collected to a resolution of 3.5 Å with a R_{merge} of 29.2% from a crystal belonging to the $P12_11$ space group. Unit-cell parameters are a = 54.93, b = 164.74, c = 106.89 Å and ß=98.49 (article submitted to Acta Cryst. F).

Table 2. ID23-1/2					
Nsar					
Protein	N. Crys.	Conditions	Cryo	Loop / Capillary	Results
Nsar	10	1-3: JCSG C9 Cond C1	20% Glycerol	10 crystals in loop	Poor diffraction
		4-5: JCSG C9 Cond C3	20% Glycerol		
		6 & 9: JCSG C9	25% Glycerol		
		7-8: JCSG C9 Cond C4	20% Glycerol		
		10: C. Screen H7	No cryo		
D-acylase M7					
Protein	N. Crys.	Conditions	Cryo	Loop / Capillary	Results
M7	7	1-2: 0.2M Na Formate & 20% PEG 3350	20% Glycerol	loops	Full data set
		3-4: 0.2M Ammonium Formate & 20% PEG 3350	20% Glycerol		
		5-7: 0.1M Na citrate pH 5.0 & 20% PEG 6000	20% Glycerol		

Table 2 (continuation). Resume of collected data at ID23-1/2 from AllBali crystals.					
ID23-1 (28-Jun-2014)					
Protein	N. Crys.	Conditions	Cryo	Loop / Capillary	Results
AllBali	10	1-2: PEG-ION 14 D1	20% Glycerol	10 crystals in loop	1 full data set
		3-4: PEG-ION 14 A2	20% Glycerol		
		5-6: PEG-ION 14 B4	20% Glycerol		
		7-8: PEG-ION 14 D3	20% Glycerol		
		9-10: PEG-ION 14 B2	20% Glycerol		

Future perspectives: We will focus or efforts on improving crystals from Nsar and to improved crystal quality of AllBali.

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. lyszoyme, 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 3). Crystals of GST diffracted to a maxima resolution of 2.0 Å while Hyl crystal reached 1.5 Å and lysozyme co-crystallized with C60 at 1.6 Å.

Table 3. Resume of collected data at ID23-1/2

ID23 Hyl					
Protein	N. Crys.	Conditions	Cryo	Loop / Capillary	Results
Hyal SelMet	10	1-3: 30% PEG 8000& 0.2M Ammonium sulphate (no agarosa)	20% Glycerol	loop	3 Full data sets.
		4, 9 & 10: 20% PEG 8000, 0.1M Na Cacodylate pH 6.5 & 0.2M Mg Acetate (no agarosa)	20% Glycerol		
		5, 6, 7 & 8: 20% PEG 8000, 0.1M Na Cacodylate pH 6.5 & 0.2M Mg Acetate (con agarosa)	20% Glycerol		
Lsyzozyme+C60	7	NaCl20%, 50 mM NaAc pH 4.5	20% Glycerol	loop	1 full data set.

Future perspectives: Data processing is on going and future experiments are been designed.

In this BAG at the beamline ID23-1/2 the UAL lab collected data from crystals of several proteins.

i) Protein miss-folding and disease. From this subject we measure two mutants crystals from the third PDZ domain of the PSD95 (PDZ3-PSD95): D332G and D332P. These mutants replace the aspartate residue that is converted to a succinimide residue at the position 332, which is found at the loop between β2-β3 strands. The glycine residue is expected to increase the flexibility of the loop, while the proline reside is expected to show the opposite effect. Crystals from the D332G mutant were obtained in two different crystallization conditions and show different diffraction pattern.

ii) Proline rich sequences (PRMs) binding domains. We have collected data from several crystals of the TSG101-UEV domain. We have improved the crystal diffraction of the TSG101-UEV/Ebola9-peptide and we have manage to collect for the first time data from the TSG101-UEV/Leukaemia virus-peptide (Figure 1). Besides we have improved the data of a new crystal form of the TSG101-UEV (previous crystals diffracts at >3 Å resolution). We also brought crystals of the VP40 and TSG101-UEV/VP40 complex but crystals didn't diffract or result in crystal diffraction of the TSG101-UEV alone.

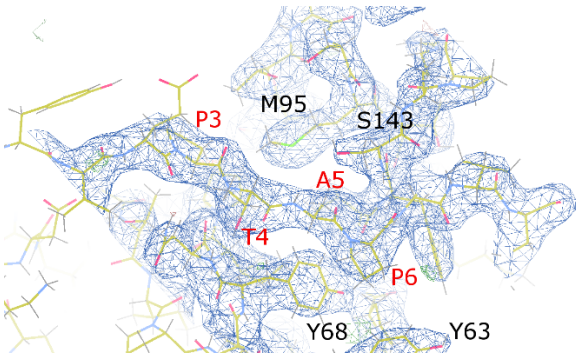

Figure 1.- Coot screenshot of the PTAP-late domain motif of the Leukaemia virus-peptide bound to the TSG101-UEV domain.

We also measured crystals from a complex between the third WW domain of Nedd4 with a high affinity peptide obtained by phage display technique, but these crystals only diffracts at >8 Å and we didn't collect these data.

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

ii) Ring1B. We have recently published the physico-chemical characterization of the N-terminal segment of this protein ("The isolated N terminus of Ring1B is a well-folded, monomeric fragment with native-like structure" Martínez-Gómez AI et al. Protein Eng Des Sel. 2014 Jan;27(1):1-11. doi: 10.1093/protein/gzt056). 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. At this BAG we have collected MAD data from a cobalt derivative. Crystals show serious decay and it is difficult to obtain a full data set.

Table 4.- Data collected by the UAL laboratory.

ESRF Experiment	Beamline: ID23-1/2			
Xtal (Protein)	# samples	Xtal Conditions	Cell	Resolution
PDZ3-PSD95-D332G	2	0.1 M Na-citrate (pH 5.6), 19% 2-Propanol, 19% PEG 4000, 5% Glycerol	$I4_122$, 89.9 89.9 84.8	1.8 Å
	1	2M $AmSO_4$; 10% Glicerol	$P4_3$, 41.4 41.4 47.0	2 Å
PDZ3-PSD95-D332P	2	1.6M NH_4-dihydrogenphosphate, 0.1 M TRIS-HCl (pH 8.5) 0.08M, Glycerol 20%	$C2_1$, 151.18 31.8 71.5 90.00 99.15 90.00	1.8 Å
TSG101-UEV	2	0.1M AcONa pH 4.6, 25% PEG 4K, 0.2M $(NH_4)_2SO_4$	$P3_2$ 1, 170.65 170.65 38.71	2.4 Å
TSG101-UEV/Ebola9-peptide	2	0.1M Hepes pH 7.0, 25% PEG 4K, 0.1M $(NH_4)_2SO_4$	I4, 104.6 104.6 73.5	2 Å
TSG101-UEV/Leukaemia virus-peptide	1	0.1M Hepes pH 7.0, 25% PEG 4K, , 0.2M $(NH_4)_2SO_4$	C 2 2 2_1, 78.9 119.6 41.9	2.4 Å
N-Ring1B	3	pH5-6, 15 % PEG 4k, 5 mM $CoCl_2$	$P2_1$, 42.32 96.04 63.31 90 97.85 90	>2 Å