ESRF	**Experiment title: Macromolecular Crystallography at South-East Andalusia**	**Experiment number**: MX-1541
Beamline: BM14U	**Date of experiment**: From: 27 April 2014 to: 28 April 2014	**Date of report**: 25/07/14
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 BM14U (27-04-2014 / 28-04-2014):

This up-dated report corresponds to the data collected at BM14U during the third round of Mx1541. We brought 50 samples from the different team grouped as CSIC-UGR. All the samples were tested and the main results are listed below.

Crystals from CSIC-UGR:

i) Ancestral Lactamases/Thioredoxins

Summary of results:

We have collected several data sets from different ancestral lactamases. Although ancestral bacterial lactamases, ENCA and GNCA, has already been solved we have kept them in the loop in a search for better diffraction quality since both structures were solved from home source collected data. We are also seeking to obtain the structure of GNCA bounded to different inhibitors. In this case GNCA crystals were soaked or co-crystallized with sulbactam. Preliminary results showed that the binding pocket is empty. Table 1.1 details the statistics for the data collection and current refinement of crystal number 5.

Table 1. Resume of collected data at BM14U from ancestral proteins.

BM14U (27-Apr-2014)					
Protein: Lactamase	N. Crys	Conditions	Cryos	Loop / Capillary	Results
GNCA+Inh4	2	NaF pH 4.0	20% Glycerol	1 crystals in loop	1 Full data set
GNCA+Inh5	2	NaF pH 4.0	20% Glycerol	1 crystals in loop	1 Full data set
GNCA+Inh1	1	NaF pH 4.0	20% Glycerol	1 crystal in loop	1 Full data set
GNCA+Inh2	1	NaF pH 4.0	20% Glycerol	1 crystal in loop	1 Full data set
GNCA+Inh3	1	NaF pH 4.0	20% Glycerol	1 crystal in loop	1 Full data set
Protein: Thioredoxin	N. Crys	Conditions	Cryos	Loop / Capillary	Results
TrxK90L	10	1-10: 20% PEG 8000 & 0.05M K Phosphate	No cryo 20% PEG 200	10 crystals in loops	Bad resolution

Table 1.1. Data collection and refinement statistics of GNCA-Inh5 complex.	
Resolution range (Å)	66.7 - 1.4 (1.45 - 1.4)
Space group	I 41
Unit cell	94.323 94.323 91.562 90 90 90
Total reflections	360332 (21042)
Unique reflections	75266 (5702)
Multiplicity	4.8 (3.7)
Completeness (%)	95.69 (72.53)
Mean I/sigma(I)	29.73 (3.43)
Wilson B-factor	13.90
R-merge	0.03019 (0.3568)
R-meas	0.03393
CC1/2	1 (0.845)
CC*	1 (0.957)
Refinement	
R-work	0.1956 (0.2423)
R-free	0.2136 (0.2844)

Statistics for the highest-resolution shell are shown in parentheses.

Future perspectives: New crystals from different ancestral proteins (lactamases and thioredoxins) are been produced and structural models from crystals will be attempted.

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). Crystals of PctB have been obtained in the presence of glutamine and a full data set at 3.2 Å was collected during this session. Crystal belongs to the same space group than the PctB-Arg aduct ($P3_121$). The structure will be solve by MR.

Table 2. Resume of collected data at BM14U from PctB-Arg crystals.				
BM14U (27-Apr-2014)				
Protein	N. Crys.	Conditions	Cryos	Results
PctB+Gln	8	1-3: 2.0M ammonium sulphate & 0.1M Tris/HCl pH 8.5	20% Glycerol	1 Full data set.
		4-8: 2.0M lithium sulphate & 0.1M Hepes/NaOH pH 7.5	20% Glycerol	
			No cryo	

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

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.

Summary of results:

At this stage crystals of M7 and Nsar have been obtained. In this session Nsar and M7 crystal were tested (Table 3) for X-ray diffraction.

Table 3				
Nsar				
Protein	N. Crys.	Conditions	Cryos	Results
Nsar	10	1-2: 0.2M Ammonium tartrate dibasic pH 7.0 & 20% PEG 3350	No cryo	Ice rings and/or poor diffraction
		3-4: 8% Tacsimate Ph 6.0 & 20% PEG 3350	No cryo	
		5-6: 0.8M Succinid acid pH 7.0	25% Glycerol	
		7-8: 0.1M Na Hepes pH 7.0 & 10% PEG 6000	No cryo	
		9-10: 1.5M Na Chloride & 10% Ethanol	25% Glycerol	

Table 3 (continuation)				
M7				
Protein	N. Crys.	Conditions	Cryos	Results
M7	15	1-2: 0.1M Tris pH 8.0 & 40% MPD	No cryo	1 Full data Poor diffraction
		3-4: 0.2M Ammonium acetate pH 7.0 & 20% PEG 3350	No cryo	
		5: 0.2M Ammonium citrate dibasic pH 5.1 & 20% PEG 3350	No cryo	
		6-7: 0.2M Ammonium sulfate, 0.1M MES pH 6.5 & 30% PEG MME 5000	No cryo	
			25% Glycerol	
		1-3: 0.2M Sodium Iodide pH 7.0 & 20% PEG 3350	No cryo	
		4-5: 0.2M Sodium thiocyanate pH 6.9 & 20% PEG 3350	No cryo	
		6-8: 0.2M Potassium thiocyanate pH 7.0 & 20% PEG 3350	No cryo	
			10% Glycerol	

Future perspectives: Crystal improvement is already on going.

iii) CheW-RecA complex. In collaboration with the group of Dra. Susana Campoy, (University Autonoma of Barcelona) we are attempting to crystallize and solve the structure of the complex CheW-RecA of Salmonella enterica serovar Typhimurium since initial co-immunoprecipitation assay have shown that both protein interact strongly.

Summary of results: In Table 4.

Table 4				
ID29 Formamidase				
Protein	N. Crys.	Conditions	Cryos	Results
CheW-RecA	3	1-3: 30% PEG 400, 0.2M $CaCl_2$ & 0.1M Hepes/NaOH pH 7.5	No cryo	Salt crystals

Future perspectives: New screenings are been run in search for successful conditions.