Experiment title: Macromolecular Crystallography at South-East Andalusia	Experiment number: MX-1406
BAG-Report	**Date of report**: 21/10/13

Beamlines: dates (Shifts: 3x)		**Local contact(s)**:	*Received at ESRF:*
ID14-1:	3-04 nov 2012	D. Nurizzo (didier.nurizzo@esrf.fr)	
ID14-4:	24-25 feb 2013	A. Mccarthy (andrewmc@embl-grenoble.fr)	
BM14U:	17-18 jul 2013	H. Belrhali (belrhali@embl.fr) B. Manjasetty (babu@embl.fr)	
ID23-1:	20-21 abr 2013	G. Leonard leonard@esrf.fr)	

Names and affiliations of applicants (* indicates experimentalists):

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Full report:

In this report we resume the main results of the data collected at beam lines ID14-1 and 4, BM14U and ID23-1. The report is organized by protein system with a small introduction, a summary of the results including published/submitted or in preparation articles, and perspectives, if applicable. A full list of article and PDB deposited from this period is listed at the end of the report.

Problems/issues:

- **ID14-1 (03-Nov-2012):** There was a small incidence at ID14-1 with the blocking of the sample changer that force us to manually remove the cap from time to time yet we manage to test and collect all the samples.
- **ID14-4 (24-Feb-2013):** One Dewar did not arrive and the full session was dedicated to 50 samples.

McpS bounded to several ligands

Introduction:

McpS is a chemoreceptor able to recognize specifically 6 of the 7 intermediates of the TCA cycle at the root exudates of some plants. We have solved the structure of the Ligand Binding Domain of McpS (McpS-LBD) together with two of its main co-factors, malic acid and succinate, at 1.8 and 1.9 Å respectively (PDB ID. 2YFA and 2YFB, data collected at BM16 and ID14-4). We have shown that McpS represents a novel small-molecule binding domain (Pineda *et al.* 2012 and Gavira *et al.,* 2012) supported by X-ray structures form data collected at the ESRF.

1. Evidence for chemoreceptors with bimodular ligand binding region harboring two signal-binding sites. *PNAS,* (2012). 109, 18926-31. Pineda-Molina, E.; Lacal, J.; Reyes-Darias, J. A.; Ramos, J. L.; J. M. Garcia-Ruiz; Gavira, J. A.; Krell, T. *doi: 10.1073/pnas.1201400109*

2. Crystallization and crystallographic analysis of the ligand-binding domain of the Pseudomonas putida chemoreceptor McpS in complex with malate and succinate. *Acta. Cryst.,* (2012). F68, 428-431. J. A. Gavira, J. Lacal, J. L. Ramos, J. M. García-Ruiz, T. Krell and E. Pineda-Molina. *doi: 10.1107/S1744309112004940*

Summary of results:

Following this investigation line we are producing co-crystal of McpS soaked with identified low affinity ligands such as citrate, benzoate, etc. The full pool of structures together with the ligand free protein will be essential to understand the chemotaxis mechanism. The results are summarized in table 1 and table 1.1.

Table 1

ID14-1 (03-Nov-2012) (*Benzoate (Bz) Malate (Ml) Acetate (Ac) Citrate (Ct) Tartrate (Tr)*)				
Protein	N. Crys.	Conditions	Cryos	Results
McpS-Bz	2	1 & 2: 0.1 M MES pH 5.0, 20% PEG 4000 & 0.15 M AS	20% PEG 400 20% Glycerol	Bad cryo and bad diffraction
McpS-Tr	2	1 & 2: 0.1 M Bis tris propane pH 6.5, 20% PEG 3350	20% PEG 400	Poor diffraction
Mcps-Ct	6	1 & 2: 0.1 M sodium acetate pH 5.2, 20% PEG 4000 & 0.15 M AS	20% PEG 400	Salt crystals, anisotropic & poor diffraction or not diffraction
		3: 0.1 M sodium acetate pH 5.4, 20% PEG 4000 & 0.15 M AS	20% PEG 400	
McpS-Ml	6	1-4: 0.1 M sodium benzoate pH 6.5, 25% PEG 4000 & 0.15 M AS	20% Glycerol Paratone 20% MPD 20% PEG 400	Bad cryo, anisotropic & poor diffraction or not diffraction
		5 & 6: 0.1 M sodium benzoate pH 7.0, 20% PEG 4000 & 0.15 AS	20% Glycerol	
McpS-Ac	2	1 & 2: 0.1 M sodium benzoate pH 7.0, 20% PEG 4000 & 0.15 M AS	20% Glycerol 20% PEG 400	Salt crystals or poor diffraction
McpS-Ml/Bz	4	1: 0.1 M MES pH 5.8, 20% PEG 4000 & 0.25 M AS	20% Glycerol	Ice rings or poor diffraction
		2-4: 0.1 M MES pH 5.4, 20% PEG 4000 & 0.25 M AS	20% PEG 400 20% MPD 20% Glycerol	
ID23-1 (20-Apr-2013)				
McpS-Ml/Bz	14	1-2: 0.1M sodium cacodylate pH 5.4, 25% PEG 4000 & 0.25M AS	20% PEG 400	Salt crystals, poor diffraction or not diffraction
		3: 0.1M sodium cacodylate pH 4.4, 20% PEG 4000 & 0.15M ammonium sulphate	20% PEG 400	
		4-10: 0.1M MES pH 5.4, 20%	20% PEG 400	

		PEG 4000 & 0.25M AS		
		11-14: 0.1M MES pH 5.8, 25% PEG 4000 & 0.25M AS	20% PEG 400	
McpS-Bz/Ml	2	1-2: 0.1M sodium cacodylate pH 5.0, 20% PEG 4000 & 0.25M AS	20% PEG 400	Full data set. **Table 1.1**
McpS-Ct	14	1-10: 0.1M sodium acetate pH 5.4, 20% PEG 4000 & 0.15M AS (protein in buffer pH 6.0)	25% PEG 400	Ice rings, poor diffraction or not diffraction
		11 & 12: 0.1M sodium acetate pH 5.4, 20% PEG 4000, 0.15M AS (protein in buffer pH 8.0)	25% PEG 400	

The only useful data set was obtained at ID23-1 from crystals grown with malate as a ligand and is been process.

Table 1.1 Data collection statistics of McpS in complex wit malate.	
# images	820
Space group	C 1 2 1
Unit cell	224.90, 45.5, 51.4 90, 95.3, 90.0
Completness (Inner, Outer, Overall)	95.80, 96.50, 96.90
Rsymm (Inner, Outer, Overall)	5.00, 49.00, 15.40
Resolution	100.0-14.18, 3.25-3.17,100.0-3.17
Detector resolution (**Å**)	2.51

Future &perspective:

This project is undergoing for crystal improvement of the McpS in complex with Benzoate (Bz), Citrate (Ct) & Tartrate (Tr) in combination with Malate (Ml), Succinate (Su) or Acetate (Ac).

Intertwined dimeric structures of the SH3 domain of the c-Src tyrosine kinase

Introduction:

SH3 (Src Homology 3) domains are widespread proline-rich recognition modules, found in many different proteins, in diverse numbers and combinations. These domains are implicated in deregulated signaling pathways during cancer development and are also associated to other pathologies such as AIDS, osteoporosis, or inflammatory processes. Our research group interest is focus in the determination of high resolution crystallographic structures of the SH3 domains and their complexes in order to characterize the molecular components of binding specificity between these domains. Besides, in the case of the c-Src-SH3 domain and as results of previous measurements in the BM16 and ID14-4 beamlines at the ESRF, we have solved the structure of this domain (Camara-Artigas *et al.*, 2009) and several mutants and their complexes with poly-proline rich peptides from class I and II (Bacarizo *&* Camara-Artigas, 2013). In the structure of c-Src-SH3 domain the principal feature is that the asymmetric unit contains two c-Src-SH3 molecules associated as an intertwined dimer. Our goal in these measurements will be to determine the structures of several mutants of this domain to complete our study on the molecular basis of the domain swapping process and its biological implications. Comparison of the dimeric and monomeric structures of this swapped domain will help to unravel the structural implications of domain swapping that leads, in some cases, to the formation of amyloids fibrils (Bacarizo *et al.*, 2013).

1. Camara-Artigas,A.,Martin-Garcia,J.M., Morel,B.,Ruiz-Sanz,J., and Luque, I. (2009). Intertwined dimeric structure for the SH3 domain of the c-Src tyrosine kinase induced by polyethylene glycol binding. *FEBS Lett* 583,749-753.

Summary of results:

We were able to test many crystal of c-Src-SH3 and collected more than 11 full data sets. In Table 2 we summarized those set that are been use to solve the structures by MR.

Table 2. Resume of collected data at ID14-1 and 4 of the c-Src-SH3 domain.

0ID14-1 (03-Nov-2012)				
Mutation+Ligand	Xtal Conditions	Cryo	Cell	Resolution
Src-SH3 domain				
Q128E	0.1 M AcoNa pH5, 1.5 M AmSO4, 10% PEG 200, 10% Glicerol, 10 mM NDSB	None	P6122; 47.02 47.02 126.01	1.79
H122R/Q128E	0.1 M Hepes pH 7.5, 1.8 M AmSO4, 10% Glicerol, 5mM NiCl,10mM MBCD	None	P3121; 36.02 36.02 81.58	1.74
Src-WT/NS5A	0.1 M Hepes pH7, AmS04 2M, 0.86 mM MBCD, NaCl 100 mM	10% Glicerol	I41; 62.32 62.32 27.27	1.57
Src-WT	0.1 M Hepes pH7.5, AmS04 1.8M,10% Glicerol, NiCl 5mM	None	P212121; 43.67 45.36 58.02	1.53
Src-WT/APP12	0.1 M AcONa pH4, 1.6 M AmSO4, Glicerol 10%	None	C2; 65.90 32.85 31.64	1.21
E93V/APP12	0.1M AcoNa pH5, 1.9 M AmSO4, 10% Glicerol	None	P31; 36.94 36.94 83.40	1.20
Src-2x	2.2M AmSO4, 0.1M AcONa pH4, 10% Glicerol, 5% PEG 300, 0.01 M NDSB	None	P6322; 68.39 68.39 46.41	1.49
ID14-4 (24-Feb-2013)				
H122R/Q128K	Hepes pH 7.5, Ammonium sulphate+ $NiCl_2$ + MBCD	10% Glycerol	P 31 2 1; 36.05 36.05 81.21	1.2
H122R/Q128K	Hepes pH 7.5, Ammonium sulphate+ $NiCl_2$ + MBCD	100% MiTeGen oil.	P31 2 1; 36.10 36.10 81.36	1.5
H122R/Q128K	Cacodilate pH 6.5,Ammonium sulphate+ $NiCl_2$ + MBCD	5% PEG 300	P 31 2 1; 35.71 35.71 81.13 90.00 90.00 120.00	1.35
H122R/Q128K	MES pH 6, Ammonium sulphate+ $NiCl_2$+MBCD	10 % PEG 300	P 31 2 1; 35.54 35.54 80.94 90.00 90.00 120.00	1.5
T96G	Cacodilato pH 6.5, Ammonium sulphate+ $NiCl_2$+ 10 % Etilenglicol	10% PEG 300	C21 21 21; 40.13 356.98 50.31 90.00 90.00 90.00	1.6
Q128R+APP12	Sodium acetate pH4, Ammonium sulphate, Sodium chloride	10 % PEG 300	C2; 65.7994 32.8390 31.5787 90.000 104.378 90.000	1.1
WT+VSL12	Hepes pH 7.5, Ammonium sulphate+ $NiCl_2$+10% Glycerol	None	P 2 2 2; 35.1778 41.1235 43.2250 90.00 90.00 90.00	1.3
WT+VSL12	Hepes pH 7.5, Ammonium sulphate+ $NiCl_2$+10% Glycerol	5% PEG 300	P 2 2 2; 32.88 40.94 43.88 90.00 90.00 90.00	1.0
Q128E+APP12	Sodium acetate pH4, Ammonium sulphate, Sodium chloride + 10% Glycerol	10%PEG300	C2; 65.25 32.70 32.12 90.00 105.03 90.00	1.3
Src-SH3/RT	Sodium acetate pH4, Ammonium sulphate, Sodium chloride+PEG 300 5%+ Glycerol 10%	None	C2; 84.35 42.32 52.71 90.00 90.786 90.00	1.8
α-spectrin SH3 domain				
N47A	Sodium acetate pH5, Ammonium sulphate+Proline 2M	100% MiTeGen oil	C2; 171.021 182.060 123.747 90.00 133.05 90.00	2.8

The data set obtained from the double mutant of the c-Src-SH3 domain (H122R/Q128K) at this beam time, together with data collected at the Spanish synchrotron radiation facility ALBA (Barcelona, Spain) allow us to determine the structure of this mutant at several pHs at resolution near 1 Å. The results will be exposed in a poster presentation in the ALBA user meeting (September 2013) and manuscript is in preparation under the title "A cloning artefact generating an ATCUN site as a helpful tool in protein crystallography".

The structures of the complexes of the VSL12 and APP12 c-Src-SH3 domains will be soon published together with the thermodynamic data.

Other data at Table 1 have been already improved in other beam time allocations.

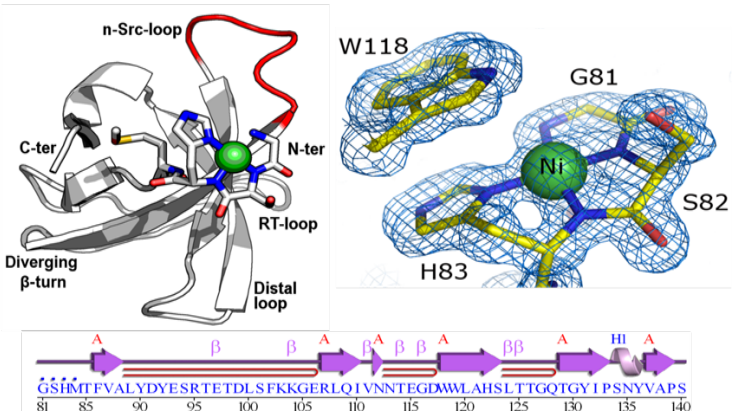

Figure 1.- **ATCUN motif in the c-Src-SH3 domain.** The c-Src-SH3 domain was expressed in *E. Coli* BL21 (DE3) strain (Novagen) with an *N*-Terminal 6x-His-tag and an engineered thrombin cleavage site. The ATCUN site is formed by the residues of the cloning artifact (Gly-Ser-His-Met) that are in principle able to bind Ni^{2+}, Cu^{2+}, Cd^{2+} or Zn^{2+}.

2. Bacarizo, J. and Camara-Artigas, A. (2013). Atomic resolution structure of the c-Src SH3 domain in complex with two high affinity peptides from class I and II. *Acta Crystallographica Section D 2013 May;69(Pt 5):756-66*

3. J. Bacarizo, M. Andujar-Sánchez, E. Ortiz-Salmerón , J.L. Neira and A. Cámara-Artigas. A single mutation affects the amyloid formation in the SH3 domain of the c-Src tyrosine kinase (to be submitted)

4. A. Cámara-Artigas, J. Bacarizo Roa, S. Martínez-Rodríguez, F. Gil, J. Benach and J. Juanhuix. A cloning artifact generating an atcun site as a helpful tool in protein crystallography (to be submmited)

5. A**.** Cámara-Artigas, S**.** Martínez-Rodríguez, E**.** Ortiz-Salmerón and J**.** M. Martin-Garcia. Domain swapping in a chimeric c-Src-SH3 domain takes place through two hinge loops . (to be submited)

Future & perspective

This study has been also extended to other SH3 domains were the presence of amyloid fibrils has been detected, as for example the N47A mutant of the α-spectrin SH3 domain. Besides we continue or structural studies in other SH3 to determine the structural determinant of the binding and the role of water molecules implied is such process. In this way we have cloned the SH3 from Osteoclast-Stimulating Factor (OSF), which is an intracellular signaling protein, produced by osteoclasts themselves, that enhances osteoclast formation and bone resorption. It is thought to act via the Src-related signaling pathway and contains SH3 and ankyrin-repeat domains, which are involved in protein-protein interactions.

PDZ domains

Introduction:

Our group have solved previously the PDZ3 from the PSD95 using data obtained at the ESRF (Camara-Artigas, Murciano-Calles et al. 2010) and is interested on the development of intertwined structured in these domains. For these reason we are working right now with the second PDZ domain from Zonula Occludens in wich the presence of domain swapping has been described previosly (financial support "Thermodynamic and structural analysis of the PDZ domains. Studies on the molecular aspects that drive the conformational equilibrium and interactions with ligands in the regulation of cellular networks of protein-protein interactions", P10-CVI-5915, from the regional Andalusian Governament and FEDER from the EU).

1. Camara-Artigas, A., J. Murciano-Calles, J. A. Gavira, E. S. Cobos and J. C. Martinez (2010). "Novel conformational aspects of the third PDZ domain of the neuronal post-synaptic density-95 protein revealed from two 1.4A X-ray structures." J Struct Biol 170(3): 565-569.

Summary of results:

The most relevant results for this protein are shown in table 3 below.

Table 3. Resume of collected data at ID14-4 of the PDZ in complex with ZO2.

ID14-4 (24-Feb-2013)				
Mutation+Ligand	Xtal Conditions	Cryo	Cell	Resolution
PDZ domains				
PDZ_ZO2	Sodium acetate pH4, Ammonium acetate, PEG4K	None	P41 21 2; 44.98 44.98 275.3 90.00 90.00 90.00	3.0

Future & perspective:

The diffraction of these crystals needs to be improved and we are working also in the crystallization of several complexes of this PDZ domain and others included in the study supported by the grant P10-CVI-5915.

Diffraction studies of ancestral thioredoxin.

Introduction:

Thioredoxins belong to a broad family of oxidoreductase enzymes ubiquitous in all living organisms. The archetypical active site (CXXC) and the Trx fold are well conserved throughout evolution, indicating that Trxs enzymes were likely present in primitive forms of life. Over the past two decades statistical methods have been developed to computationally reconstruct ancestral protein sequences. Laboratory resurrection of these ancestral proteins allows exploring aspects of ancient life that cannot be inferred from fossil records alone (Perez-Jimenez et al., 2011). We have produce seven Precambrian thioredoxin enzymes (Trx), showing that the resurrected enzymes have melting temperatures up to ~32 °C higher than those of extant Trx (DCS analysis) among other interesting features. We have already crystallized several of those anciant TRXs and the structures hasve been solved from ESRF data collection and deposited at the PDB (2YJ7, 2YNX, 2YPM, 2YN1, 2YOI and 4BA7).

1. Perez-Jimenez, R., Ingles-Prieto, A., Zhao, Z.M., Sanchez-Romero, I., Alegre-Cebollada, J., Kosuri, P., Garcia-Manyes, S., Kappock, T.J., Tanokura, M., Holmgren, A., et al. (2011). Single-molecule paleoenzymology probes the chemistry of resurrected enzymes. Nature structural & molecular biology 18, 592-596.

Sumary of results:

From the results obtained in ID14-1 we were able to complete the set of structures and published the results in Structure in 2013 (Ingles-Prieto, et al., 2013). See table Only ATrx324 (AECA) crystals were of low quality producing XRD data to a resolution of 2.65 A.

Table 4. Resume of collected data at ID14-1 of the ATrx324 (AECA).

ID14-1 (03-Nov-2012)					
Protein	N. Crys.	Conditions	Cryos	Loop / Capillary	Results
Trx324	7	1: E10	No cryo	Loops	Partial data set. **Table 4.1**
		2 & 3: C4	25% PEG 400		
			No cryo		
		4: C12	No cryo		
		5 & 6: D10	No cryo		
		7: A1	No cryo		

Table 4.1. Data collection and refinement statistics.	
Protein name	AECA
PDB identifier	**3ZIV**
Data collection	
Space Group	P 1 21 1
Cell dimensions	
a, b, c (Å)	37.6, 48.8, 91.1
β (^{0})	93.2
ASU	3
Resolution (Å) *	45.51 - 2.65 (2.74 - 2.65)
R_{sym} (%)*	6.5 (59.9)
I/σ_I *	16.21 (3.24)
Completeness (%)*	97.8 (97.9)
Unique reflections*	9592
Multiplicity*	4.0 (4.0)

Refinement	
Resolution (Å)	45.51 - 2.65
R_{work}/R_{free} (%)	18.22/27.39
No. atoms	2390
Protein	2382
Water	8
Ligands	0
Average B-factors (Å^2)	80.00
R.m.s deviations	
Bond lengths (Å)	0.008
Bond angles (^{0})	1.15
Ramachandran (%)	
Favored	96.0
Outliers	0.0

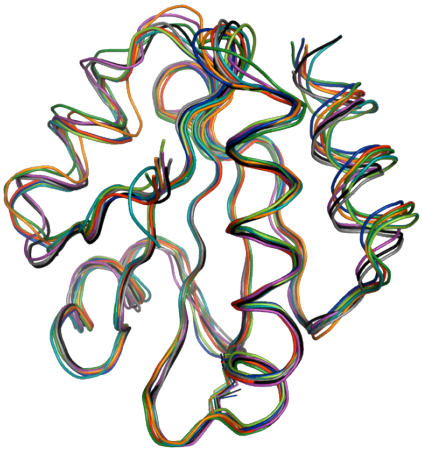

Figure 2.- **Conservation of thioredoxin fold.** Superposition of the seven ancestral thioredoxin structural models.

Other ancestral thioredoxin are been currently purified and their crystallization and structure determination will be undertaken in the near future.

2. A. Ingles-Prieto, B. Ibarra-Molero, A. Delgado-Delgado, R. Perez-Jimenez, J. M. Fernandez, E. A. Gaucher, J. M. Sanchez-Ruiz and J. A. Gavira. Conservation of Protein Structure over Four Billion Years. Structure, (2013), doi: 10.1016/j.str.2013.06.020

Future & perspective:

New constructs of both ancestral thioredoxin and specific mutants extant *E. Coli* thioredoxins are been produced and will undergo crystallization and structure determination. Therefore this project will continue during the next period.

Structural determination of the TodT/TodSm from *S. Putida*

Introduction:

We have obtained crystals of the full-length protein TodT with and without DNA at several conditions. Initial crystallization conditions have been found by the counter-diffusion method in the "Laboratory of Crystallisation Studies" in Granada (Spain). Further test has been performed using the hanging drop method. Crystal from counterdiffusion technique have been use to test for crystal quality in this period (Table 5). So far we have collected a full data set (Table 5.1) that will be used for searching an MR solution.

Summary of results:

As mentioned in the proposal we have been able to produce crystal of the REC domain of TodS previously. We keep on working trying to improve crystal size and quality.

Table 5				
ID14-1 (03-Nov-2012)				
Protein	Crystals #	Conditions	Cryos	Results

TodT	1	1: 0.2 M lithium sulphate, 0.1 M sodium acetate pH 4.5 & 50% v/v PEG 400	20% PEG 400	No diffraction
ID23-1 (20-Apr-2013)				
TodT	5	1 & 2: 1.0 M ammonium sulfate, 0.1M Bis Tris pH 5.5 & 1% w/v PEG 3350	No cryo	Full data set. **Table 5.1**
		3-5: Gel 2.6 cond 0.1M Hepes/NaOH pH 7.5, 20% PEG 4000 & 10% Isopropanol	No cryo	
ID14-1 (03-Nov-2012)				
Protein	N Crys.	Conditions	Cryo	Results
TodSm	10	1: 0.1 M MES pH 5.8, 20% PEG 4K & 0.25 M AS	20% Glycerol	Ice rings or poor diffraction
		2-4: 0.1 M MES pH 5.4, 20% PEG 4K & 0.25 M AS	20% PEG 400	
			20% MPD	
			20% Glycerol	
		5 & 6: 0.2 M lithium sulphate, 0.1 M NaAc pH 4.5, 50% PEG 400, 0.33% w/v 2,5-pyridinedicarboxylic acid, 0.33% w/v 4-nitrobbenzoic acid, 0.33% w/v mellitic acid & 0.02 M hepes sodium pH 6.8	20% PEG 400	
		7: 0.2 M lithium sulphate, 0.1 M Tris pH 8.5 & 40% PEG 400	No cryo	
		8: 0.2 M lithium sulphate, 0.1 M NaAc pH 4.5 & 50% PEG 400	No cryo	
		9: 0.2 M lithium sulphate, 0.1 M NaAc pH 4.5, 50% PEG 400, 0.25% 5-sulfoisophthalic acid monosodium salt, 0.25% anthraquinone-2,6-disulfonic acid disodium salt, 0.25% N-(2-acetamido)-2-aminoethanesulfonic acid, 0.25% tetrahydroxy-1,4-benzoquinone hydrate & 0.02 M Hepes sodium pH 6.8	20% PEG 400	
		10: 0.2 M lithium sulphate, 0.1 M NaAc pH 4.5, 50% PEG 400, 0.25% benzidine, 0.25% pheylglyoxal monohydrate, 0.25% sulfaguanidine, 0.25% sulfanilamide & 0.02 M hepes sodium pH 6.8	20% PEG 400	
ID23-1 (20-Apr-2013)				
TodSm	3	1-3: 0.1M sodium acetate pH 5.0, 20% PEG 400, 15% PEG 4000 & 10% PEG 8000	No cryo	Salt crystals or no diffraction

Table 5.1

Images	767
Space group	P 41 21 2
Unit cell	76.6 ,76.6, 36.6
Completness (Inner, Outer, Overall)	99.70, 98.00, 99.80
Rsymm (Inner, Outer, Overall)	2.00, 83.10, 5.60
Resolution	100.0-6.13 1.41-1.37 100.0-1.37
Detector resolution (Å)	1.51

Future & perspective:

TodT results are been used for RM structure determination while improvement of TodSm is undergoing in the laboratory. Different strategies including cross seeding are been tested.

Structural determination of the transcriptional regulator PtxS from *S. Putida*

Introduction:

We have obtained crystals of native PtxS by the counter- diffusion method. Diffraction test at BM16 has shown crystals diffracting up to 6Å. In order to increase protein stability we have performed co-crystallization experiments with PtxS bound to a consensus palindromic DNA sequence to which PtxS has been demonstrated to bind (Pineda et al., 2012).

1. E. Pineda-Molina, A. Daddaoua, T. Krell, J. L. Ramos, J. M. García-Ruiz and J. A. Gavira, In situ X-ray data collection from highly sensitive crystals of Pseudomonas putida PtxS in complex with DNA,Acta Cryst. (2012). F68, 1307.

Summary of results:

Crystals of the PTXS-ADN complex were grown in capillaries by the counterdiffusion method. To avoid crystal damage, previously observed with this system, crystals were cryo-protected into the capillary by diffusing the cryo-protectant. Portions of capillaries containing individual crystal were flash-frozen in liquid nitrogen and storage. Diffraction experiments were performed from Se-methionine derivatized protein from hexagonal shaped crystals. Unfortunately crystals lack of sufficient quality for data collection.

Table 6					
ID23-1 (20-Apr-2013)					
Protein	N Crys.	Conditions	Cryos	Capillary	Results
PtxS	2	1 & 2: 30% PEG 8000, 0.1M sodium cacodylate pH 6.5 & 0.2M NaAc	15% PEG 400	2 crystals in capillary	Poor diffraction

Future & perspective:

We are currently improving crystal quality.

Ancestral Lactamases

Introduction:

Experimental resurrection of ancestral proteins allows molecular evolution to be re-enacted in the laboratory and provides access to previously unexplored regions of the properties/sequence space. ß-lactamases, the enzymes responsible for the primary mechanism of resistance towards ß-lactam antibiotics, are widely distributed throughout the Eubacterial genomes and are believed to have originated several million years ago. We resurrected Precambrian ß-lactamases corresponding to several nodes in the evolutional tree. Despite of the extensive sequence changes involved (up to about 50%) we have found that ancestral Gram-negative micro-organisms are folded, highly stable (with denaturation temperature enhancements close to 35 degrees in some cases) and able to confer antibiotic resistance to modern micro-organisms (Risso et al., 2013). 2-3 Gyr old ß-lactamases are actually best described as “generalists”, with a moderate capability to hydrolyze different types of antibiotics (including third-generation). We plan to obtain 3D model structures from different nodes complete this study.

1. Valeria A. Risso, Jose A. Gavira, Diego F. Mejia-Carmona, Eric A. Gaucher, and Jose M. Sanchez-Ruiz, Hyperstability and Substrate Promiscuity in Laboratory Resurrections of Precambrian β-Lactamases. JACs 2013 135 (8), 2899-2902

Summary of results:

We have collected several data sets from different ancestral lactamases. Although ancestral bacterial lactamses, 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. PNCA was also collected al Proxima 1 (SOLEIL, PDB ID. 4C6Y) but we still search for a high resolution data set.

Table 7					
ID14-1 (03-Nov-2012)					
Protein	N. Crys	Conditions	Cryos	Loop / Capillary	Results
ENCA	3	1: 0.1 M tris/HCl pH 7.0 & 5.0 M sodium formate	20% glycerol	3 crystals in capillary	Salt crystals or poor diffraction
		2 & 3: 2.0 M lithium sulphate & 0.1 M Hepes/NaOH pH 7.5	20% Glycerol Paratone		
GNCA	3	1-3: 0.1 M sodium acetate pH 4.5 & 5.0 M sodium formate	50% Glycerol 20% Glycerol	3 crystals in loop	Not collected, low diffraction.
GPBCA	3	1-3: 30% PEG 4000, 0.1 M sodium acetate pH 4.6 & 0.2 M ammonium acetate	No cryo 20% PEG 400	3 crystals in loop	Poor diffraction
PNCA	6	1-6: 30% PEG 8000 & 0.1 M sodium acetate pH 6.5	20% PEG 400 20% Glycerol	6 crystals in capillary	Salt crystals or poor diffraction
ID23-1 (20-Apr-2013)					

GNCA	2	1 & 2: 2.0M lithium sulphate & 0.1M Hepes/NaOH pH 7.50	20% Glycerol 25% PEG 400	2 crystals in capillary	2 full data set. **Table 7.1**
GPBCA	1	1: 2.0M lithium sulphate & 0.1M Hepes/NaOH pH 7.50	20% Glycerol	1 crystal in capillary	2 full data sets. **Table 7.1**
PNCA	6	1-6: 20% PEG 8000, 0.1M sodium cacodylate pH 6.5 & 0.2M zinc acetate	20% Glycerol	4 crystals in capillary/2 crystals in loop	Full data set. **Table 7.1**
BM14U (17-Jul-2013)					
ConsPNCA	13	1-7: 25% PEG 4000, 0.1M sodium acetate pH 4.6 & 0.2M ammonium sulphate	15% Glycerol	12 crystals in loop/1 crystal in capillary	4 full data sets. **Table 7.2**
		8-10: 20% PEG 8000 & 0.05M potassium phosphate	15% Glycerol		
		11-13:30% PEG 4000, 0.1M sodium acetate pH 4.6 & 0.2M ammonium sulphate	15% Glycerol		
GNCA	1	1: 20% PEG 8000, 0.1M sodium cacodylate pH 6.5 & 0.2M zinc acetate	15% Glycerol	1 crystal in loop	No diffraction
GPBCA	6	1-6: 20% PEG 8000, 0.1M sodium cacodylate pH 6.5 & 0.2M zinc acetate	15% Glycerol No cryo	6 crystals in loop	Bad diffraction

Table 7.1

Protein	GNCA	GPBCA	PNCA
Space group	P 6 2 2	P 62	P 2 2 2
Unit cell	110.4 (90.0) 110.4 (90.0) 90.4 (120.0)	111.1 (90.0) 111.1 (90.0) 111.1 (120.0)	36.0 (90.0) 93.2 (90.0) 153.8 (90.0)
Completeness: Inner, Outer, Overall	99.20, 100.00, 99.80	98.60, 97.00, 99.50	97.40, 97.50, 99.00
Rsymm: Inner, Outer, Overall	4.30, 83.20, 10.40	3.90, 65.30, 4.90	6.90, 97.10, 18.90
Resolution	100.0-6.71 1.54-1.5 100.0-1.5	100.0-6.75 1.55-1.51 100.0-1.51	100.0-12.61 2.89-2.82 100.0-2.82
Detector resolution (Å)	1.45	1.70	2.23

Table 7.2 Data collection and refinement statistics. Statistics for the highest-resolution shell are shown in parentheses.

PDB ID.	4C75
Protein	ConsPNCA
Resolution (Å)	47.88 - 2.2 (2.279 - 2.2)
Space group	P 21 21 21
Unit cell	80.715 118.834 118.939 90 90 90
Total reflections	432575 (42699)
Unique reflections	58758 (5810)
Multiplicity	7.4 (7.3)
Completeness (%)	99.99 (100.00)
Mean I/sigma(I)	13.13 (3.15)
Wilson B-factor	25.97
R-merge	0.131 (0.6625)
R-meas	0.1411
CC1/2	0.995 (0.784)
CC*	0.999 (0.938)
R-work	0.1764 (0.2135)
R-free	0.2269 (0.2803)
Number of non-hydrogen atoms	8718
macromolecules	7915
ligands	77
water	726
Protein residues	1048
RMS(bonds)	0.006
RMS(angles)	1.00
Ramachandran favored (%)	98
Ramachandran outliers (%)	0
Clashscore	4.78
Average B-factor	37.20

Future & perspective:

We are currently working to produce crystal of better quality from GPBCA and preparing new constructs of other ancestral protein.

Choline sulphatase from *Sinorizobium meliloti* (CSSM)

Introduction:

Choline sulphatase catalyzes the conversion of choline-O-sulfate and, at a lower rate, phosphorylcholine, into choline. This enzyme belongs to a biosynthetic pathway for the production of glycine betaine starting from choline, which is a potent osmoprotectant accumulated by Sinorhizobium meliloti and other microorganisms to cope with osmotic stress. The crystal structure of this enzyme is unknown.

Summary of results:

We have obtained crystals of the wild type CSSM enzyme (alone, and co-crystallized with the product and the substrate of the reaction) and of H104 mutant. We have collected several data sets from these crystals cryoprotected in 10% PEG300.

Table 8

BM14U (17-Jul-2013)

Protein	N. Crys	Conditions	Cryos	Loop / Capillary	Results
CSSM+Choline	10	1M LiSO4 0.1M Hepes pH 7.5	10% PEG300	crystals in loop	Collected, ~2.3Å
CSSM-H104A mutant+Choline	3	1M LiSO4 0.1M Hepes pH 7.5	10% PEG300	crystals in loop	Collected, ~2 Å
CSSM	11	1M LiSO4 0.1M MOPS pH 7.0 1M LiSO4 0.1M MOPS pH 7.5 1M LiSO4 0.1M Hepes pH 7.5	10% PEG300	crystals in loop	Collected, ~2.5 Å
CSSM+subtrate	7	1M LiSO4 0.1M MOPS pH 7.0 1M LiSO4 0.1M Tris pH 8.0 1M LiSO4 0.1M Tris pH 8.5	10% PEG300	crystals in loop	Collected, ~2.5 Å

Future & perspective:

Datasets are been used for MR structure determination, although we are having problems since the asymmetric unit may allocate 8 monomers but so far we have been able to position only six.

Structural determination of *Pseudomonas* chemotactic transducer A and B

Introduction:

Pseudomonas aeruginosa is not only an important opportunistic pathogen and causative agent of emerging nosocomial infections but can also be considered a model organism for the study of diverse bacterial mechanisms that contribute to bacterial persistence. In this context the elucidation of the molecular mechanisms responsible for the switch from planktonic growth to a biofilm phenotype and the role of inter-bacterial communication in persistent disease should provide new insights in *P. aeruginosa* pathogenicity

We are interested in the stcructural characterization of some of the *P. aeruginosa* chemoreceptors such as PctA and PctB (*Pseudomonas* chemotactic transducer A and B) which are involved in the chemotaxis of these bacteria in response to the binding of L-amino acids. However the binding spectrum of both receptors is quite different despite they are proposed to share a similar structure.To elucidate the way of binding of these co-factors, we have produced crystals of PctA and PctB pre-incubated with several of their natural ligands. Preliminary results form ID14-4 (ESRF) have already been pubished (Rico-Jimenez, et al., 2013)

1. M. Rico-Jiménez, F. Muñoz-Martínez, T. Krell1, J. A. Gavira and E Pineda-Molina. “Purification, crystallization and preliminary crystallographic analysis of the ligand binding regions of the PctA and PctB chemoreceptors from Pseudomonas aeruginosa in complex with amino acids.” *Acta Crystallographica Section F* (Accepted).

Summary of results:

X-ray diffraction experiments are crucial to test their diffraction properties under different cryo- conditions and pursuit their structural resolution. Below we sumarize the tested crystal of PctA and PactB with different aminoacids.

Tabla 9					
ID14-1 (03-Nov-2012)					
Protein	N. Crys	Conditions	Cryos	Loop / Capillary	Results
PctB-Asn	4	1-4: 2.0 M AS, 0.1 M Hepes pH 7.5 & 4% PEG 400	15% Glycerol	4 in capillary	Ice rings and poor diffraction
PctB-Ile	1	1: 2.0 M sodium formate & 0.1 M sodium acetate pH 4.6	No cryo	1 in capillary	Full data. **Table 9.1**
PctB-Trp	1	1: 2.0 M sodium formate & 0.1 M sodium acetate pH 4.6	No cryo	1 in capillary	Ice rings and poor diffraction
ID23-1 (20-Apr-2013)					
PctA-Arg	4	1-4: 2.0M AS & 0.1M Tris-HCl pH 8.50	20% Glycerol in 3.0M AS	4 in capillary	Ice rings and poor diffraction
PctA-Gln	3	1-3: 2.0M AS & 0.1M Tris-HCl pH 8.50	20% Glycerol in 3.0M AS	3 in capillary	Ice rings and poor diffraction

Table 9.1	
Images	266
Space group	P 21 21 21
Unit cell	70.1, 76.8, 115.3
Completness (Inner, Outer, Overall)	99.20, 99.90, 99.70
Rsymm (Inner, Outer, Overall)	3.30, 70.00, 7.20
Resolution	100.0-4.61 2.22-2.14 100.0-2.14
Detector resolution (Å)	1.51

Future & perspective:

We are producing crystals with other amino acids and it is expected to have a MR solution of PctA from previous collected data and used it to determine the PctB 3D model. Binding site for each ligand will be the main focus from this series of expected structures.

Production resume

Articles:

- A. Ingles-Prieto, B. Ibarra-Molero, A. Delgado-Delgado, R. Perez-Jimenez, J. M. Fernandez, E. A. Gaucher, J. M. Sanchez-Ruiz and J. A. Gavira. “Conservation of Protein Structure over Four Billion Years.” *Structure*, (2013), doi: 10.1016/j.str.2013.06.020.
- M. Rico-Jiménez, F. Muñoz-Martínez, T. Krell1, J. A. Gavira and E Pineda-Molina. “Purification, crystallization and preliminary crystallographic analysis of the ligand binding regions of the PctA and PctB chemoreceptors from Pseudomonas aeruginosa in complex with amino acids.” *Acta Crystallographica Section F* (Accepted).
- Bacarizo, J. and Camara-Artigas, A. (2013). “Atomic resolution structure of the c-Src SH3 domain in complex with two high affinity peptides from class I and II.” *Acta Crystallographica Section D* 2013 May;69(Pt 5):756-66
- J. Bacarizo, M. Andujar-Sánchez, E. Ortiz-Salmerón , J.L. Neira and A. Cámara-Artigas. “A single mutation affects the amyloid formation in the SH3 domain of the c-Src tyrosine kinase.” (to be submitted)
- A. Cámara-Artigas, J. Bacarizo Roa, S. Martínez-Rodríguez, F. Gil, J. Benach and J. Juanhuix. “A cloning artifact generating an atcun site as a helpful tool in protein crystallography.” (to be submmited)
- A. Cámara-Artigas, S. Martínez-Rodríguez, E. Ortiz-Salmerón and J. M. Martin-Garcia. “Domain swapping in a chimeric c-Src-SH3 domain takes place through two hinge loops.” (to be submited)

- V. A. Risso, J. A. Gavira, E. A. Gaucher and J. M. Sanchez-Ruiz. “Phenotypic differences between consensus variants and laboratory resurrections of Precambrian proteins.” (to be submited)

Deposited PDB list:

3ZIV, “*Crystal Structure of Ancestral Thioredoxin Relative to last Archaea- Eukaryotes Common Ancestor (AECA) from the Precambrian Period*”

4C75, “*Consensus (all-con) beta-lactamase class A*”

4HVV,*"Crystal structure of the T98E c-Src-SH3 domain mutant in complex with the high affinity peptide APP12"*

4LE9 *"Crystal structure of a chimeric c-Src-SH3 domain"*

4JZ3 *"Crystal structure of the chicken c-Src-SH3 domain intertwined dimer"*

4JZ4 *"Crystal structure of chicken c-Src-SH3 domain: monomeric form"*