



Experiment title: Macromolecular Crystallography at South-East Andalusia

Experiment number:
MX-1938

Beamline: ID30A-3	Date of experiment: From: 08 Julio 2018 at 09:30 to 09 Julio 2018 at 08:00	Date of report: 24/10/2018
Shifts: 3	Local contact(s): GIACHIN Gabriele	<i>Received at ESRF:</i>
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Partial Report of Mx1938 (ID30A-3):

This up-date report corresponds to the six round of data collection at ID30A-3 within the Mx1938 proposal. We brought 110 samples from the team grouped as CSIC-UGR (60 samples) and from the Almeria University (50 samples). All the samples were tested and the main results are summarized below.

Crystals from CSIC-UGR (Table 1):

i) LBD-TlpQ bound to different ligands. We have already determined the structure of TLPQ-LBD bound to histamine (PDB ID. 6FU4) from Se-met data collected at ESRF. The article has been accepted in mBio but we still were searching for identified ligands. We tested 7 crystals but all of them of low quality.

Future perspectives: This project is finished.

ii) LysR-type transcriptional regulator (Admx) from *rizobacterium plymuthica*. AdmX is a transcriptional activator of the adm gene cluster that produce andrimid. In collaboration with the EEZ (CSIC) we have crystallized the ligand-binding-region bound to IAA (3-indolacetic acid) and tested for crystals improvement with no succeed.

Future perspectives: We are producing the Se-met derivatives.

iii) L-carbamoylase from *Sinorhizobium meliloti* (SmeLCar). L-specific carbamoylases are biotechnological important enzymes allowing the use of the hydantoinase process for the production of different optically pure L-amino acids. We solved in the past the first structure of an L-carbamoylase (PDB 35NF), but we have been unsuccessful in obtaining liband-bound structrues with this enzyme. We have crystallized a new L-carbamoylase of unknownn structure. Only a dataset at a modest resolution was obtained. MR trials have been unsuccessful.

Future perspectives: Crystal improvement/optimization is being carried out.

iv) Hydantoin racemase from *Ensifer meliloti* (HR). Hydantoin racemase enhances the enzymatic tandem known as “hydantoinase process”, utilized worldwide in the industrial production of tons of optically pure D- or L-amino acids (precursors of different commercially available antibiotics, such as ampicillin or amoxicillin). We solved the first structure of the C181A mutant of HR from a dataset collected at ID30A-3 in a previous round of the Mx1938 proposal, and several other ligand-bound structures during this bag-proposal. In this expedition, we have diffracted 3 crystals belonging to the WT enzyme, soaked with two different substrate/inhibitors, and different new ligand-soaked crystals of the HRC181A mutant. We have been successful in obtaining the Hg²⁺- and IBH-bound structures.

Future perspectives: Final cycles of model building and refinement corresponding to different free and bound structures obtained during the Mx1938 proposal are being carried out. The corresponding structures will be deposited asap, and the corresponding paper will be prepared in the near future.

v) Aminomutase: aminomutases are important enzymes for the biosynthesis of Beta-amino acids. We previously crystallized this enzyme and could solve its structure from a dataset obtained at ALBA synchrotron. However, it lacked different important regions containing putative catalytic residues. We have again crystallized this protein, and have bought 4 crystals. Only one crystal diffracted, at a resolution of approx. 2.8 Å. Initial inspection of the structure shows no Fe-S, but allows envisioning the presence of the previously unobserved loops.

Future perspectives: Crystal improvement/optimization is being carried out.

Table 1. Data collected by the CSIC-UGR team.				
Protein	Samples	Conditions	Cryo	Notes
TlpQ	7	C14+1mM Spermidine	15% Gol	2 Full data sets at 2.4 Å.
AdmX	20	PPP9, PPP8, C24 & C18	10-30% PEG 200	2 Full data sets, worse than at 2.6 Å.
SmeLcar (SER35)	12	C9, C18 KCHRI	15% GOL, no cryo	Poor diffraction, only 1 full dataset 2.5 Å
HR	17	A.S5 + A.S9	15% GOL	9 full dataset (different ligands)
Aminomutase	4	C44 PEGION	20% GOL, 20 PEG200	1 full dataset 2.7 Å

Crystals from Almeria (Table 2):

i) PDZ2-PSD95. This domain was crystallized alone and in complex with the n-NOS-PDZ, but only few and small PDZ2-PSD95 crystals were obtained. No diffraction was observed.

Future perspectives: We are working in the improvement of the crystal and the co-crystallization of the complex.

ii) Synthetic construct of GP41 (SC-GP41). Several crystals belonging to different constructions of the SC-GP41 in complex with several high affinity peptides have been obtained. We brought in this beamtime some crystals of the complex cov3ABC-W, but the crystals were small and didn't diffract. We count already with a crystal structure of this complex solved at 2 Å resolution.

Future perspectives: Our intent is to improve the quality of the diffraction.

iii) SH3 domain from an oncogenic c-Src tyrosine kinase (VSRT). We have measured twelve crystals belonging to VSRT soaked in urea solutions. These crystals diffracted at a resolution of ~2 Å. The solution of the structure and analysis is under way.

iv) Chimeric constructions of the c-Src and Fyn SH3 domain. We have cloned some chimeric constructions of the c-Src-SH3 domain where the RT- (SF-RT), n-Src (SF-Src) and both (SF-2X) loops belonging to this SH3 domain have been interchanged by those present in the homologous Fyn-SH3 domain and vice versa (FS-RT, FS-Src and FS-2X). We have measured crystals from FS-SRC and SF-SRC chimeras in presence of the chemical denaturant urea at acidic and neutral pHs. Crystals diffracted at high resolution (~1.5 Å) but not molecules of urea were observed. We have concluded that the procedure to obtain the crystals affect to the binding of the denaturant.

Future perspectives: We are working to improve the procedure to obtain new crystals.

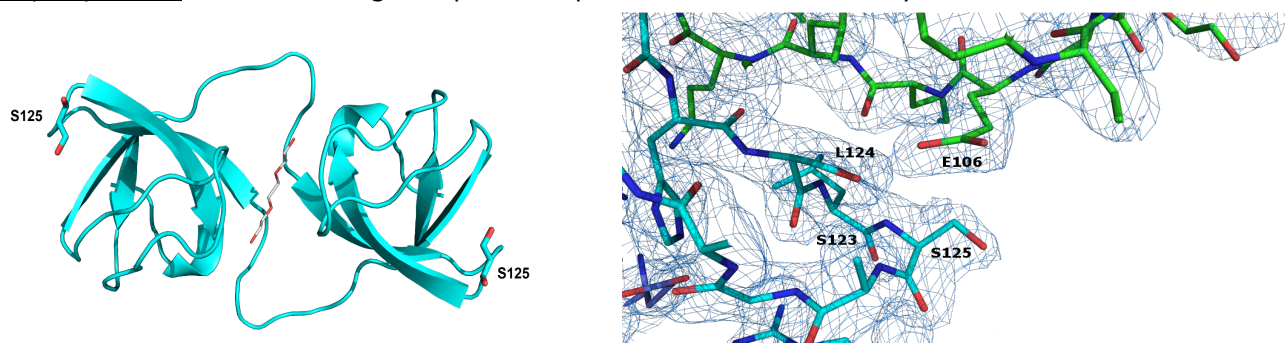


Figure 1. c-Src-SH3 T125S structure. Cartoon representation of c-Src-SH3 T125S which belongs to the intertwined dimer, the PEG molecule is between the two monomers (Left). Density maps of the MR Solution where the mutation T125S is showed (Right).

v) Chimeric constructions of the c-Src and c-Abl SH3 domain. Same as the previous chimeras, we have cloned the chimeric constructions: SA-RT, SA-Src, SA-2X, AS-RT, AS-Src, AS-2X. We have measured three crystals of AS-2X and one of AS-SRC, but no diffraction was observed.

Future perspectives: We are working to improve these crystals.

vi) c-Src-SH3 crystals. We have measured 8 crystals belonging to the mutant Q128E, 3 crystals of the T125S mutant and 3 crystals belonging to WT. We have solved the structure of all these mutants crystallized using ammonium sulphate. All these crystals belong to the intertwined dimer. These crystals diffract at moderate resolution ($\sim 2\text{\AA}$). We soaked the crystals in different solutions with the purpose to improve the quality of the diffraction.

Future perspectives: We have observed a slight improvement in the resolution but want to go better.

vii) Fyn-SH3 crystals. We have measure three crystals of this domain, but no diffraction was observed.

Future perspectives: We are working in crystals improvement.

Table 2. Data collected by the Almeria team.				
PROTEIN	SAMPLES/DIFFRAC.	CONDITIONS	DIFFRACTION (\AA)	SPACE GROUP/CELL
PDZ2-PSD95	4/0	0.8M NaH_2PO_4 , 0.8M KH_2PO_4 , 0.1M TRIS/HCl pH8.0	-	No diffraction.
SC-GP41	1/0	0.2M Li_2SO_4 , 0.1M MES pH6.0	-	No diffraction.
VSRT	12/7	2.0 M $(\text{NH}_4)_2\text{SO}_4$, 0.1M TRIS/HCl pH8.0	1.8-2.2	P322: 38 38 66
AS-SRC	1/0	1M Li_2SO_4 , 1mM DTT, 0.1M Na Ac pH4.0	-	No diffraction.
AS-2X	3/0	3M $(\text{NH}_4)_2\text{SO}_4$, 40 mM NaCl, 0.1M TRIS/HCl pH8.0	-	No diffraction.
SRC-WT	3/3	2.0 M $(\text{NH}_4)_2\text{SO}_4$, 0.1M HEPES pH7.0	1.5-2.1	P6122: 47 47 127
SRC Q128E	8/8	2.0 M $(\text{NH}_4)_2\text{SO}_4$, 0.1M MES pH6.0	1.6-2.4	P6522: 47 47 127
FYN-WT	3/0	1.5M Na formiate, 0.1M TRIS/HCl pH8.0	-	No diffraction.
SRC T125S	3/3	3.0M Na formiate, 0.1M MES pH6.0	~ 2.1	P 65 2 2: 47 47 127
FS-SRC	6/3	1.5 M $(\text{NH}_4)_2\text{SO}_4$, 0.1M NaAc pH5.0	1.5	P212121: 28 32 59
SF-SRC	6/5	2.0 M $(\text{NH}_4)_2\text{SO}_4$, 0.1M HEPES pH7.0	1.5	P1 211: 45 40 56