ESRF	**Experiment title: Macromolecular Crystallography at South-East Andalusia**	**Experiment number**: MX-1830
Beamline: ID30A-1	**Date of experiment**: From: 25 July 2017 at 09:30 to 25 July 2017 at 17:00	**Date of report**: 12/08/17
Shifts: 1	**Local contact(s)**: NURIZZO Didier / BOWLER Matthew	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists): Jose A. GAVIRA-GALLARDO[1], Ana CAMARA-ARTIGAS[2*], Sergio MARTINEZ-RODRIGUEZ[3], Mª Teresa CONEJERO-MURIEL[1], FERNANDEZ Raquel[1]

1. Laboratorio de Estudios Cristalográficos, IACT, CSIC-UGR, Spain.
2. Dpto. Química y Física, University of Almeria, Spain.
3. Dpto. Química Física, University of Granada, Spain.

Partial Report of MX1830 ID30A (25-07-2017):

This partial report corresponds to the data collected at ID30A-1 programed for the 24th of July, problem with the transport company delay the experiment one day.

We sent a Dewar with 50 samples from the Almeria University (UAL) but only 40 samples were measured. All the crystals belong to different projects of the UAL protein crystallography lab, some of them in collaborations with different groups of the UGR. All these projects are supported by grants belonging to the Spanish Government. Among samples not belonging to any actual project we have tested some crystals belonging to a phycocyanin from the cyanobacteria *S. Platiensis*.

1. c-Src-SH3 crystals. We have measured 3 crystals belonging to the double mutant H122T/Q128K. We have solved the structure of this double mutant crystallized using ammonium sulphate: previous crystal show the presence of a pseudo porphyrin ring at the amino terminal bound to Ni^{2+}. The main crystal contact is facilitated by a cyclodextrin molecule. We have replaced the nickel salts and the cyclodextrin additives by a non-detergent sulphobetaine (NDSB). Crystals diffract at atomic resolution and structure solution is under way.

2. 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- and/or n-Src loops belonging to this SH3 domain have been interchanged by those present in the homologous Fyn-SH3 domain. In this way, we have obtained three different chimeras: SF-RT, SF-Src and SF-2X, which correspond to the replacement of the RT loop alone, the n-Src loop alone and replacement of both loops, respectively. Also we have obtained the chimeric constructions of the Fyn-SH3 where the loops have been replaced for those preset in the c-Src-SH3 domain. We have obtained crystals from SF-Src that diffract at atomic resolution (~1Å). In the case of the FS chimeric constructions (Fyn SH3 domain where the loops have been interchanged by those in the c-Src-SH3 domain), we have measured two crystals of the FS-Src in complex with the peptide VSL12, both diffracting to atomic resolution. Unfortunately, only one crystal

has been measured with the righ completeness. Fortutatelly this set of data correspond to the higher resolution crystal (1 Å).

3. Chimeric constructions of the c-Src and c-Abl SH3 domain. Same that previos chimeric proteins, we have interchanged the c-Src-SH3 domain loops by those present in the Abl-SH3 domain. In this case, the Abl-SH3 domain show a different way of interaction between the canonical binding motif PxxP. The presence of negatively charged residues in the RT loop of the c-Src SH3 allows the orientation of the polyproline motif through salt bridges, while the interaction in the Abl Sh3 domains is preferential linked to hydrogen bond interactions. Same that with the Fyn SH3 domain we have obtained three different chimeras: SA-RT, SA-Src and SA-2X, which correspond to the replacement of the RT loop alone, the n-Src loop alone and replacement of both loops, respectively. We have solved previously the structures of the chimeric constructions SA-2X and SA-RT at different pHs. The crystals of the SA-RT have been obtained in presence of three different polyproline rich motif (PRM), the peptides VSL12, APP12 and NS5A. Most of the crystals diffract at atomic resolution and although the crystal cell is alike to that reported for the crystals obtained in absence of the peptides, the electron density show the presence of the peptide bound to two molecules of the SH3 domain at the same time. This behavior has been observed previously in other SH3/PRM structures (as an example, see PDB entries 1FYN and 3UA7). Some crystals diffract at resolution higher than 1 Å, unfortunately the last shell show a low completeness that reduces the real resolution of the data.

4. Third PDZ domain PSD95. We have measured several crystals of this domain, but data are lower quality than other measured previously. Data have not been processed.

5. covABC. These crystals belong to a synthetic construction of the amino-terminal domain of the GP41. No diffraction was observed.

6. Third WW domain of Nedd4. We have previously obtained data at 3.5 Å from this small modular domain. Unfortunately, any attempt to improve crystal quality fail.

Results obtained from this single and incomplete shift are summarized in Table 1. Crystal left to measure will be processed at the end of August.

Table 1.- Data collected by the UAL lab on July 24th , ID30A

Crystal	Samples/ Diffraction	Crystallization	Diffraction	Space group/cell
c-Src-SH3 H122T/Q128K	3/3	Ammonium sulphate + NDSB pH 7.0	1.1-1.35 Å	P3121 / 36 36 82 90.0 90.0 120.0
SF-Src	4/3	Ammonium sulphate + Betaine or NaSCN pH 7.0	1-1.3 Å	P1/ 35 41 45 74 69 70; P21/ 28 40 43 90 104 90
SF-Src+APP12	1/1	Ammonium sulphate pH 4	1-1.3 Å	C2/ 67 32 58 90 96 90
FS-Src+VSL12	2/2	Ammonium sulphate pH 4-5	1 Å (1.4 Å data set low completeness)	P212121/ 33 33 63 90 90 90
SA-RT	12/10	Ammonium sulphate pH 6-8	0.96-1.2 Å	C2221 /41 56 52 90 90 90
PDZ3 domain	8/2	Ammonium sulphate	1.3-3.6 Å	P212121/ 30 55 66 90 90 90
covABC-W	2/0	Sodium Chloride	No	-
Phycocyanin SP	7/0	PEG4K/8K pH 6.5-7.0	No	-
WW3-Nedd4	1/0	Ammonium sulphate	No	-