



**Experiment title: Macromolecular Crystallography at South-East Andalusia**

**Experiment number:**  
MX-1629

<b>Beamline:</b> ID23-2	<b>Date of experiment:</b> From: 05 July 2015 to: 06 July 2015	<b>Date of report:</b> 06/08/15
<b>Shifts:</b> 3	<b>Local contact(s):</b> POPOV Alexander	<i>Received at ESRF:</i>

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#### **Partial Report of Mx ID23-2 (05-07-2015 / 05-07-2015):**

This is up-dated report of the data collected at ID23-2 during the 5<sup>th</sup> round of MX-1629. We send to the ESRF 100 samples from the teams CSIC-UGR and UAL, 50 each, but the Dewar coming from Granada was not delivery in time. It arrived Monday the 6<sup>th</sup>. Thanks to David Flot and Matthew Bowler we manage to leave the dewar in place after formalizing an on-the-flight petition of time for ID30A-1.

#### Crystals from UAL (Almería):

In this BAG at the beamline ID-23-2 the UAL lab collected data from 50 crystals. Unfortunately, most of the crystals diffracts weakly or didn't diffract. Table 1 shows a resume of the data collection. Briefly:

<b>Table 1.-</b> Data collected by the UAL laboratory.				
ESRF Experiment		Beamline: ID23-2	T <sup>a</sup> : 100 K	BAG: MX-1629
Protein	Samples	Conditions	Cell	Resolution
Choline sulfatase	4	1.4 M LiSO <sub>4</sub> ; 0.1M Hepes pH6.5	C121/127.1 205.8 117.3 (90.0 110.2 90.0)	Data good at resolution >3.5 Å
Abl n-Src mutant	1	4.3 M Sodium chloride 0.1 M Sodium HEPES pH 7.5	No diffraction	-
Tsg101-UEV/Nef L-domain	4	0.1M Hepes pH 7; 20% PEG 6K; 5mM BaCl <sub>2</sub>	No diffraction	-
Tsg101-UEV/Ubiquitin	1	0.1M AcONa pH 4.6; 1.5M AmSO <sub>4</sub> ; 1mM CuCl <sub>2</sub>	No diffraction	-
Tsg101-UEV/Proline	10	0.1M Hepes pH7; 0.25M AmSO <sub>4</sub> ; 25% PEG 4K; 100mM Prolina	P21/ 38.921 168.223 195.548 90 92.905 90	Data good at resolution >2.5 Å. Structure under refinement
Nedd4-WW3 domain	30		No diffraction	-

- Choline sulphatase (4 crystals).** We brought to the ESRF crystals of this protein obtained in presence of Choline sulphate that grows in presence of 1.4M LiSO<sub>4</sub> at pH 6.5. We have already solved the structure this protein which show a modified cysteine residue at the active site. Currently we have solved the structures of WT and the active-site mutant His104Ala with and without the substrate choline. We want to complete the information about this protein collecting data from the choline sulphate complex but the crystal only diffracts at >3.5 Å resolution, which makes difficult to model the active site.
- Proline rich sequences (PRMs) binding domains (46 crystals).** We brought to the ESRF 30 crystals of the third WW domain of the Nedd4. These crystals grown in 11 different conditions but none of the crystals

diffracted. We have measured also 10 crystals of the TSG101-UEV domain obtained in presence of proline. This additive improve the crystallization of the protein: crystal were obtained in only 1-2 day as compared with several weeks needed in absence of this additive. The structure is under refinement (Rwork/Rfree= 0.22/0.28) (Figure 1).

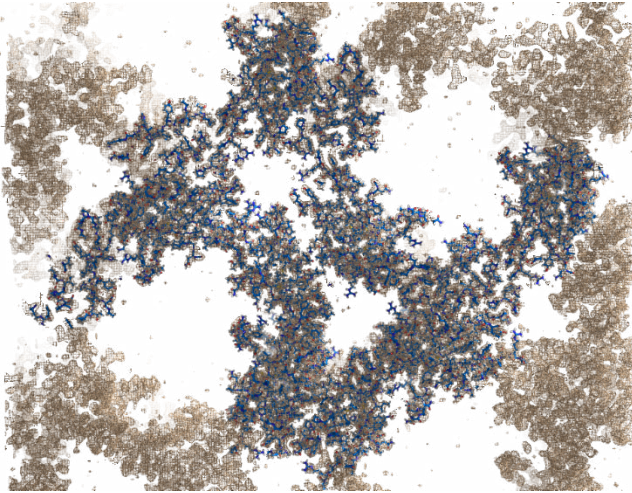


Figure 1.-Cell unit of the TSG101-UEV domain crystallized in presence of proline. The AU is composed by 12 molecules of the UEV domain of the Tsg101. The crystal show a pseudo-hexagonal symmetry.

Future & perspective: We continuous working in the different proteins collected in this table to reach the goals exposed in the proposal of this BAG.