ESRF	**Experiment title: Macromolecular Crystallography at South-East Andalusia**	**Experiment number**: MX-2064
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Partial Report of MX2064 ID30A-3

This partial report corresponds to the second data collection experiment of the Mx2064 carried out at ID30A-3. We tested 100 samples from the Glasgow (Table 1) and Almeria (Table 2) teams.

Crystals from Institute of Infection, Immunity and Inflammation-University of Glasgow (Table 1):

The phage-inducible chromosomal islands (PICIs) are a family of highly mobile genetic elements (MGE) that contribute to horizontal gene transfer, host adaptation, and virulence. They are molecular parasites that exploit phages as helpers, using a variety of elegant strategies to manipulate the phage life cycle for promote their own spread.

Following infection by a helper phage the PICI genome excises, replicates using its own replicon, and is efficiently packaged into infectious particles composed of phage virion proteins.

Staphylococcus aureus pathogenicity islands (SaPIs) are the prototype members of the family and contain four distinct modules that mediate their regulation, excision and integration, autonomous replication and phage packaging exploitation (ERP cycle). For study the ERP cycle in SaPIS we have crystallized several protein complexes:

i) STL repressor of SaPI1 in complex with antirepressor 80α Sri in Hominis Species.

After infection by a helper phage, a phage anti-repressor protein relieves Stl-mediated repression of the SaPI, initiating the ERP cycle. We obtained several crystals of the antirepressor Sri (Hominis) in complex with STL (Staphyloccocus Hominis) to characherize the de-repression mechanism. In this round, we tested 8 crystals with different cryoprotectants and precipitants and collected one data set at 4 Å, belonging to the P 2(1)2(1)2(1) space group.

Future perspectives: Improve the crystal´s quality to increase resolution.

ii) STL SaPI1 in complex with Staphylococcus Aureus DNA. In order to characterize the repression of the island, we co-crystallized the complex of STL/DNA for the first time. We tested 42 crystals using different cryoprotectants and precipitant agents, but we got 15 Å of maximum resolution in the best of the case.

Table 1. Data collected by the Institute of Infection, Immunity and Inflammation-University of Glasgow

Protein	Samples	Conditions	Cryo	Resolution
STL_hom/Sri hom	8	0.2 M NaAcetate, 0.1 M Na-citrate pH 5.5, 5 % w/v PEG 4000	35% PEG200, 30% PEG 400	1 data set at 4.0 Å

STL_SaPI1/DNA	2	0.2 M NaCl 0.1 M Na/K -phosphate pH 6.5, 25 % w/v PEG 1000	40% PEG200, 10% MPD	Not collected, max. 15 Å
STL_SaPI1/DNA	2	0.1M Hepes pH 7, 22%PEG 500	20-40% PEG 400, 5%MPD, 20%GOL	No data set.
STL_SaPI1/DNA	10	0.1M Hepes pH 7.5, 22%PEG 500, 0.5M NaCl	40% PEG400	Not collected, max. 18 Å
STL_SaPI1/DNA	5	0.1M Tris pH 8, 22%PEG 500, 0.5M NaCl	30%Glycerol	Not collected, max. 20 Å
STL_SaPI1/DNA	5	0.1M Tris pH 8.5, 22%PEG 500, 0.5M NaCl	40% Sucrose	Not collected, max. 18 Å
STL_SaPI1/DNA	10	40% (v/v) PEG 400 100 mM Na-citrate pH 5.5, 200 mM Magnesium chloride	Included	Not collected, max. 18 Å

Crystals from Almeria (Table 2):

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, but the crystals were small and did not diffract, or did it at low resolution (~3.0 Å).

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

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 SF-2X, SF-RT and SF-SRC chimeras, the latest ones were soaked in bromophenol blue and in presence of the chemical denaturant urea at acidic and neutral pHs. Crystals diffracted at high resolution and medium resolution (~1.6-2.3 Å).

Future perspectives: We are working to find the procedure to obtain crystals with highest resolution.

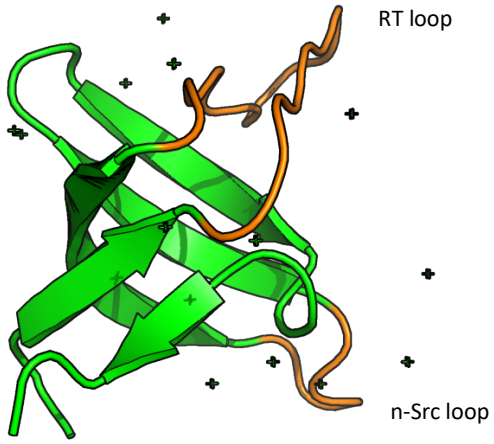

Figure 1. AS-2X structure. Cartoon representation of the Abl-SH3 domain where the RT- and n-Src loops (in orange) belonging to this SH3 domain have been interchanged by those present in the homologous c-Src-SH3 domain

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 3 crystals of AS-2X, which 2 diffracted at moderate resolution (~1.9-2.2Å), 3 crystals of AS-SRC, which 1 diffracted at moderate resolution (~2.2Å) and 2 crystals of SA-Src, but no diffraction was observed.

Future perspectives: We are working to improve these crystals.

vi) c-Src-SH3 crystals. We have measured 2 crystals belonging to the mutant T125S mutant. We have solved the structure of these mutants crystallized using ammonium sulphate. All these crystals belong to the intertwined dimer. These crystals diffract at moderate resolution (~1.9-2.2Å). We soaked the crystals in bromophenol blue 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.

iii) Lysozyme. We have measured 19 crystals of lysozyme soaked in different dyes at acidic, neutral and basic pHs. These crystals diffracted at a resolution of ~1.5-3.0 Å. We have concluded that a better resolution is needed to study the binding of the dyes.

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

Table 2. Data collected by the Almeria team.				
Protein	**Samples/diffrac.**	**Conditions**	**Diffraction (Å)**	**Space group/cell**
SC-GP41	14/6	0.4M Na-K tartrate tetrahidratado/ 2M Formiato-Na, 0.1M AcONa pH4.6/ 20% PEG6K, 0.1M TRIS pH8.0	-	-
SF-2X	3/3	1.5 M $(NH_4)_2SO_4$, 0.1M NaAc pH5.0, 5%PEG300, 5mM □ciclodextrin	1.6	P212121-24 33 60
SF-RT	3/2	2.0 M $(NH_4)_2SO_4$, 0.1M TRIS/HCl pH8.0, 5% glycerol	2.5	P4- 48 48 187
SF-Src	4/2	2.0 M $(NH_4)_2SO_4$, 0.1M HEPES pH7.0, 5%PEG300	1.9	P2- 28 41 44
AS-2X	3/2	2.0 M $(NH_4)_2SO_4$, 0.1M HEPES pH7.0	2.2	P41- 42 42 30
AS-Src	3/1	2.0M $(NH_4)_2SO_4$, 5%PEG300, 10% glycerol, 40 mM LiCl, 0.1M NaAc pH5.0	2.3	P222- 23 45 88
SA-Src	2/0	0.8M $(NH_4)_2SO_4$, 0.1M NaAc pH 5.5	-	-
SRC T125S	2/2	2.0 M $(NH_4)_2SO_4$, 0.1M HEPES pH7.0, 5% glycerol	1.9	P6122-47 47 126
Lysozyme	26/19	0.3-0.8M NaCl, pH 4.5-8.0	1.5-3.0	P41212- 78 78 38 P222- 30 56 73