ESRF	**Experiment title: Macromolecular Crystallography at South-East Andalusia**	**Experiment number**: MX-1938
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Shifts: 3	**Local contact(s)**: **LEONARD Gordon**	*Received at ESRF:*

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Partial Report of Mx1938 ID30B:

This up-date report corresponds to the third round of data collection at ID30B within the Mx1938 proposal. We brought 140 samples from the team grouped as CSIC-UGR, UAL and from our recently incorporated Bag-member from the GBRC at Glasgow University. All the samples were tested and the main results summarized below.

Crystals from CSIC-UGR:

i) LBD-McpH. In previous experiments we got diffraction data at 2.7 Å resolution but did not find MR solution. We are at present improving crystal quality prior MAD/SAD data collection. We tested two remaining crystals but none of them diffracted X-ray at a reasonable resolution. Further crystal improvement is required.

ii) L-amidase from *Pseudomonas* sp (PseAmid). L-specific amidases are industrially attractive enzymes, due to its potential for the production of optically pure L-amino acids starting from racemic mixtures of amino acid-amides, which are cheap precursors. We have cloned, expressed purified and crystallized the enzyme from *Pseudomonas*. Tested crystals diffracted X-ray up to 1.8 Å belonging to the orthorhombic space group $P2_12_12_1$. Structural determination is on-going by MR with R/Rfree of 18.5%/23.4% respectively.

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

iii) 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). In conjunction with a stereoselective hydantoinase and a stereospecific carbamoylase, hydantoin racemase allows generalizing the use of this enzymatic cascade to 5-monosubstituted hydantoin substrates for which chemical racemization is not favored. Despite the industrial application of hydantoin racemase dates to more than three decades ago, no structural information for this enzyme was available till date. We have been able to solve the first structure of the C181A mutant of HR from a dataset collected at ID30A-3 in the previous round within the Mx1938 proposal (Fig. 1A; C 2 with a = 92.25, b=137.08, c=57.19 and β=116.17). We have brought in this round 20 crystals of the same mutant grown in different conditions, some of them soaked with different ligands; we have also obtained from datasets collected in this round the structure of the HR bound to different inhibitors, from crystals belonging to a different SP than that of the free form (Fig, 1B; $H3_2$ with a=b=144.75 and c=57.35)

Future perspectives: Crystals of the WT enzyme have been already grown and flash-cooled for the next round of beam-time. Soaking of HR C181A mutant crystals with different substrates have also been prepared and flash-

cooled. The corresponding paper is being prepared, awaiting for the structures of the WT HR and different new complexes.

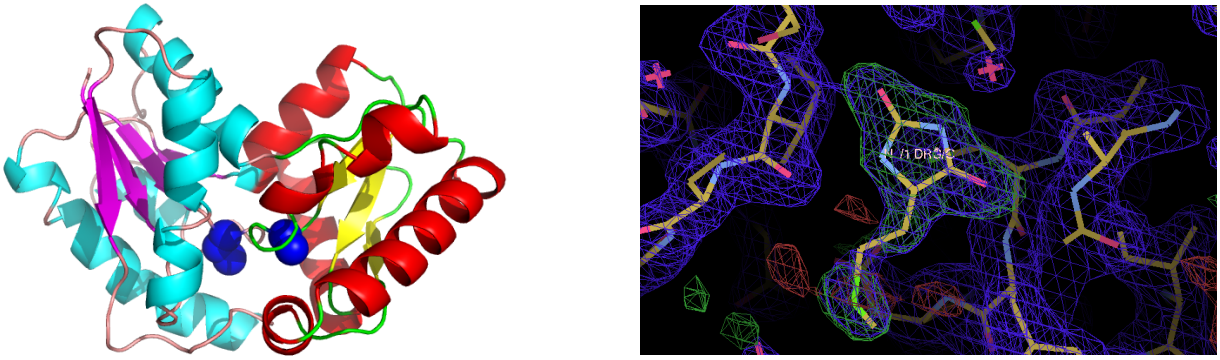

Figure 1. Left) Overall structure of the V-shaped monomer belonging to HR from *E. meliloti*. The two α-β-α domains have been represented in different colours; C76 and A181 residues, belonging to the catalytic centre, are shown as blue spheres. Right) Close-up of the catalytic centre of HR C181A mutant bound to the inhibitor LMTEH.

iv) Formamidase from *Bacillus cereus* (BceAmiF). During the review process of the corresponding manuscript, the reviewers asked for new experiments to try ascertaining the modification clearly shown in the catalytic cysteine of this enzyme (from structures at atomic resolution obtained previously from datasets collected at the ESRF). After extensive but unsuccessful efforts to try determining its nature, we decided to soak this enzyme with different modification-agents, which could support our hypothesis on the modification of this enzyme. Datasets up to 1.4 Å have been collected. MR of the different datasets is ongoing to ascertain whether new ligands are bound in the catalytic centre.

Future perpectives: Decision to be taken after solving the different datasets collected, but this project might be finalized.

Table 1. Data collected by the CSIC-UGR team.

Protein	Samples	Conditions	Cryo	Notes
McpH	2	AS 6	No cryo	No datasets.
PseAmid	8	C28	20% GOL	4 full datasets
HR	20	C14, C15, C18, C23	15% GOL	6 full datasets
BceAmiF	20	PPP4, PPP7, C28	15% GOL	12 full datasets.

Crystals from Institute of Infection, Immunity and Inflammation-University of Glasgow:

i) Small terminase subunit (TerS) in complex with phage inhibitor protein (ppi). In this round we collected several datasets of the PPi *E.Coli* labeled with Se-Meth and in native conditions at 3 and 2.4 Å respectively. The crystals belong to a tetragonal $P4_12_12_1$ space group and thanks to the anomalous SAD data collection we were able to solve the phase problem obtaining the for the first time the structure of the PPi protein (**Figure2**).

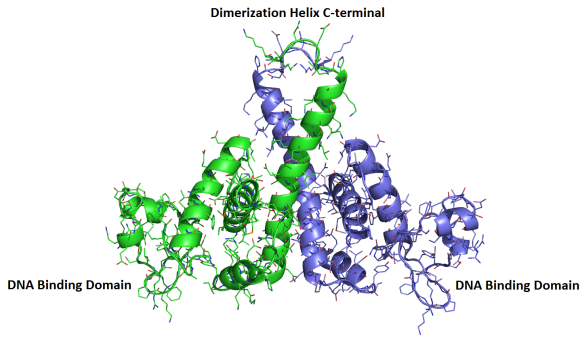

Figure2. PPI structure of 3 Å (Se-Meth) and 2.4 Å (Native) collected at ESRF. PPi structure were solved solving the phase problem using the B3x6 SAD dataset. The structure shows a long C-terminal alpha helix that produces the biological dimer.

ii) STL repressor of SaPI1 in complex with antirepressor 80α Sri. 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 (80α) in complex with STL (SaPI1) to characherize the de-repression mechanism. In this round, we tested several crystals with different cryoprotectants and precipitants and collected one data set at 2.7 Å. We obtained better resolution than in previous rounds and with the Se-Meth model obtained previously the structure is ready for deposit.

Table 2. Data collected by the Institute of Infection, Immunity and Inflammation-University of Glasgow				
Protein	**position**	**Conditions**	**Cryo**	**Resolution**
PPi-SeMeth	B3x6	0.1M Hepes pH 7.5; 10% PEG 6K	25% Glycerol	3.0 Å
PPi-native	B1x5	15% PEG 8K	30% Glycerol	2.5 Å
PPi-native	B1x4	15% PEG 8K	30% Glycerol	2.4 Å
Sri/STL-native	B2x10	0.4M AmPh	30%PEG 200	2.7 Å

Crystals from UAL:

i) 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, SF-2X, which correspond to the replacement of the RT loop alone, the n-Src loop alone and replacement of both loops, respectively. We have performed the same protocol with the Fyn-SH3 domain, where we have replaced the loops by those present in the c-Src-SH3. We have measured crystals from FS-RT chimera that diffract at high resolution (~1.5 Å). Besides, we have measured crystals of the FS-Src in presence of the chemical denaturant urea at acidic pHs resulting in near atomic resolution diffracting crystals.
ii) Chimeric constructions of the c-Src and c-Abl SH3 domain. Same as the previous chimeras, 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 distant Abl-SH3 domain. Although we have measured 10 crystals, we only collected data from one crystal and those data are bad quality and are not processable.
iii) SH3 domain from the oncogenic c-Src tyrosine kinase. We have measured six crystals belonging to the SH3 of the oncogenic c-Src tyrosine kinase. Some crystals diffract at high resolution and the structure has been solved at 1.4 Å.
iv) Chemotaxis protein CheY mutants. We have measured 14 crystals of different mutants of the CheY in presence of magnesium.
v) Synthetic coleid-coil based on the gp41 protein of the HIV. These studies are conducted to decipher the structural determinants of the interaction that allow the HIV to infect the cells and how these interactions can be inhibited. We have measures 10 crystals of a complex of a synthetic coleid-coil based on the gp41 amino-terminal region in complex with a high affinity peptide. The structure has been solved at 2 Å.
Results are summarized in Table 3.

Table 3. Data collected by the UAL team.				
Crystal	**Samples/Diffraction**	**Crystallization**	**Diffraction**	**Space group/cell**
c-Src-Fyn-SH3 RT	2/2	Ammonium sulphate + pH 4.5	~1.5Å	C21/ 73 47 43 90 97 90
c-Src-Fyn-SH3 SRC	3/2	Ammonium sulphate + pH 8	1.3-1.5Å	P21/ 28 41 44 90 105 90
c-Src-Fyn-SH3 SRC	2/1	Ammonium sulphate + pH 5	1.8 Å	P6522/ 47 47 125 90 90 120
c-Src-Abl-SH3 SRC	10/1	Ammonium sulphate + pH 5+ PEG300	? Å	No processing
v-Src- SH3	6/3	Ammonium sulphate + pH 7		P3121/ 37 37 66 90 90 120
CheY	3/2	PEG 6K+ MgCl2+pH 7.5	1-1.3 Å	P212121/ 33 33 63 90 90 90
covABC-W	10/4	PEG 4K+ pH 5-7	2 Å	P212121/ 30 75 96 90 90 90