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Partial Report of MX2281 ID30B

This partial report corresponds to the first data collection experiment of Mx2281 carried out at ID230B. We tested 100 samples from the Granada (URG and CSIC) (Table 1) and Almeria (Table 2) teams.

Crystals from Granada CSIC & UGR (Table 1):

i) Insulin (Ins). Recombinant human insulin is one of our model systems. We obtain the crystal in different types of hydrogel affecting the properties including dissolution rate, hardness, protein stability and crystal quality. For insulin crystals obtained in FMOC-dipeptide hydrogel were measured diffracting to 1.4 Å.

Future perspectives: On going project.

ii) hGo (Human Glycolate oxidase). Salicylic derivatives have shown to inhibit human glycolato oxidase (hGO) and, for this reason, they are considered drug candidates for the treatment of primary hyperoxaluria type I (PH-1). PH-1 is a rare disease affecting the hepatic metabolism of glyoxilate in which patients present systemic accumulation of oxalate crystals and lacks of pharmacological treatment. Salicylates have recently proved to be good inhibitors of GO and, very importantly, they show good phenotypic activities in hyperoxaluric hepatocytes. The determination of the structure of hGO-salicylates complexes will throw light to the binding mode of these compounds and will help the determination of their mechanism of inhibition. This will allow the establishment of reliable structure-activity relationships and, therefore, the development of new hits with improved activity. We have already obtained crystals of the protein without any inhibitor and co-crystallization and soaking will be done. We hope to determine the structure by MR and therefore native data sets at high resolutions is the main goal.

Future perspectives: Binding experiments are being conducted to ascertain the best ligands to be soaked/co-crystallized. Crystal improvement/optimization is being carried out.

iii) ATrx (Ancestral reconstructed thioredoxin). Our previous experimental characterization of the folding reaction of Precambrian and E. coli thioredoxins have revealed the role of residues in the 70-79 loop region in modulating the folding rate of thioredoxin. To get further insight into the nature of the interactions involved, we plan to study a set of modern thioredoxins, based on a search in the NCBI Reference Sequence Database to capture sequence diversity in an evolutionary context. Each reconstructed enzyme needs to be characterized and the 3D structure determined for comparison. Phases will be determined by MR. Only one of the variant produced diffracting crystals. The best data set produce data to a resolution of at 2.85 Å which have been used to solve the structure.

Future perspectives: Article is in preparation.

iv) EVH1 domain from human SPRED-1. EVH1-domains are evolutionary conserved protein domains of around 115 amino acids found in different proteins implicated in signalling, nuclear transport and cytoskeletal

events. They recognize and bind specific proline-rich sequences (PRSs), usually with low affinity, but tightly regulated by the high specificity encoded into residues in the protein:peptide interface. We have successfully produced the EVH1 domain from human SPRED-1, a protein responsible for Legious syndrome (a rare, genetic skin pigmentation disorder). We brought 9 crystals obtained in our fist crystallization set-up for this protein. Unfortunately, all of them resulted in salt crystals,

Future perspectives: New crystallization experiments have been conducted in order to obtain protein crystals.

v) D-acylase (QF3, 1 crystal**)**. We have demonstrated the application of the "amidohydrolase process" for the production of different non-natural D- and L-amino acids. D-acylases allow the production of different optically pure amino acids using an enzymatic DKR together with an NSAR. We have cloned and purified different putative D-acylases to evaluate their substrate promiscuity. We brought the only crystal we have obtained from this enzyme. Unfortunately, it resulted to be a salt crystal.

Future perspectives: New crystallization experiments have been conducted in order to obtain protein crystals.

Table 1. Data collected by the CSIC-UGR.

Protein	Samples	Conditions	Cryo	Resolution
ancTrx	16	HR-I: C11, C33	15% GOL	5 data sets, the best at 2.5 Å
Ins (FMOC)	4	Gel crystals	0-15% GOL	4 data sets, the best at 1.4 Å
hGo (L1-L5)	20	C37 & C38	15% GOL+Ligands	Several data sets, the best at 2.9 Å.
EVH1	9	HR-II: C27, J1: C3, J3: A6, B2	15% GOL	
QF3	1	C18	15% GOL	No diffraction.

Crystals from Almeria (Table 2):

i) 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. In this beamtime, we brought some crystals of the covNHR-VQ. The crystals were very small (<20 μm) and didn't diffract. Also, we try to improve the quality of the complex covNHR-VQ with CHR C34 the peptide, but the crystals diffracted at low resolution.

Future perspectives: To improve the quality of the covNHR-VQ crystals. Also, we are working with new constructions and we plan to measure these crystals soon.

ii) Chimeric constructions of the c-Src. We have cloned several chimeric constructions of the c-Src-SH3 domain where the RT-, n-Src and both loops belonging to this SH3 domain have been interchanged by those present in the homologous Fyn-SH3 domain and the non-homologous Abl-SH3 domain (SF-RT, SF-Src-SF-2X; SA-RT, SA-Src, SA-2X) and viceversa (FS-RT, FS-Src and FS-2X; AS-RT, AS-Src, AS-2X). We measured crystals obtained at different pHs and in presence and absence of PEG of low molecular weight which has been proven to induce the dimerization of the protein by domain-swapping. Besides, we have measured crystals from SF-SRC chimeras in presence of the chemical denaturant urea at pH 8.0. Although the crystals diffracted at high resolution (~1.5 Å), not molecules of urea were observed, and we concluded that the procedure to obtain the crystals affect to the binding of the denaturant. In addition, we have measured crystals of the chimeric constructions soaked with bromophenol blue in order to study the effect of the dye in the diffraction quality and radiation damage. Crystals of the C-Src and C-Abl chimeric constructions were low quality and doesn't diffract or diffracted at low resolution. As part of these studies, we are also cloned some nucleation site mutants of these SH3 domains. The E128Q mutant of Fyn diffracted at a resolution of ~2Å. The solution of the structure and analysis is under way.

Future perspectives: We continue to crystallize new constructions in diverse conditions to study the behavior of the loops and residues in the protein nucleation site in the propensity of these proteins to form intertwined dimers or/and amyloid fibres.

iii) Lysozyme crystals. We have measured 30 crystals of lysozyme (crystallized between pH 5.5-7.5) soaked on presence of the dye bromophenol blue and other related compounds in order to study the binding of dyes to lysozyme and their effect in radiation damage. The analysis of these structures is under way.

Table 2. Data collected by the Almeria team.

Protein	Samples	Conditions	Diffraction (Å)	Space group/cell
SC-GP41 (Cov mut)	4/0	20% PEG 6000, 0.1M TRIS/HCl pH 8.0	-	No diffraction
SC-GP41 (Cov VQ libre)	2/0	10% PEG 6000, 2.0M NaCl	-	No diffraction
SF-SRC	3/2	2.5 M AS, 0.1M TRIS/HCl pH8.0, 3M Urea	~1.5	P1 211: 45 40 56
FS-SRC	4/4	2.0 M AS, 0.1M NaAc pH 5.0	~1.0-1.5	P212121: 28 32 59
SA-SRC	4/1	0.8 M AS, 0.1M NaAc pH 5.0	~3	P1
F-1	3/2	3.5M HCOONa, 0.1M NaAc pH 4.5	~1.8	C121: 73 45 42
Lysozyme	30/30	0.2M NaCl, 0.1M NaAc pH 5.5-0.1M phosphate pH 7.5	~1.5	P212121: 30 56 73 P43212: 77 77 38