Report of activity of MX386 at ID14.1

Data collections on XendoU crystals.

Several data sets of crystals of a novel metal-dependent endoribonuclease from *X. laevis* (XendoU) were collected. We have already solved the structure of XendoU by MIR phasing, but we are still lacking information about the active site and the metal binding site. We have previously found an electron density peak in the putative active site that we attributed to a phosphate ion, as the crystals grew in phosphate buffer. During this beamtime we collected data sets of crystals soaked in bis-tris buffer at pH 5.5 and HEPES buffer at pH 7.0 to confirm the attribution of the electron density peak to a phosphate ion. Soaking was performed also in the presence of Mn^{2+} ions and 5'UMP, an analogue of the substrate. One data set was also collected from a crystal of XendoU co-crystallised with an oligoribonucleotide of twenty bases mimicking the native substrate. Here we report the data collections statistics. The resolution of the collected data sets is quite poor.

a. XendoU crystal soaked in bis-tris pH 5.5 in the presence of 5'UMP

Space Group	C2
Cell dimensions	a=169.55Å; b=53.86Å; c=137.74Å; β=118.88°
Resolution	50.0 - 3.0Å
Total no. of observations	80,529
No. of unique observations	22,201
Multiplicity	3.6
Data completeness	99.9% (100.0%)
Mean I/σ(I)	12.8
Rmerge	0.09 (0.38)

b. XendoU crystal soaked in bis-tris pH 5.5 in the presence of 5'UMP and Mn^{2+}

Space Group	C2
Cell dimensions	a=166.84Å; b=53.22Å; c=135.86Å; β=118.74°
Resolution	50.0 - 3.2Å
Total no. of observations	62,889
No. of unique observations	17,603
Multiplicity	3.6
Data completeness	99.7% (99.9%)
Mean I/σ(I)	11.0 (2.5)
Rmerge	0.13 (0.47)

c. XendoU crystals soaked in HEPES pH 7.0 in the presence of 5'UMP and Mn^{2+} (two data sets)

Space Group	C2	C2
Cell dimensions	a=167.57Å; b=53.56Å; c=137.88Å; β=119.43°	a=166.43Å; b=53.14Å; c=137.36Å; β=119.73°
Resolution	50.0-3.0Å	50.0-3.0Å
Total no. of observations	74,351	31,104
No. of unique observations	21,268	18,890
Multiplicity	3.5	1.6
Data completeness	98.2% (98.1%)	89.5% (88.9%)
Mean I/σ(I)	13.0 (3.1)	9.7 (2.2)
Rmerge	0.10 (0.45)	0.09 (0.38)

d. Co-crystal of XendoU with an oligoribonucleotide

Space Group	C2
Cell dimensions	a=171.29Å; b=53.15Å; c=137.18Å; β=119.61°
Resolution	50.0-3.1Å
Total no. of observations	36,405
No. of unique observations	14,626
Multiplicity	2.5
Data completeness	74.1% (75.5%)
Mean I/s	7.3 (2.1)
Rmerge	0.13 (0.47)

All the structures were solved by MR using MolRep since the native cell is slightly different and refined with Refmac5.

A decrease or absence of electron density peaks confirmed the positioning of the phosphate ion binding site. However no metals, nor 5'UMP, nor oligo-ribonucleotide were detected in the maps, raising the question of a direct metal binding to the protein, and encouraging to repeat the experiment using 3'UMP and searching for different crystallization conditions (possibly with lower ionic strength). However the data coming from the crystal grown in the presence of the oligo-ribonucleotide were productive since they clearly showed electron density for a loop between amino acids 44-56 (Fig.1), not visible in the native data.

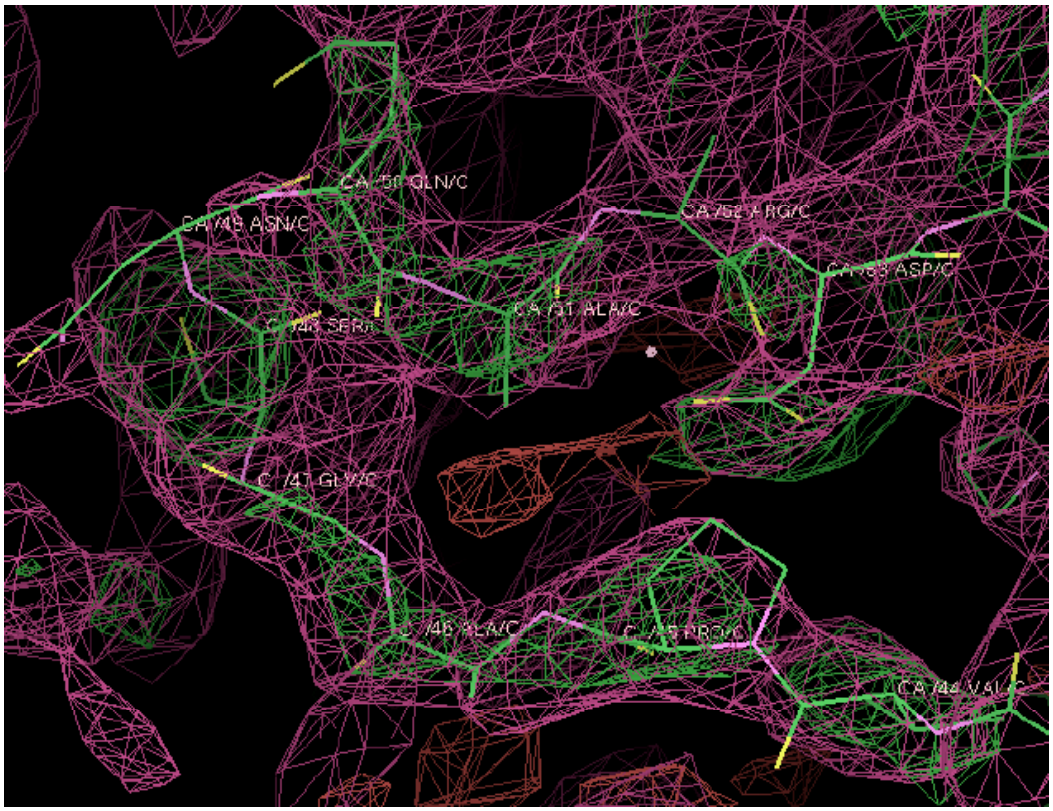

Figure 1. 2Fo-Fc (magenta) and Fo-Fc (green) maps at 0.5 σ from data set (d).

Data collections on CcmH crystals.

We are working on the structure of CcmH protein. At present the structure is under refinement and we are now trying to solve the structure of the reduced form of the protein. In fact CcmH contains one disulphide bridge with a putative functional role in the maturation of c-type cytochrome in vivo. Our aim is to establish whether the protein undergoes structural rearrangement during the oxidation-reduction cycle.
We tested several crystals soaked with DTT at different concentrations (40 mM - 2 mM). Many of these crystals did not diffract well probably due to the presence of the reducing agent. Finally we were able to collect one good data set of a CcmH crystal soaked with 5mM DTT for 3 hours.

CcmH (resolution 30.0-2.2 Å)	
Space group	P21212
Unit cell dimensions (Å)	a= 63.284, b=40.350, c= 63.956
Completeness (last shell) %	92.5 (85.1)
Mosaicity	1.4
Rsym (last shell)	0.081 (0.258)

Data collection of Glutathione-S Transferase from *Ochrobactrum anthropi*

GST	Data collection statistics
Max resolution (Å)	3.0
Space group	$P6_122$
Unit cell (Å)	a=b=58.5940 c=212.3660
Mosaicity	0.45
Completeness	98.9 %
Multiplicity	12.6
Rmerge	0.094

This is the first crystal of the GST from *O. anthropi* ever collected. The structure of the protein has been solved by Molecular Replacement and is currently under refinement.

Data collection of Glutathione-S Transferase (Sigma class) from *Xenopous laevis*

GST	Data collection statistics
Max resolution (Å)	3.2
Space group	P3
Unit cell (Å)	a=b=68.0137 c=185.2283
Mosaicity	0.63
Completeness	99.0 %
Multiplicity	11.4
Rmerge	0.063

This is the first crystal collected of this protein. We are currently trying to improve our crystallization setup to produce crystals diffracting at higher resolution.