



	<b>Experiment title:</b> X-ray diffraction studies on the transcriptional regulator PtxS	<b>Experiment number:</b> Mx1269
<b>Beamline:</b> ID23-1	<b>Date of experiment:</b> from: 04 jul 2011 to: 05 jul 2011	<b>Date of report:</b> 1, March 2012
<b>Shifts:</b> 2	<b>Local contact(s):</b> Ricardo Ferraz leal ( email: ricardo.leal@esrf.fr )	<i>Received at ESRF:</i>
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## Report:

### Diffraction studies of PtxS-ADN

Following our previous studies we were able to produced crystals of the PtxS Se-methionine derivate. As in previous experiments all attempts to extract the crystals from the capillaries, for cryo-protection purpose, failed. Therefore capillaries were cut in portions and glued to SPINE caps. We have to say that by the time the experiment was run we have only two capillaries with crystals. Those capillaries were used to set-up four SPINE-caps. Due to same mistake during the preparation protocol or the handling of the Dewar, all capillaries were lost and therefore data collection was impossible.

### Other diffraction studies.

Following our criteria of “full use of time” the time was employed to test or collect data on systems approved in previous proposal with allocated time.

### **McpS bounded to citrate (MX1017/MX1016/MX1106)**

As pointed it out Mx1017 experimental report “Crystal of the protein MCPS were already collected at BM16 under the CRG proposal 16-01-727 in presence of succinate, malic acid and citrate. Only those grown in the presence of succinate and malic acid diffracted to a resolution of 2.4 Å. All attempts to solve the structure by MR failed. Seleno-methionine crystals of MCPS in complex with malic acid were obtained under similar crystallization condition and data collection was attempted from cryo-protected crystals at BM16 by tuning the wavelength to the pick, inflexion and remote of selenium. Seleno-methionine crystals were isomorphs with the previous diffracted one in presence of both succinic and malic acids. Phases were obtained by SAD from data collected at the Se-peak. At Id14-4 we continued collecting data of the derivatized protein.

With data collected at both BM16 (MX1017 and CRG-1610-727) and Id14-4 (MX-1016) we have solved the structure of MCPs together with malic acid (PDB ID. 2YFA), by SAD, and succinate (PDB ID. 2YFB), by MR (Submitted to PNAS and accepted in Acta Cryst. F).

Here we collected a complete data set of the complex MCPS bound to citrate to a maximum resolution of 2.75 Å. Crystal belongs to the P21212 space group with unit cell dimensions of 96.90, 98.20, 99.30 Å. MR has found a solution and refinement is in progress while attempts to improve crystal quality is also on going.

### **Diffraction studies of ancestral thioredoxin (Mx1106/reportMx1103/Mx1180)**

The structure of Trx205 and Trx352 is been solved by MR and coordinates and structures factors are ready for deposition at the PDB (statistics are shown in table below).

	Trx205	Trx352
Wavelength (Å)		
Resolution range (Å)	58.15 - 2.11 (2.18 - 2.11)	36.6 - 1.3 (1.37 - 1.3)
Space group	F 4 3 2	C 1 2 1
Unit cell	193 193 193 90 90 90	58.4 47.8 73.8 90 98.5 90
Total reflections	140420	169922
Unique reflections	18214	46574
Multiplicity	7.7 (7.9)	3.6 (1.8)
Completeness (%)	99.90 (100.00)	94.40 (89.80)
I/sigma(I)	11.20 (1.70)	18.40 (7.90)
Wilson B-factor	38.73	11.11
R-sym	0.136 (2.002)	0.047 (0.066)
R-factor	0.1816	0.1561
R-free	0.2119	0.1810
Number of atoms	1816	4166
Protein residues	210	212
Water molecules	65	406
RMS(bonds)	0.009	0.012
RMS(angles)	1.21	1.34
Ramachandran favored (%)	100	99
Ramachandran outliers (%)	0	0
Clashscore	11.06	10.18
Average B-factor	47.70	15.50