



	Experiment title: High resolution studies on the TodT/TodS two-component system of the toluene dioxygenase (TOD) pathway and of several ancestral thioredoxin (Atrx)	Experiment number: Mx1180
Beamline: ID29	Date of experiment: from: 05 feb 2011 to: 06 feb 2011	Date of report: 2, March 2012
Shifts: 3	Local contact(s): Gordon Leonard (email: leonard@esrf.fr)	<i>Received at ESRF:</i>
Names and affiliations of applicants (* indicates experimentalists): Dr. Jose a. Gavira. LEC-IACT (CSIC-U.Granada)* Dr. Estela Pineda (Main Proposer). LEC-IACT (CSIC-U.Granada)*		

Report:

Diffraction studies of the TodT/TodS two-component system of the toluene dioxygenase (TOD) pathway

Several crystals of both systems TodT (with and without DNA) and TodS were tested for diffraction at ID29 but any of them were of sufficient quality for data collection. Crystals were of sufficient size (30 μ) and of good shape but lack of internal order or it suffers from the cryo protection protocol. We are currently working on improving the number of crystal in order to wider the cryo-conditions tests.

Diffraction studies of ancestral thioredoxin (Mx1106/reportMx1103)

We have examined crystal of several ancestral thioredoxin i.e. Trx212, Trx352 and Trx324. We have tested more than 40 crystals and collected several data sets on Trx352 and Trx212 while crystal of Trx324 are still reluctant to diffract X-ray to a sufficiently high resolution. Data sets from Trx352 showed a maximum resolution limit worst than 2.4 Å while crystal of Trx212 diffracted X-ray to a maximum resolution of 1.3 Å. The structure of Trx212 is been solved by MR and coordinates and structures factors are ready for deposition at the PDB (statistic is shown in table below).

	Trx212
Wavelength (Å)	
Resolution range (Å)	34.09 - 1.3 (1.35 - 1.3)
Space group	P 1 21 1
Unit cell	36.1 62.9 42.9 90 109 90
Total reflections	121246
Unique reflections	43330
Multiplicity	2.8 (2.8)
Completeness (%)	97.30 (97.80)
I/sigma(I)	11.60 (2.50)
Wilson B-factor	12.43
R-sym	0.056 (0.428)

Refinement	
R-factor	0.1710
R-free	0.2003
Number of atoms	3879
Protein residues	210
Water molecules	355
RMS(bonds)	0.011
RMS(angles)	1.36
Ramachandran favored (%)	100
Ramachandran outliers (%)	0
Clashscore	17.34
Average B-factor	16.90

Diffraction tests:

In preparation to future data collection beam-time we have also done several test on crystal of PtxS (Mx1269) in complex wit DNA and of McpS (MX1017/MX1016/MX1106) in complex with benzoate and citrate.