	Experiment title: High resolution studies on the TodT two-component system of the toluene dioxygenase (TOD) pathway for the metabolism of toluene in Pseudomonas putida DOT-T1E	**Experiment number**: MX1103
Beamline: ID23 2	**Date of experiment**: from: 23/06/2010 to: 24/06/2010	**Date of report**: 27, August 2010
Shifts: 3	**Local contact(s)**: Dr. David FLOT	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Dr. Estela Pineda (Main Proposer). LEC-IACT (CSIC-U.Granada)

Dr. Jose a. Gavira. LEC-IACT (CSIC-U.Granada)

Report:

Diffraction studies of TodT and TodS

This beam-time was initially intended to collect full data sets of both TodT and TodS from small crystals and therefore the proposal was submitted to ID23 2 (see proposal mx1103). At the same time we put-in a second proposal (assigned mx1106) for the data collection of different systems. Both proposal were accepted and schedule for May (15^{th}/16^{th}) and June (23th/24^{th}) respectively. Focus in the first one (mx1106) we did not have enough time to obtain sufficient fresh crystals of two DOT-T1E components. As described in the proposal protein stability is a big issue and during this rushing period we were not able to obtained crystals of TodS and only marginal amount of crystal of TodT.

Several crystals of the complex TodT-ADN were tested but any of them were of sufficient quality for data collection.

The remaining beam-time was used to complete the data collection of several systems (see below) initially intended for the previous proposal (mx1106) but that were not collected due to a communication problem between the detector and the computer/software controlling it. We lost almost a shift trying to fix this problem and moving from ID14 to ID23.

Diffraction studies of ancestral thioredoxin

In the previous run we collected complete data sets for two of ancestral thioredoxins, ATrx250, ATrx325. At ID23 2 we were able also to collect several complete data sets for the ancestral Trxs, ATrx205 and ATrx324. We have been able to find a MR solution for 205 but fail with 324, probably due to the low crystal quality.

	ATrx205	**ATrx324**
Wavelength (Å)		
Space group	P1	$P2_1$
Cell parameters (Å)	27.73 62.04 78.11 84.87 87.19 89.95	37.61 48.80 91.07 90.000 93.237 90.000
Resolution range (Å)	46.39-1.9 (2.0-1.9)	48.78-2.48 (2.62-2.48)
Observed reflections	73205	89443
Independent reflections	38662	11561
Data completeness (%)	94.1 (91.0)	98.3 (96.6)
R_{merge} (%)†	10.1 (41.0)	8.5
Average *I/σ (I)*	9.6 (3.0)	16.7 (2)
Multiplicity	1.9 (1.9)	7.7 (5.8)
Mol/Asym	6	
Matthews coefficient ($Å^3$ Da^{-1})	1.89	
Solvent content (%)	34.83	
Refinement		
R-work	20.2	No MR solution
R-free	26.3	

We also tested crystals of ATrx325 and collect several data sets, searching for resolution improvement but any of them were of better quality than the previos one. Ancentral thioredoxing crystals were in general of lower quality probably due to againg.

Diffraction studies of PTXS-ADN

Crystal of PTXS-ADN complex were grown in capillaries by the counterdiffusion method. To avoid crystal damage, previously observed with this system, crystals were cryo-protected into the capillary by diffusing the cryo-protectant. Portions of capillaries containing individual crystal were flash-frozen in liquid nitrogen and storage.

Space group	P3		
Unit cell			
	Overall	InnerShell	OuterShell
Low resolution limit	59.13	59.13	2.47
High resolution limit	2.39	9.26	2.39
Rmerge	0.189	0.065	0.882
Total number of observations	1230893	21242	115546
Total number unique	142405	2432	13960
Mean((I)/sd(I))	8.9	24.0	2.5
Completeness	100.0	98.9	100.0
Multiplicity	8.6	8.7	8.3

So far all attempts to solve the structure by MR have failed. We are in the process of producing Seleno-Met variant to solve the structure by SAD/MAD.