	Experiment title: **Determination of the 3-D structure of Glyoxylate carboligase (GCL)**	**Experiment number**: MX-379
Beamline: ID14-4	**Date of experiment**: from: 30/11/04 to: 1/12/04	**Date of report**: 1/9/05
Shifts: 1	**Local contact(s)**: Gordon Leonard	*Received at ESRF:*

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Report (final):

Full MAD diffraction data were recorded at the ID14-4 beamline. Data were processed with XDS and CCP4 programs. Three crystals were mounted during the experiment at ESRF but only one could be properly indexed because of the peculiar non-crystallographic symmetry of this crystal form. The asymmetric unit contains 6 monomers (a tetramer and a dimer) with the dimer being translated by 0.3*c* (i.e. exactly along the *c* by a fraction 0.3 of the *c* axis

The crystals belong to the tetragonal space group $P4_12_12$, with unit cell dimensions, a,b= 188.18 Å, c= 249.40 Å. Table 1 summarizes the data collection statistics.

Structure determination and refinement

Three out of the six monomers in the asymmetric unit were placed by molecular replacement using Phaser, with a search model consisting of superimposed models of 6 ThDP enzymes of known structures and sequence similarity to GCL in the range of 25-30%. Initial phases produced from this partial model were used

to detect Se atoms in the anomalous difference map. The highest 100 peaks (over 3σ) served for MAD phasing at 2.8 Å resolution complemented by solvent-flattening using the program SHARP (the phasing power and figure of merit before solvent flattening were 1.41 and 0.39, respectively). The map thus produced was of high quality and allowed backbone tracing of GCL followed by manual fitting of the nearly complete sequence (residues 2-593) using the program O. Refinement under strict non-crystallographic and geometric restraints was carried out using the program Refmac. Local anneal-omit maps were calculated to verify the correctness of the model using the program CNS. The final R_{work}/R_{free} values are 0.219/0.253 and the model geometry is excellent according to analysis by the Molprobity server, with no Ramachandran outliers and 98.6% of residues in Ramachandran favored regions. The final model consists of six copies of GCL monomers, FAD, ThDP/Mg^{2+} and DTT, 4 Mg^{2+} ions, one PEG molecule and 362 water molecules.

Accession codes. Protein Data Bank The atomic coordinates and structure factors for GCL have been deposited under accession code 2PAN.

Table 1. Data collection statistics of GCL crystal

Beamline	ESRF ID 14-4		
Space group	$P4_12_12$		
Range (Å)	40.0-2.8 (3.0-2.8)		
a=*b* (Å)	188.18		
c (Å)	249.40		
Wavelength (Å)	Peak 0.97897	Inflection 0.97946	Remote 0.93929
Total number of reflections	977032	1035690	1046103
Number of unique reflections	210042	210608	210109
Completeness (%)	99.7 (99.8)	100 (100)	99.7 (100)
I/σ	10.9 (4.9)	10.63 (3.89)	8.92 (2.53)
R_{sym} (%)	7.9 (23.5)	9.0 (33.4)	12.7 (57.3)
R_{meas} (%)	8.9 (27.4)	10.1 (38.5)	14.2 (65.5)