ESRF	**Experiment title:** High resoltuion data collection mutase	**Experiment number**: BAG-LS1861
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Report:

The biosynthesis of the cell wall is an attractive target for structure-assisted drug design of novel anti-bacterial drugs mainly because there are a large number of compounds in the cell wall that are not found in mammalian and other higher organisms. One such compound is galactofuranose (Gal*f*). Gal*f* is a component of the LPS in many gram-negative bacteria, found in the O antigens of many species including *Klebsiella pneumonia* and *Escherichia coli* Galactofuranosyl residues are also an important component of the arabino-galactan complex that forms part of the mycolitic layer in the cell walls of mycobacteria [Weston, 1998 #6]. Gal*f* is incorporated into cell walls from UDP-Gal*f*, formed by the enzyme UDP-galactopyranose mutase (mutase) from UDP-galactopyranose (Gal*p*). The chemistry involved in this ring contraction mechanism is completely unprecedented and is the source of great interest. Complexes of UDP-galactopyranose mutase with 4 different inhibitors/substrates were prepared by soaking crystals with 5-10 mM of ligand for 1-2 hrs. No in-house data collection is possible due to the very weak diffraction from the crystals (see previous reports). Crystals were frozen and data collected at 30 sec. Exposures, 0.5 ° oscillation, 180 images. The resulting data are listed in the Table I below. Ligand 4 only gave mosaic crystals to at best 4 Å resolution. The data sets for the other three complexes were solved and refined, however no electron density could be seen for any of the ligands. We have therefore concluded that we require to crystallize the enzyme from a different species to determine co-complexes.

Table I

UDP-galactopyranose mutase + ligands	**Ligand 1**	**Ligand 2**	**Substrate**
Resolution (Å)	48.8 – 2.5	74.5 – 2.7	52.7 – 2.5
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Cell constants (Å; º)	56.33 90.29 131.93 90.0, 90.0, 90.0	56.39 96.84 131.71 90.0, 90.0, 90.0	55.40 95.16 127.92 90.0, 90.0, 90.0
Total measurements	63902	81405	74686
Unique reflections	18743	25616	23509
Average redundancy	3.4	2.8	3.2
I/s	5.4	3.5	4.0
Completeness (%)	98	99.4	97.9
R_{merge}	9.6	13.5	15.5

3-Methylaspartase (E.C. 4.3.1.2) catalyses the reversible anti-elimination of ammonia from L-*threo*-(2*S*,3*S*)-3-methylaspartic acid to give mesaconic acid as well as a slower *syn*-elimination from the (2*S*,3*R*)-epimer, L-*erythro*-3-methylaspartic acid. The *anti*-elimination reaction occurs in the second step of the catabolic pathway for glutamic acid in *Clostridium tetanomorphum*. The reverse reaction is of particular interest because the addition of ammonia to substituted fumaric acids is highly stereoselective and gives highly functionalised amino acids. Access to these synthetically useful compounds by conventional synthesis is extremely difficult and not well developed. However, engineering of 3-methylaspartase is greatly hindered by the lack of structural information and the absence of homologues in the Protein Data Bank. The crystal structure will facilitate future efforts to engineer 3-methylaspartase by directed evolution. The structure will help unravel the complex mechanism of this transformation. We have obtained native crystals and collected the data summarised in Table 1.

This work has been accepted for publication in Acta Cryst D. 'Over-expression, purification, crystallization and data collection of 3-methylaspartase from *Clostridium tetanomorphum*' by Miryam Asuncion, *et al.*,

Table I

Protein	***C. methanomorphum* methyl aspartase**
Resolution (Highest Shell, Å)	50.6 – 2.0 (2.11 – 2.0)
Space group	$P2_12_12$
Cell constants (Å; º)	67.2, 109.9, 110.3; 90.0, 90.0, 90.0
Total measurements	226095
Unique reflections	53841
Average redundancy	4.2 (4.0)
I/s	7.0 (3.4)
Completeness (%)	96.8 (98.1)
R_{merge}	7.4 (19.9)

All data has been collected at 0.933 ?. Processing was done with MOSFLM/SCALA.