	Experiment title: BAG	**Experiment number**: LS-2087
Beamline: ID29	**Date of experiment**: from: 20th July to: 21st July	**Date of report**: Feb 18th
Shifts:	**Local contact(s)**:	*Received at ESRF:*

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Report:

Aldolase

Following the landmark paper from Wilson & Wong in Science, the mechanism of the class I aldolase has once again attracted considerable interest. The mechanism shown in the science paper is based on being able to trap covalent intermediates of substrate during turnover. The technique was also highlighted by Sygush in PNAS paper where they trapped a pyruvate complex. The Wong & Wilson mechanism may not be general. We have been working on evolved aldoalses to alter their product profile and some of these may prove very useful in understanding the basic mechanism of aldolases. To this end we have solved the structure of a KDPG aldolase from *Thermatoga maratima* (previous report). On this experiment we optimised the conditions for preparing the pyruvate covalent intermediate of this enzyme.

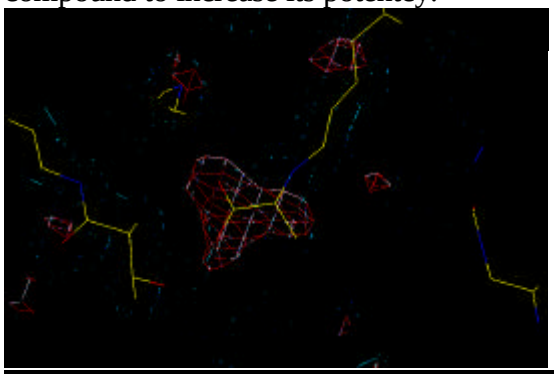
Values in Parenthesis refer to the highest resolution shell, covalent lysine

Resolution (Å)	37.27-2.36 (2.36 – 2.2Å)
Space Group	$P2_12_12$
Unit-cell Parameters (Å, º)	a = 103, b = 135, c = 43.3,$\alpha = \beta = \gamma = 90$
Total measurements	292,414
Unique reflections	65,259
I/σ	5.8 (1.5)
Average redundancy	2.7 (2)
Data completeness (%)	98 (85)
R_{merge} † (%)	9.8 (36.3)

† R_{merge} = ΣΣ I(h)j – (I(h)) / ΣΣ I(h)j where I(h) is the measured diffraction intensity and the summation includes all observations

Drug design Mycobacteria Tuberculosis

We are funded by the Wellcome Trust to explore the mechanism of enzymes of the rhamose biosynthesis but not inhibitor design. The rhamnose pathway is an attractive target for therapeutic intervention and in collaboration with Professor Lee (Univ Tenessee) and Professor McNeil (Colorado) we have designed the first generation of inhibitors of one the enzymes in the rhamnose pathway. The compounds inhibit the enzyme and show activity in whole cell assay against *M. tuberculosis*. We collected a data set to 1.85Å on one such co-crystal. The structure reveals that the compound is bound at the active site. It provides a clear template for us to adjust this compound to increase its potentcy.



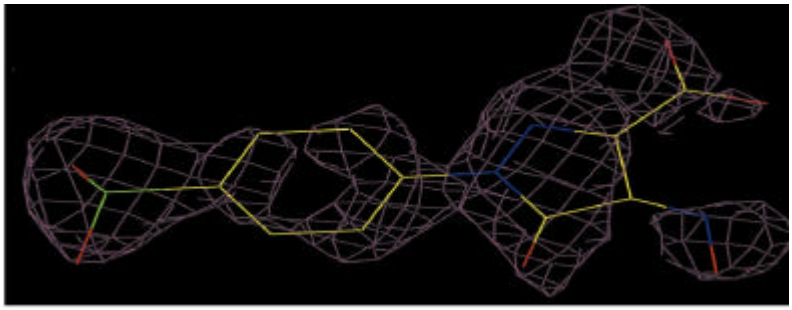


Figure 1(left) Covalent intermediate attached to Lys in aldolase (Fo-Fc map 2.4s, 0.22eÅ^{-3}. Figure 2 (right) Electron density of the first inhibitor of RmlC. The map is an Fo-Fc map contoured at 2.1s (0.2eÅ^{-3}).

Methyl Aspartase Ammonia Lyase

We had collected native data and solved the structure using data from the ESRF. Previous reports give details of these experiments. On this experiment we used a series of co-crystallisation and soaking experiments to locate the catalytic metal ion site. We were able to obtain a Mg^{2+} complex to 1.9Å which was crucial to our understanding of the enzyme function. This work has been published on-line in the Journal of Biological Chemisty (http://www.jbc.org/cgi/reprint/M111180200v1.pdf) and will appear in print in March.

Data collection	Metal
Wavelength (Å)	0.934
Resolution (Highest Shell, Å)	55.05 – 1.90 (1.95 – 1.90)
Space group	$P2_12_12_1$
Cell constants (Å; º)	a=67.0, b=108.8, c=109.6; a=ß=?=90
Total measurements	731140
Unique reflections	63452
Average redundancy	11.5 (9.0)
I/s	9.0 (4.3)
Completeness (%)	99.6 (95.1)
R_{merge}	11.4 (26.4)
R	13.5 (14.5)
R_{free}	17.5 (20.6)
rmsd bonds (Å) / angles (º)	0.021 / 1.743
PDB accession code	1KCZ

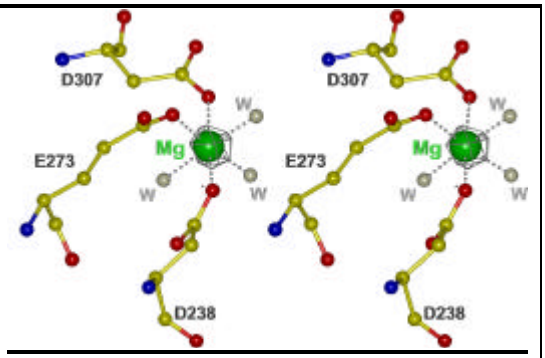


Figure 3 The divalent metal ion binding site for methylaspartase. Electron density (5s) is shown for the Mg^{2+} ion. Oxygen atoms are in red, nitrogen in blue, carbon in yellow.