s8a.m1.p1 **Crystal structure of methylmalonyl-CoA epimerase: a novel enzymatic function on an ancient metal-binding scaffold** A.A. McCarthy[1], H.M. Baker[1], S.C. Shewry[1], M.L. Patchett[2] and E.N. Baker[1], *[1]School of Biological Sciences, University of Auckland, Auckland, New Zealand and [2]Institute of Molecular Biosciences, Massey University, Palmerston North, New Zealand.*
Keywords: racemase, metal binding, evolution.

An essential step in the metabolism of odd-numbered fatty acid chains is carried out by the epimerisation of D-methylmalonyl-CoA to L-methylmalonyl-CoA, the substrate for the B_{12}-dependent enzyme methylmalonyl-CoA mutase. The epimerisation is carried out by a homodimeric (2 x 16.5 kDa) enzyme, methylmalonyl-CoA epimerase (MMCE). This enzyme is present in both bacteria and animals, where its amino acid sequence is highly conserved. Activity is dependent on the binding of divalent metal ions, notably Mn^{2+}, Co^{2+}, Ni^{2+} or Zn^{2+}. We have used MAD methods to solve the crystal structure of MMCE at 2.0 Å resolution.

Crystals of MMCE are monoclinic, a=43.6, b=78.6, c=89.4 Å, β=92.0°, with four molecules in the asymmetric unit. Three-wavelength data for the SeMet-substituted enzyme were collected at SSRL, the positions of 23 of the 24 Se atoms were found using SOLVE, and the structure was readily modelled from an electron density map at 2.0 Å resolution. Refinement with CNS gives a current R-factor of 22.7% (R_{free} = 26.1%).

The MMCE monomer has an α/β fold that is made up of two βαβββ modules representing the N- and C-terminal halves of the monomer. The β-sheets of the two modules pack edge-to-edge to create an 8-stranded β-sheet that curves around to enclose a large cleft. Back-to-back packing of two monomers creates a tightly-associated dimer. At the pH of crystallization (4.6), no metal ion is bound. However, a putative metal binding site is provided by four side chains, from His12, Gln65, His91 and Glu141, that are located in the floor of the cleft, at the centre of the β-sheet. The metal ion at this site may bind the substrate carboxylate group, as proposed for another epimerase, mandelate racemase[1].

Remarkably the fold of MMCE corresponds with that found in several proteins with quite different activities; bleomycin resistance protein (BRP), glyoxalase (GLO) and extradiol dioxygenases (EDO)[2]. The metal binding residues align precisely, consistent with evolution of these different proteins from a common metal-binding precursor but there are different connectivities between the βαβββ modules..

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s8a.m1.p2 **Structural basis of sialyl-transferase activity in trypanosomal sialidases.** A. Buschiazzo, M.F. Amaya, P. Alzari, *Unité de Biochimie Structurale – Département d'Immunologie Institut Pasteur 25, rue du Dr. Roux 75015 Paris FRANCE.*
Keywords: Chagas disease, sialidase, mutagenesis.

The intracellular parasite *Trypanosoma cruzi*, the etiologic agent of Chagas disease, sheds a developmentally regulated surface trans-sialidase which is involved in key aspects of parasite-host cell interactions. Although it shares a common active site architecture with bacterial neuraminidases, the *T. cruzi* enzyme behaves as a highly efficient sialyl-transferase. Here we report the crystal structure of the closely related *Trypanosoma rangeli* sialidase and its complex with inhibitor. The enzyme folds into two distinct domains: a catalytic β-propeller fold tightly associated with a lectin-like domain. Comparison with the modeled structure of *T. cruzi* trans-sialidase and mutagenesis experiments allowed the identification of amino acid substitutions within the active site cleft that modulate sialyl-transferase activity and suggest the presence of a distinct binding site for the acceptor carbohydrate. The structures of the trypanosoma enzymes illustrate how a glycosidase scaffold can achieve efficient glycosyltransferase activity and provide a framework for structure-based drug design.