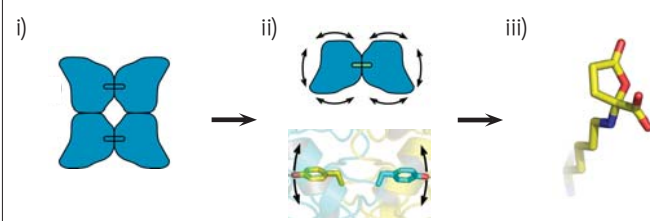


# Evolution of quaternary structure in a homotetrameric protein

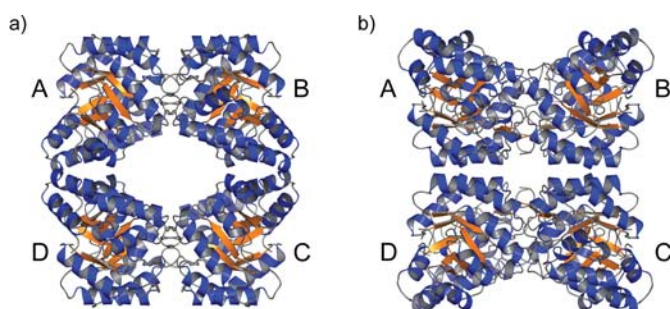
Sean R. A. Devenish<sup>1</sup>, Michael D.W. Griffin<sup>1,2</sup>, Renwick C.J. Dobson<sup>1</sup>, F. Grant Pearce<sup>1</sup>, Laurence Antonio<sup>1</sup>, Andrew E. Whitten<sup>4</sup>, Chu K. Liew<sup>5</sup>, Joel P. Mackay<sup>5</sup>, Jill Trehwella<sup>5</sup>, Geoffrey B. Jameson<sup>3</sup>, Matthew A. Perugini<sup>2</sup> and Juliet A. Gerrard<sup>1</sup>

The reason that many proteins exist as oligomeric species in their native form is one of the major unsolved problems in structural biology. In order to explore the role of quaternary structure, dimeric variants of a homotetrameric protein, *Escherichia coli* dihydrodipicolinate synthase, were engineered and their properties compared to the wild-type, tetrameric form. X-ray crystallography reveals that the active site is not disturbed when the quaternary structure is disrupted. However, the activity of the dimeric enzymes in solution is substantially reduced, and a tetrahedral adduct of a substrate analogue is observed to be trapped at the active site in the crystal form. Remarkably, heating the dimeric enzymes increases activity. We propose that the homotetrameric structure of DHDPs reduces dynamic fluctuations present in the dimeric forms, and increases specificity for the first substrate, pyruvate. By restricting motion in a key catalytic motif, a competing, non-productive reaction of a substrate analogue is avoided. Support for this idea comes from the study of a further mutation, DHDPs-Y107F, and a comparison of the quaternary structures of DHDPs from *E. coli* and *Nicotiana sylvestris*. Evolution of quaternary structure to optimise the dynamic properties of subunits might prove relevant in other protein complexes.

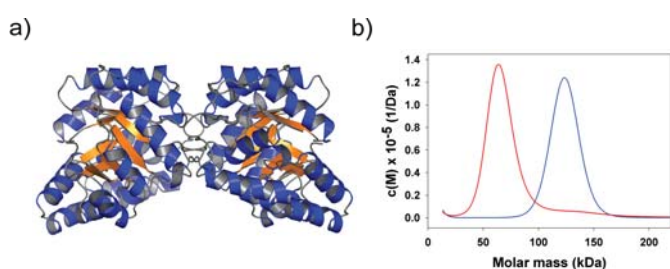
## Summary of findings



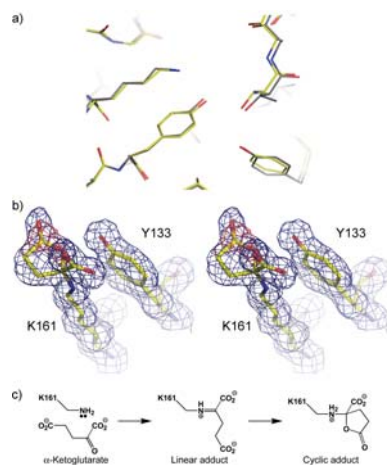
(i) Alteration of the oligomeric state of DHDPs changes the wild-type tetramer to a dimer of identical tertiary structure, but a postulated hinging motion along the monomer-monomer interface is facilitated by the reduction in oligomeric order (ii). This motion alters the position of amino acid residues that are crucial for enzyme activity and substrate specificity. As a result, dimeric forms bind a naturally occurring substrate analogue, which becomes trapped, covalently attached to the protein, inactivating the enzyme (iii). This suggests that DHDPs evolved as a tetramer in order to control the dynamics of the protein for optimal substrate selectivity and catalysis, factors which may prove relevant to other oligomeric proteins.



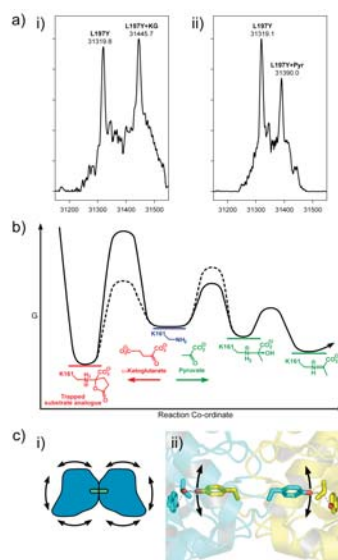
**Figure 1.** The X-ray crystal structures of DHDPs from (a) *E. coli*<sup>1</sup> and (b) *N. sylvestris*<sup>2</sup>. Each enzyme is a homotetramer of  $(\beta/\alpha)_8$ -barrels composed of two tight-dimer units (A-B and C-D), but the arrangement of the two dimer units is different.



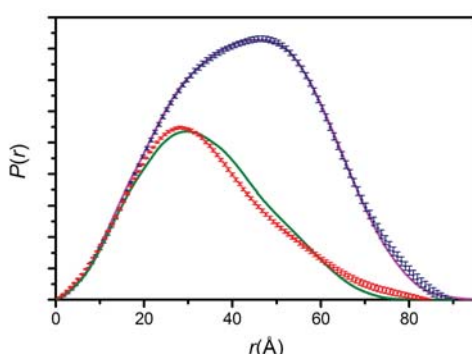
**Figure 2.** (a) The crystal structure of the DHDPS-L197Y dimer (PDB code 2OJP) showing the authentic tight dimer unit. (b) Sedimentation velocity analyses of wild-type DHDPs and the DHDPS-L197Y. The continuous mass  $[c(M)]$  distribution is plotted as a function of molar mass (kDa) for wild-type DHDPs (blue line) and DHDPS-L197Y (red line). No evidence was found of tetrameric enzyme when the experiment was repeated at 4°C, 20°C and 37°C.



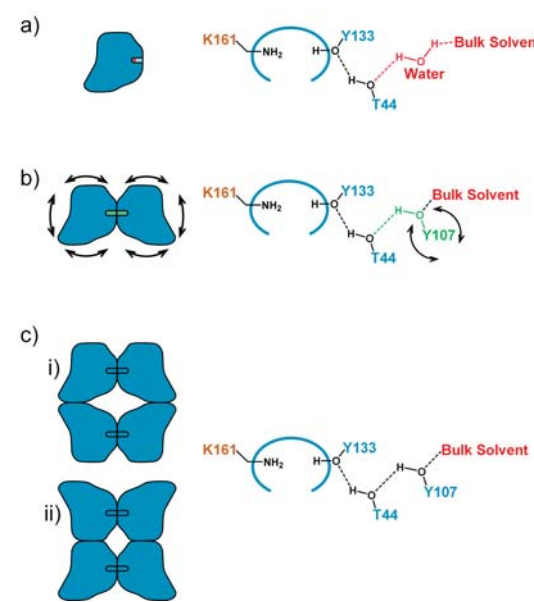
**Figure 3.** (a) Superposition of the active sites of wild-type DHDPs (grey) and DHDPS-L197Y (yellow) indicate that no significant structural changes were present in the active site of DHDPS-L197Y in the crystalline state. (b) The tetrahedral electron density was present at the active site lys161 in both DHDPS-L197Y and DHDPS-L197D, but not in the wild-type structure. A cyclic covalent adduct formed between lys161 and  $\alpha$ -ketoglutarate has been modelled into the active site.  $2F_o - F_c$  electron density is contoured at  $1\sigma$  (blue) and  $F_o - F_c$  electron density is contoured at  $3\sigma$  (green) and  $-3\sigma$  (red). (c) The formation of the cyclic  $\alpha$ -ketoglutarate adduct proceeds via formation of a linear Schiff base with lys161.



**Figure 4.** Understanding the puzzling behaviour of dimeric variants of DHDPs. (a) ESI-TOF mass spectrometry of freshly prepared DHDPS-L197Y (i) reveals a species corresponding to the mass of the protein with a covalent cyclic  $\alpha$ -ketoglutarate adduct (L197Y+KG). Incubation of this protein sample with 10 mM pyruvate (ii) shows complete displacement of the  $\alpha$ -ketoglutarate adduct and the appearance of a species corresponding to the mass of the protein with pyruvate bound as a Schiff base (L197Y+Pyr). (b) Free energy diagram for the first half of the reaction in wild-type DHDPs (solid line) and the dimeric variants (dashed line). Formation of a covalent adduct between lys161 and  $\alpha$ -ketoglutarate is more favourable in the mutant enzymes where displacement of the trapped adduct becomes the rate-determining step. (c) Breathing motion in dimeric structures (i) disrupts the catalytic triad (ii) and leads to the formation of a trapped substrate analogue.



**Figure 5.** Evidence of increased motion of the dimeric DHDPS-L197Y:  $P(r)$  profiles calculated from scattering data ( $I(Q)$  versus  $Q$ , where  $Q = 4\pi(\sin\theta)/\lambda$ ) using the program GNOM<sup>3</sup> and the calculated profiles (using the program CRYSOLOG<sup>4</sup>) from the corresponding crystal structures.



**Figure 6.** Proposed evolution of the homotetrameric forms of *E. coli* and plant DHDPs. (a) The ancient monomer has a catalytic diad and a structured water molecule to complete the proton relay. (b) Interdigitation of tyr107 from a neighbouring monomer completes the catalytic triad, but introduces problems associated with the breathing of the dimeric molecule. (c) The breathing motion is prevented by buttressing two tight-dimers together to form alternate homotetrameric forms, as seen in the bacterial (i) and plant (ii) enzymes.

**Table 1.** Heat activation of DHDPS-L197Y, DHDPS-L197D and DHDPS-Y107F. Enzymes were heated under standard conditions at 40 °C in the presence of 60 mM pyruvate before being cooled to 30 °C and tested using the coupled assay. Activity is expressed as a percentage of the activity of wild-type enzyme.

time incubated	wild-type	DHDPS-L197Y	DHDPS-L197D	DHDPS-Y107F
0 minutes	100%	0.7%	0.9%	16.8%
100 minutes	100%	10.4%	15.1%	30.3%

**Table 2.** Activation in the presence of  $\alpha$ -ketoglutarate. Enzymes were heated overnight at 30 °C in the presence or absence of pyruvate or  $\alpha$ -ketoglutarate and tested using the coupled assay. Activity is expressed as a percentage of the activity of untreated enzyme.

treatment	% activity		
	wild-type	DHDPS-L197Y	DHDPS-Y107F
not incubated	100	100	100
incubated	97	140	226
incubated in the presence of pyruvate	97	2524	289
incubated in the presence of $\alpha$ -ketoglutarate	58	972	92

**References:**  
This research is presented in full in: MDW Griffin, RCJ Dobson, FG Pearce, L Antonio, AE Whitten, CK Liew, JP Mackay, J Trehwella, GB Jameson, MA Perugini, JA Gerrard (2008) Evolution of quaternary structure in a homotetrameric enzyme, *J. Mol. Biol.* doi:10.1016/j.jmb.2008.05.038.

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- Addresses of Authors**
- School of Biological Sciences, University of Canterbury, Christchurch, New Zealand
  - Department of Biochemistry and Molecular Biology Department, Bio21 Molecular Science and Biotechnology Institute, University of Melbourne, Parkville, Victoria, Australia
  - Centre for Structural Biology, Institute of Fundamental Sciences, Massey University, Palmerston North, New Zealand
  - Bragg Institute, Australian Nuclear Science and Technology Organisation, NSW 2234, Australia
  - School of Molecular and Microbial Biosciences, University of Sydney, NSW 2006, Australia