STRUCTURAL
BIOLOGY

Volume 74 (2018)

Supporting information for article:

Structure and function of L-threonine-3-dehydrogenase from the parasitic protozoan *Trypanosoma brucei* revealed by X-ray crystallography and geometric simulations

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Table S1 TDH quaternary structure identified by PISA and confirmed visually. * ΔG_{diss} = dissociation barrier.

Model	Oligomer	Accessible surface area (Å^2)	Buried surface area (Å^2)	ΔG_{diss}* (kcal.mol^{-1})	Stable in solution?	Visual confirmation
Model: 5L9A Space group: P1 Unit cell parameters: a=46.98, b=57.72, c=70.07; α=72.46°, β= 70.38°, γ=73.20° Molecules in the asymmetric unit: 2	Dimer	25869.5	3161.9	2.5	Yes	✓
Model: 5LC1 Space group: $P2_12_12$ Unit cell parameters: a=132.03, b=276.49, c=55.74; αβγ=90° Molecules in the asymmetric unit: 6	Dimer	24444.8	5980.5	3.8	Yes	✓
	Dimer	24278.4	6117.1	3.6	Yes	✓
	Dimer	24600.3	5880.2	3.3	Yes	✓
Model: 5K4Q Space group: $P2_12_12$ Unit cell parameters: a=132.04, b=276.49, c=55.74; αβγ=90° Molecules in the asymmetric unit: 6	Dimer	24918.1	5470.9	3.3	Yes	✓
	Dimer	24832.7	5449.2	2.6	Yes	✓
	Dimer	24993.7	5428.6	2.3	Yes	✓
Model: 5K4T Space group: $P4_32_12$ Unit cell parameters: ab=91.76, c=93.60; αβγ=90° Molecules in the asymmetric unit: 1	Dimer	25239.1	5028.6	3.2	Yes	✓

Model	**Oligomer**	**Accessible surface area (Å²)**	**Buried surface area (Å²)**	**ΔG_{diss}* (kcal.mol⁻¹)**	**Stable in solution?**	**Visual confirmation**
Model: 5K4V Space group: $P2_12_12$ Unit cell parameters: a=90.40, b=131.53, c=55.02; αβγ=90° Molecules in the asymmetric unit: 2	Dimer	24519.8	4880.8	4.4	Yes	✓
Model: 5K4U Space group: $P2_12_12$ Unit cell parameters: a=133.03,b=273.06, c=55.80; αβγ=90° Molecules in the asymmetric unit: 2	Dimer	24264.3	7309.4	4.1	Yes	✓
Model: 5K50 Space group: $P2_12_12$ Unit cell parameters: a=133.03, b=273.06, c=55.80; αβγ=90° Molecules in the asymmetric unit: 6	Dimer	24167.7	4813.6	4.3	Yes	✓
	Dimer	24294.1	4772.0	3.1	Yes	✓
	Dimer	24217.8	4790.1	2.4	Yes	✓
Model: 5K4W Space group: $P2_12_12$ Unit cell parameters: a=83.45, b=136.12, c=55.69; αβγ=90° Molecules in the asymmetric unit: 2	Dimer	24587.0	5788.1	0.3	Yes	✓
	Tetramer	46831.2	13919.2	-1.6	Uncertain	x (see Supplementary Figure 12)
Model: 5K4Y Space group: $P2_12_12$ Unit cell parameters: a=133.45, b=278.63, b=56.27; αβγ=90°	Dimer	24779.8	6050.2	4.5	Yes	✓
		24400.6	6351.1	4.1	Yes	✓

Model	**Oligomer**	**Accessible surface area (Å^2)**	**Buried surface area (Å^2)**	**ΔG_{diss}* (kcal.mol^{-1})**	**Stable in solution?**	**Visual confirmation**
Molecules in the asymmetric unit: 6		24521.6	6357.1	3.1	Yes	✓

Table S2 Samples, elution volumes, predicted molecular weights and corresponding oligomeric forms, as indicated by results of SEC experiments.

Sample Contents	**Ve (ml)**	**Ve/V_0**	**Interpolated MW (kDa)**	**Multiple of MW**	**Suggested Oligomeric State**
TDH (1.8 $x10^{-1}$ mM [6.8mg/ml])	15	1.79	68.8	1.82	Dimer
TDH (1.1 $x10^{-1}$ mM [4mg/ml]) + 10mM NAD^+	15	1.79	68.8	1.82	Dimer
KBL (4.4 $x10^{-2}$ mM [2mg/ml])	14.4	1.71	87.5	1.91	Dimer
TDH (5.3 $x10^{-2}$ mM [2mg/ml]) + KBL (4.4 $x10^{-2}$ mM [2mg/ml]) - first peak	12.5	1.49	187.7	4.97 (TDH); 4.09 (KBL)	Multi-enzyme complex
TDH (5.3 $x10^{-2}$ mM [2mg/ml]) + KBL (4.4 $x10^{-2}$ mM [2mg/ml]) - second peak	16.8	1.99	34.0	0.90 (TDH); 0.74 (KBL)	Monomers (TDH and/or KBL)

Table S3 SDR and GalE-like TDH deposited in the Protein Data Bank.

Organism	Amino acids	Quaternary structure	Wild-type/mutant	Ligands	PDB ID	Literature reference(s)
Trypnanosoma brucei	332	Dimer	Wild-type	-	5L9A	This manuscript, released 22 JUN 16
	332	Dimer	Wild-type	NAD; pyruvate	5LC1	This manuscript, deposited 22 JUN 16
	332	Dimer	Wild-type	NAD	5K4Q	This manuscript, released 15 NOV 17
	332	Dimer	Wild-type	-	5K4T	This manuscript, released 15 NOV 17
	332	Dimer	Wild-type	NAD	5K4V	This manuscript, released 15 NOV 17
	332	Dimer	Wild-type	NAD	5K4U	This manuscript, released 15 NOV 17
	332	Dimer	Wild-type	NAD, L-*allo*-threonine	5K50	This manuscript, released 15 NOV 17
	332	Dimer	Wild-type	NAD, L-threonine	5K4W	This manuscript, released 04 JAN 18
	332	Dimer	Wild-type	NAD	5K4Y	This manuscript, released 17 JAN 18
Flavobacterium frigidimaris	312	Dimer	Wild-type	NAD; glycerol	2YY7	Febs J 277: 5124-5132 (2010)
Thermoplasma volcanium	317	Dimer	Wild-type	NAD	3A1N	J Biol Chem 287:12966-12974 (2012)
	317	Dimer	Wild-type	NAD; pyruvate	3A4V	
	317	Dimer	Y137F mutant	NAD; L-threonine	3A9W	
	317	Dimer	Y137F mutant	NAD; L-3-Hydroxynorvaline	3AJR	
Mus musculus	373	Dimer	Wild-type	NAD^+	4YR9	J Struct Biol 192:510-518 (2015)
	373	Dimer	Wild-type	-	4YRA	
	373	Dimer	R180K mutant	NAD	4YRB	
Cupriavidus necator	318	Dimer	Wild-type	NAD; L-threonine	3WMX	J Biol Chem 289:10445-10454 (2014)
	318	Dimer	Wild-type	-	3WMW	

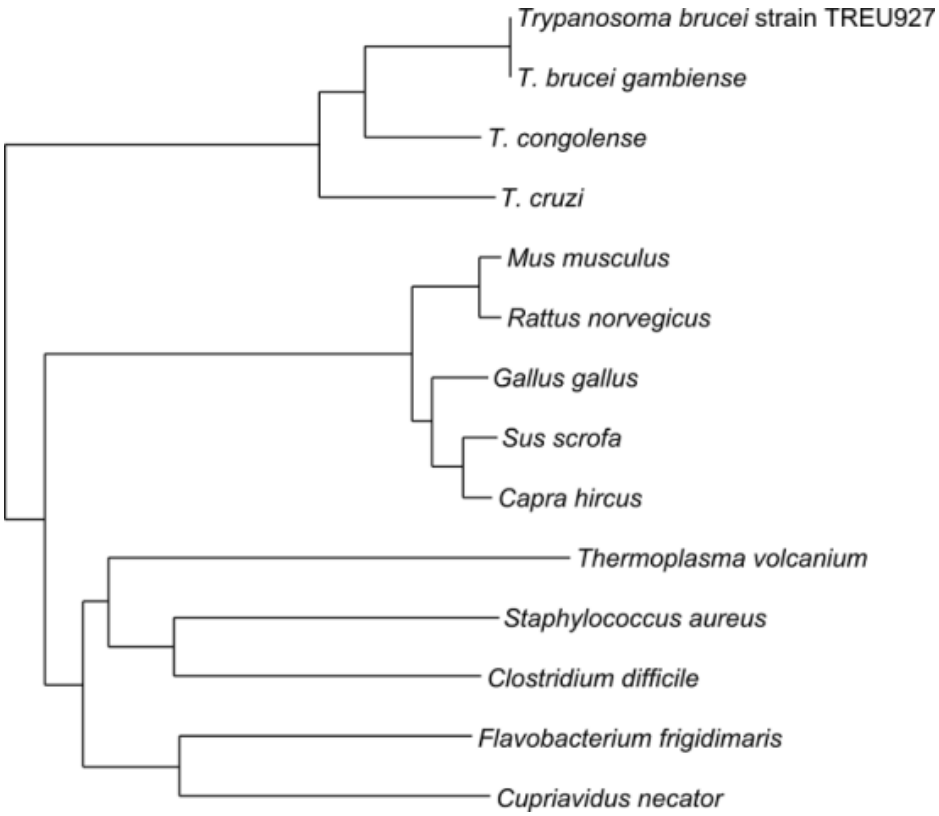

Figure S1 Phylogenetic Tree of TDH from *Trypanosoma* and other organisms. The lengths of the horizontal lines represent the relative genetic distances between the TDH genes from the named organisms. Genes connected to a common branch share a common genetic lineage. The phylogenetic tree was generated using SeaView 4.

Figure S2 Primary sequence alignment of TDH from *Trypanosoma* species and other organisms. Each residue is represented by the corresponding single-letter code, and residues are coloured according to 8 default families of amino acids sharing biochemical characteristics. Sequences of non-aligning residues are represented by a dash. Key conserved sequence segments are indicated by an arrow and bold text. The alignment was created using the software SeaView 4.

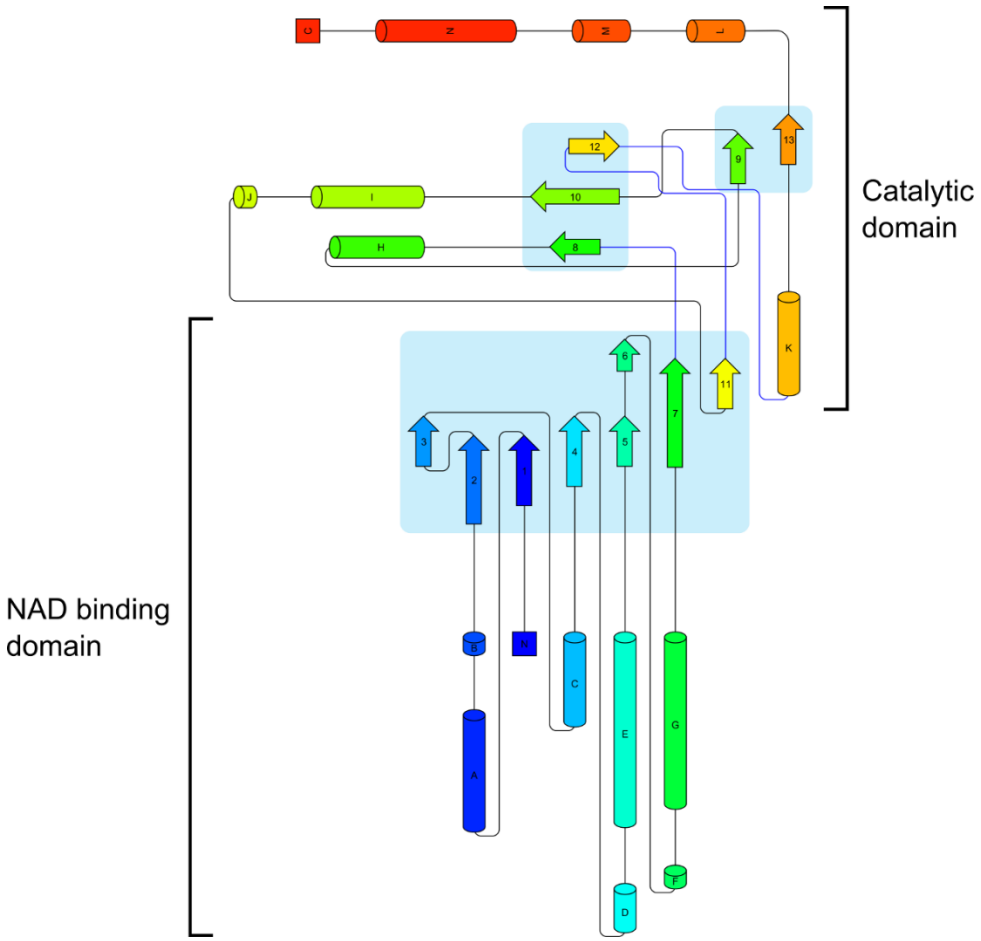

Figure S3 Protein topology diagram of TDH from *T. brucei*. From the N-terminus (N) to C-terminus (C), alpha helices are lettered from A to Z and beta strands are numbered in ascending order. The secondary structure elements are coloured from blue, starting at the N-terminus, through green, yellow and orange to red at the C-terminus. This diagram was drawn using Pro-origami software and a monomeric TDH structure (from PDB: 5K4Q).

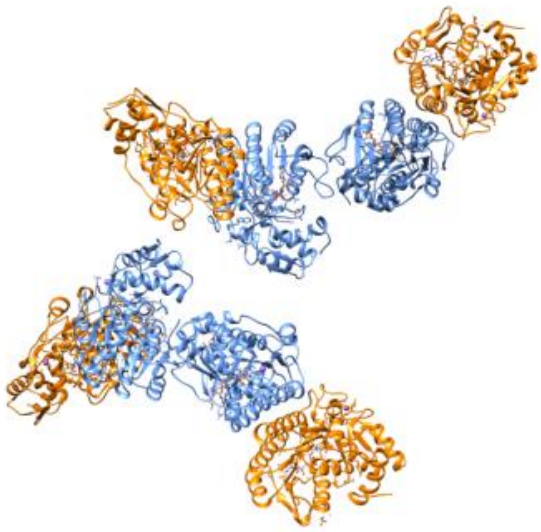

Figure S4 Visualisation of TDH structure (PDB: 5K4W) produced by pdbset, showing all symmetry-related molecules in the crystallographic unit cell. There is one dimer in the asymmetric unit, and four dimers are created by crystallographic symmetry. Subunits within the same dimer are coloured differently (blue and gold).

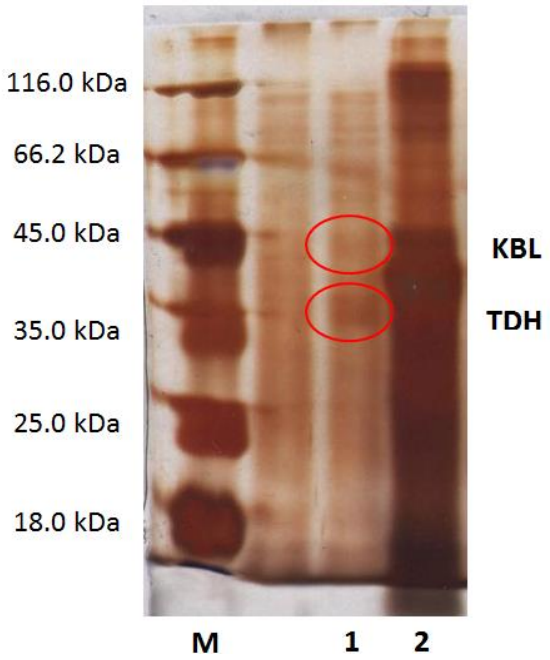

Figure S5 Silver stain of an SDS-PAGE gel indicating the presence of TDH and KBL in fractions that corresponded to peaks on the chromatogram resulting from the size-exclusion chromatography of a mixture of TDH and KBL (See Supplementary Table 2). M = molecular weight standards; 1 = fraction at 12-14ml elution (peak at 12.5ml). 2 = fraction at 16-18ml(peak at 16.75ml). The protein bands in Lane 1 are light, suggesting that the concentrations of both proteins were very low. In Lane 2, which analyses a fraction corresponding to a much larger peak, the bands are less distinguishable, due to over-staining in this lane. As the protein concentrations were high, bands corresponding to both proteins can still be seen.

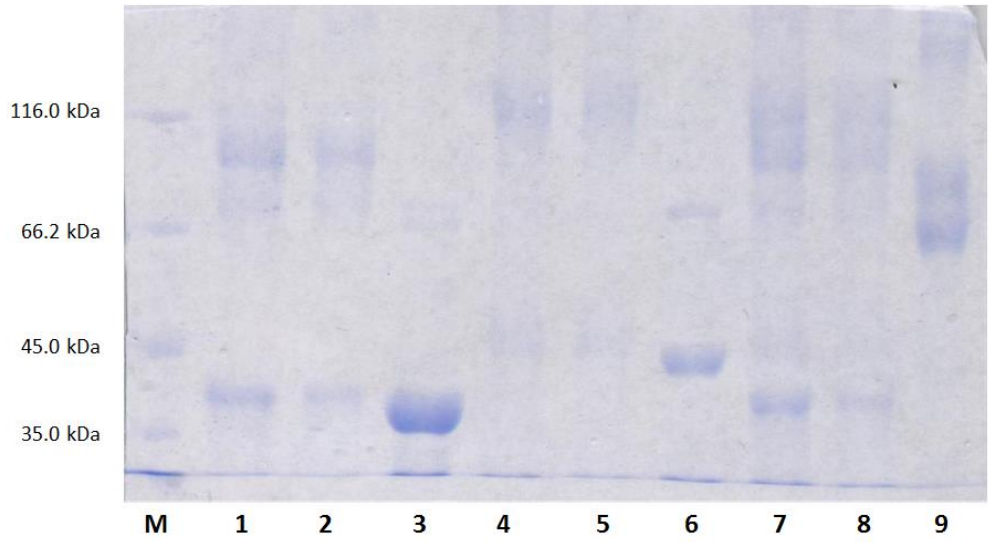

Figure S6 SDS PAGE analysis of cross-linking experiments with TDH and KBL, on a 9% polyacrylamide gel. M = molecular weight standards; 1 = TDH (2.6 x 10-2 mM[1mg/ml]); 2 = TDH (1.3 x 10-^{2} mM [0.5mg/ml]); 3 = TDH (2.6 x 10^{-2} mM [1mg/ml]) non-cross-linked control; 4 = KBL (2.2 x 10^{-2} mM [1mg/ml]); 5 = KBL (1.1 x 10^{-2} mM [0.5mg/ml]); 6 = KBL (2.2 x 10^{-2} mM [1mg/ml]) non-cross-linked control; 7 = TDH (2.6 x 10^{-2} mM [1mg/ml])and KBL (2.2 x 10^{-2} mM [1mg/ml]); 8 = TDH (1.3 x 10^{-2} mM [0.5mg/ml]) and KBL (1.1 x 10^{-2} mM [0.5mg/ml]); 9 = BSA control (1.5 x 10^{-2} mM [1mg/ml]). Note that the protein bands of samples exposed to DMS appear higher due to covalent binding with DMS, leading to a higher molecular weight. This is also the cause of there being more than one band corresponding to a particular oligomer.

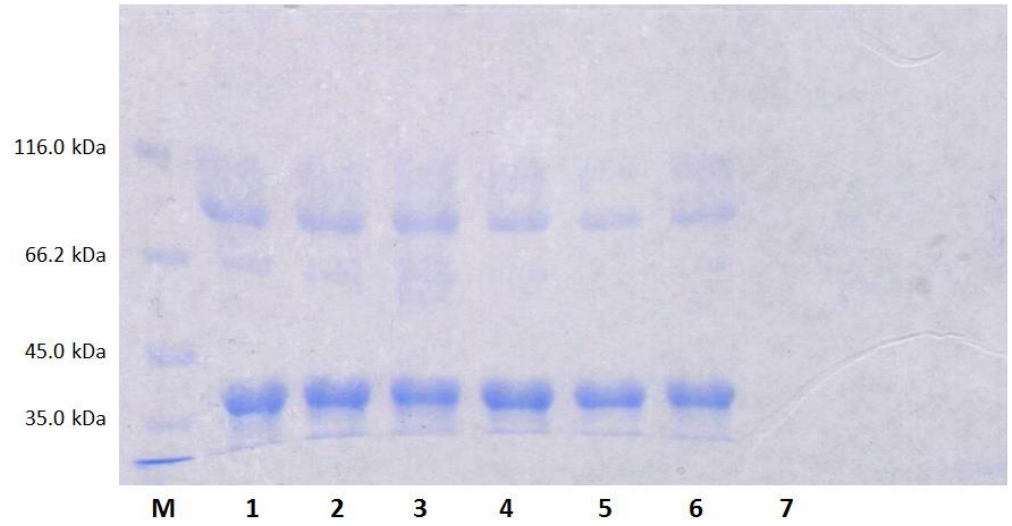

Figure S7 SDS PAGE analysis of cross-linking experiments with TDH (at 2.6 x 10^{-2} mM [1mg/ml] concentration) on a 9% polyacrylamide gel. M = molecular weight standards; 1 = TDH; 2 = TDH and 1mM NAD^+; 3 = TDH and 10mM NAD^+; 4 = TDH, 1mM NAD+ and 30mM L-threonine; 5 = TDH, 10mM NAD^+ and 30mM L-threonine; 6 = TDH, 10mM NAD^+ and 15mM L-threonine; 7 = 10mM NAD^+ and 30mM L-threonine control.

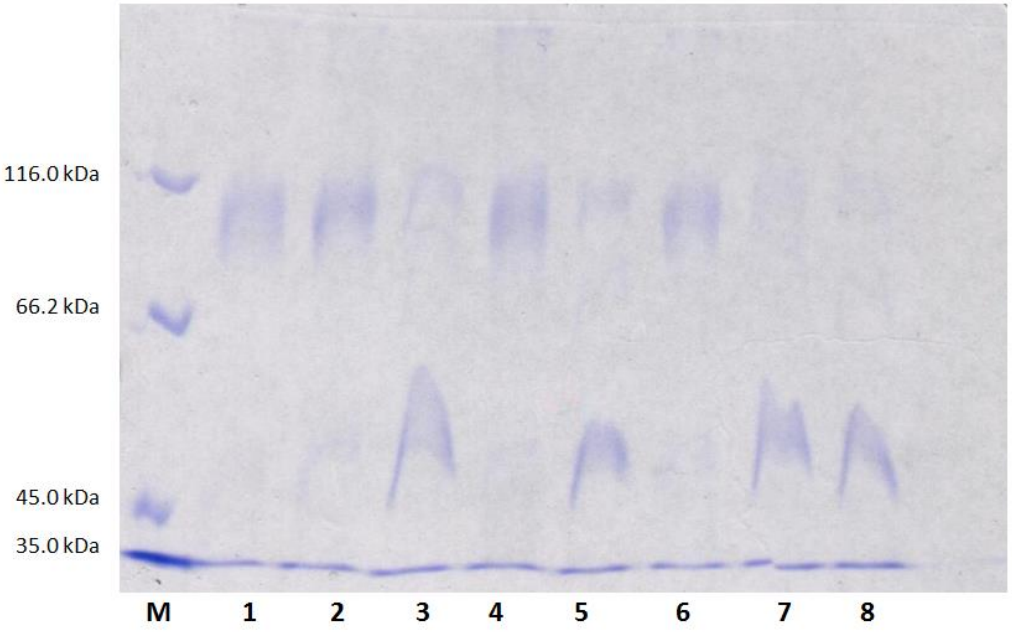

Figure S8 SDS PAGE analysis of cross-linking experiments with KBL (2.2 x 10^{-2} mM [1mg/ml]) on a 9% polyacrylamide gel. M = molecular weight standards; 1 = KBL; 2 = KBL and 1mM PLP; 3 = KBL and 30mM glycine; 4 = KBL and 5mM A-CoA; 5 = KBL, 1mM PLP and 30mM glycine; 6 = KBL, 1mM PLP and 5mM A-CoA; 7 = KBL, 1mM PLP, 5mM A-CoA and 30mM glycine; 8 = KBL, 5mM A-CoA and 30mM glycine.

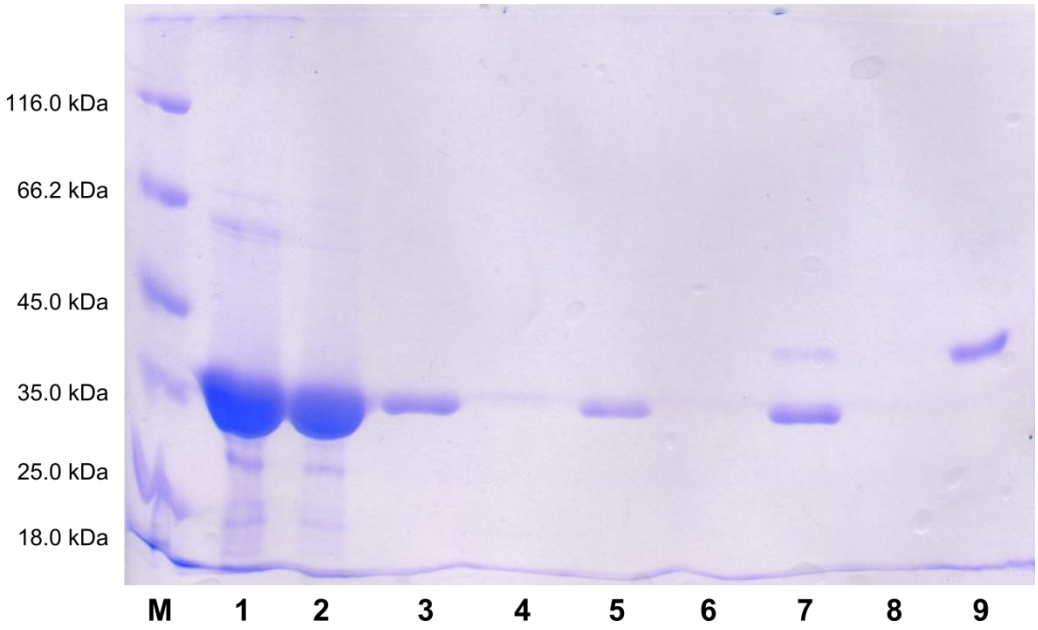

Figure S9 SDS PAGE analysis of the pull-down assay to investigate complex formation between TDH and KBL. M = molecular weight standards; 1 = original TDH sample; 2 = TDH and thrombin; 3 = TDH with His-tag removed by washing the solution through a Ni-NTA column and benzamidine column attached end-to-end; 4 = eluate collected on elution of histidine tag from Ni-NTA column; 5 = elution of thrombin (MW approx. 37 kDa) from the benzamidine column; 6 = solution flowing through Ni-NTA column as KBL is loaded; 7 = solution flowing through the Ni-NTA column as TDH is loaded; 8 = solution flowing through the Ni-NTA column as the column is washed with buffer; 9 = eluate containing KBL after applying elution buffer to the column.

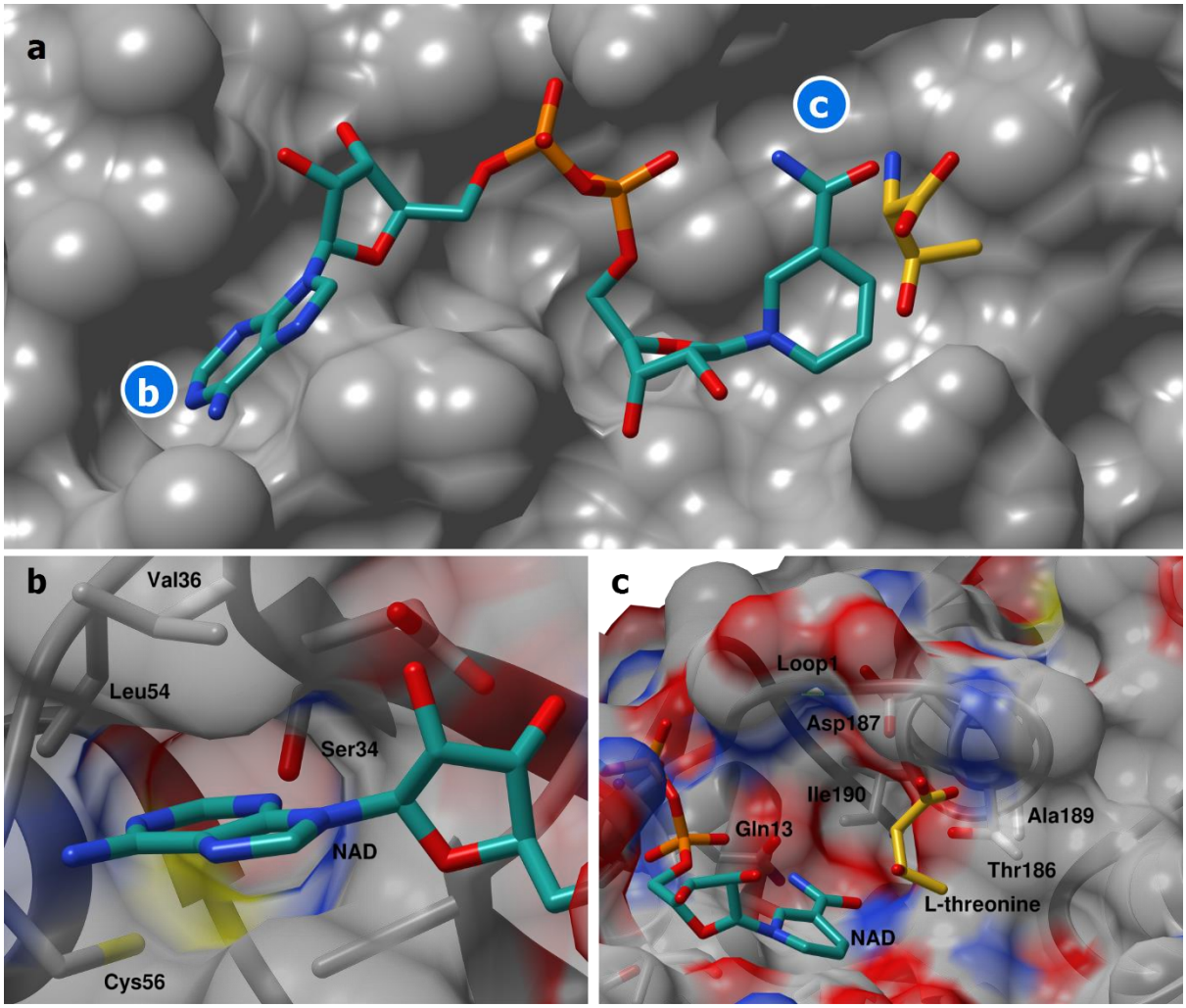

Figure S10 Additional binding pockets in the NAD binding site. Panel a shows a cross-section of TDH, focusing on the NAD binding site. The foci of panels b (additional pocket adjacent to C2 atom of adenine) and c (additional pocket close to nicotinamide amide) are highlighted in panel A.

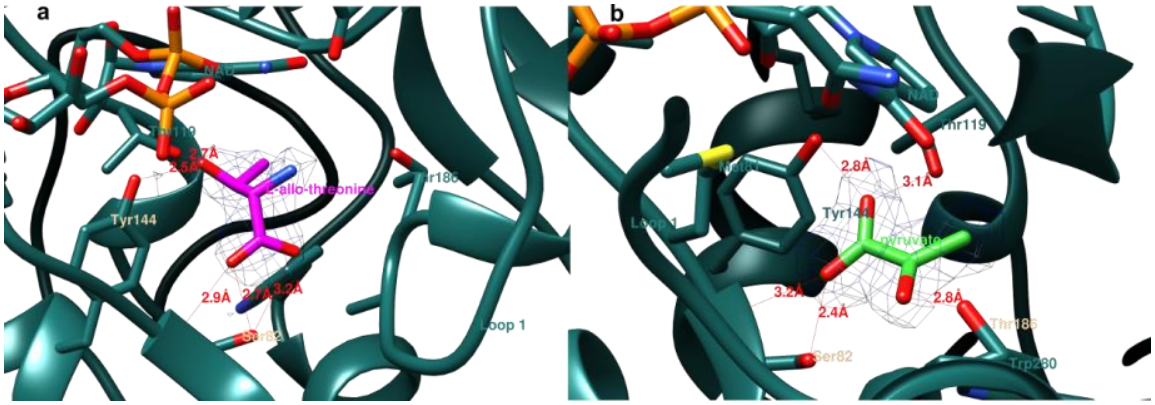

Figure S11 Binding of L-*allo*-threonine (a, PDB: 5K50) and pyruvate (b, PDB: 5LC1) to TDH in the L-threonine binding pocket. Hydrogen bonds are indicated by red lines, electron density of bound ligands is represented by a blue mesh.

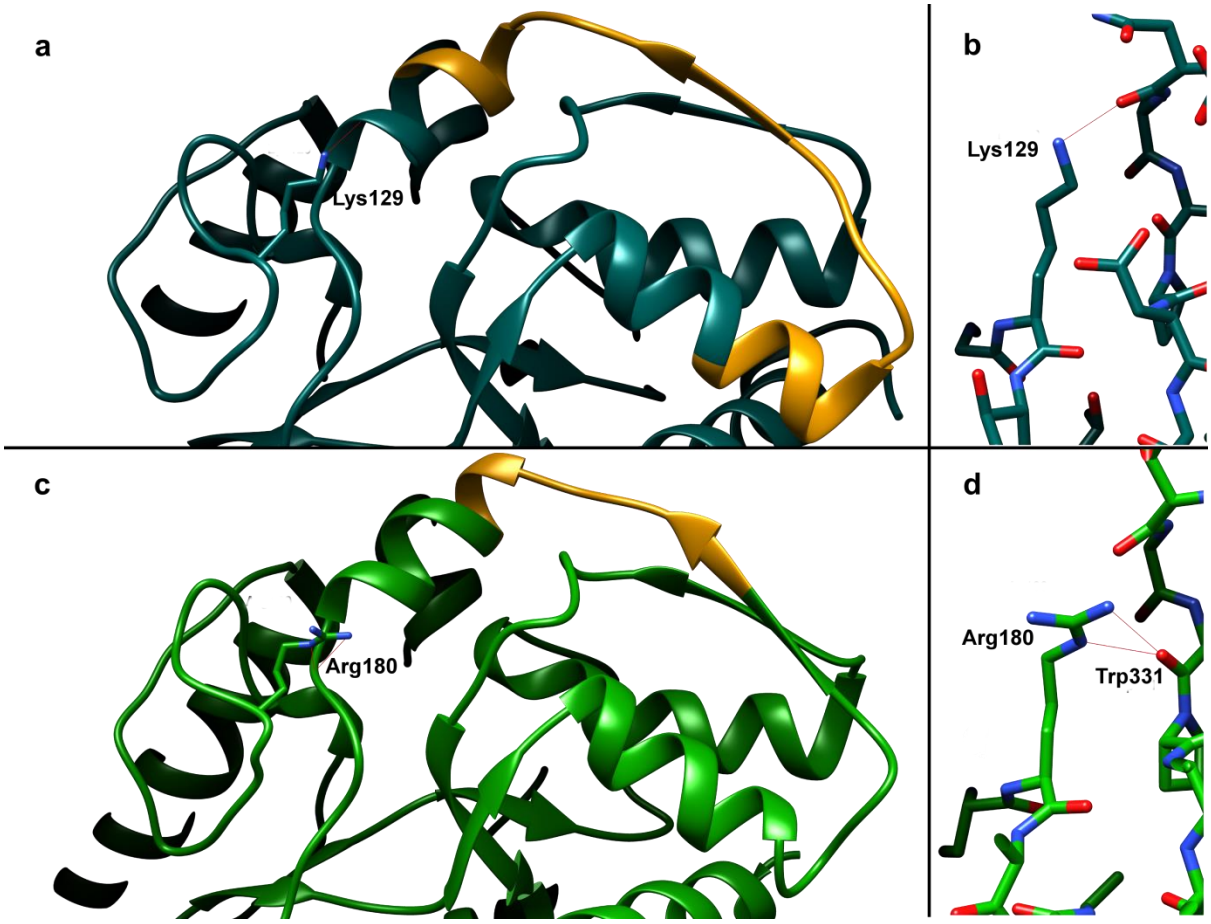

Figure S12 Equivalent positions of Lys129 and Arg180 in *Tb*TDH (top, PDB: 4YR9) and *Mm*TDH (bottom, PDB: 5K4W), respectively. Panels a and b correspond to *Tb*TDH and panels c and d correspond to *Mm*TDH. Regions identified as conformationally variable in this study (for TbTDH) and by He et al. (for *Mm*TDH) are coloured gold in panles a and b. Hydrogen bonds are represented by red lines.

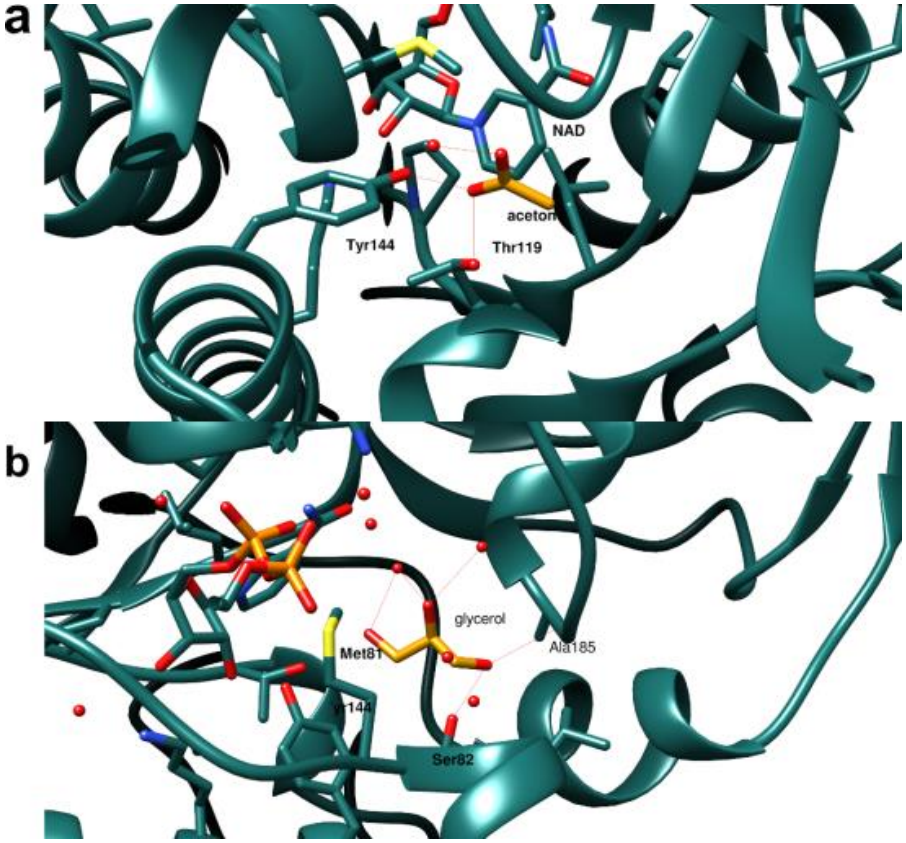

Figure S13 Binding poses of acetone (a) and glycerol (b) in the L-threonine binding pocket. These positions were observed in more than one monomer and/or structure model.

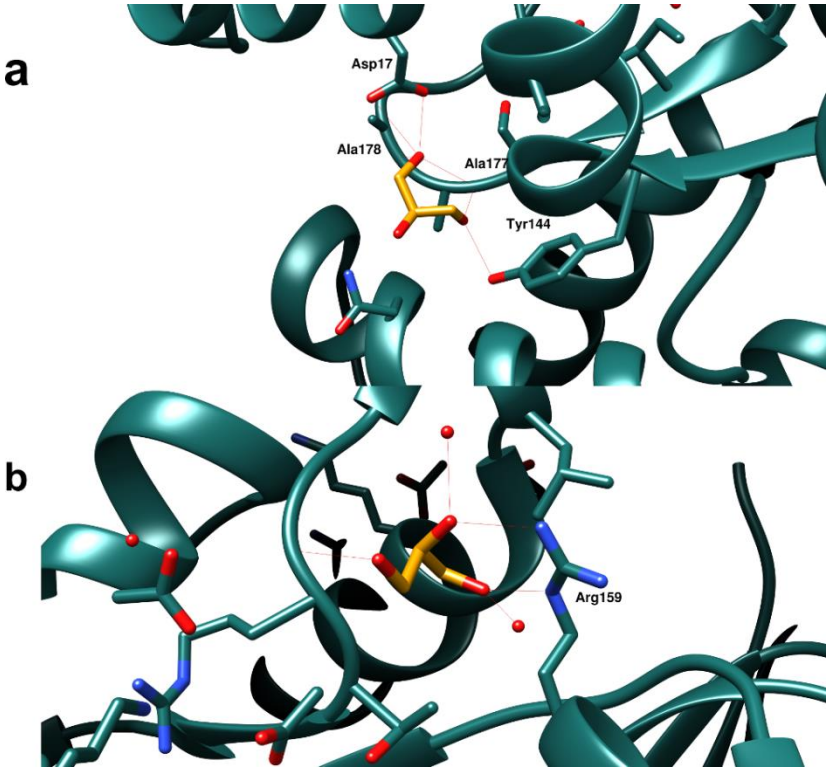

Figure S14 Additional binding positions of glycerol on TDH. Glycerol molecules were often found adjacent to residues Asp17 (a) and Arg159 (b).

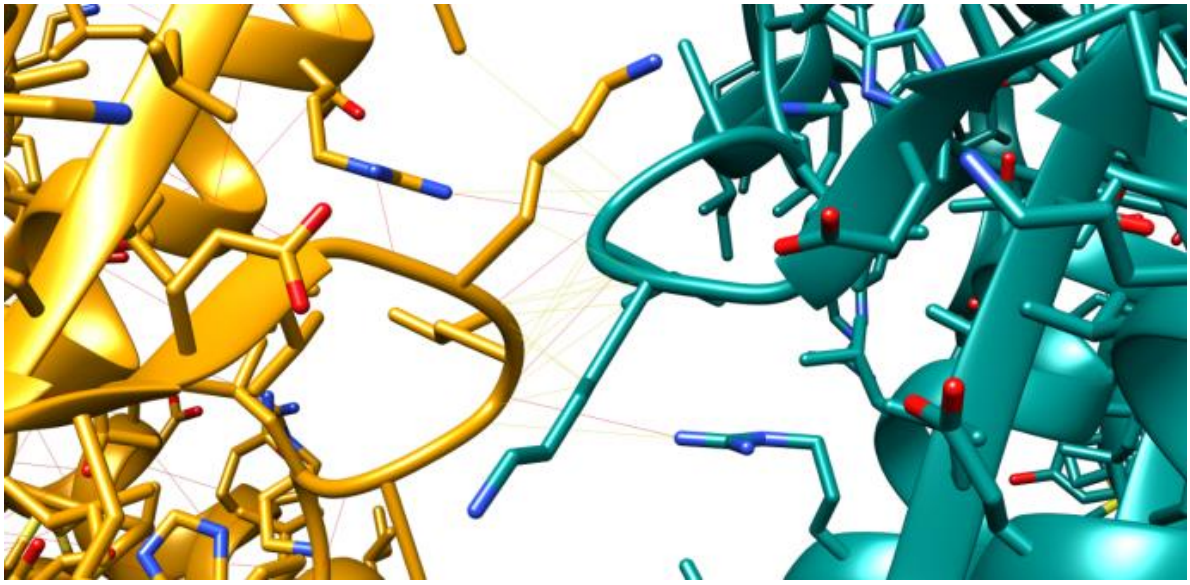

Figure S15 An interaction between two TDH monomers (PDB: 5K50) at the Loop 2 region (Pro44-Gly50). Hydrogen bonds between residues on different subunits are depicted with red lines.

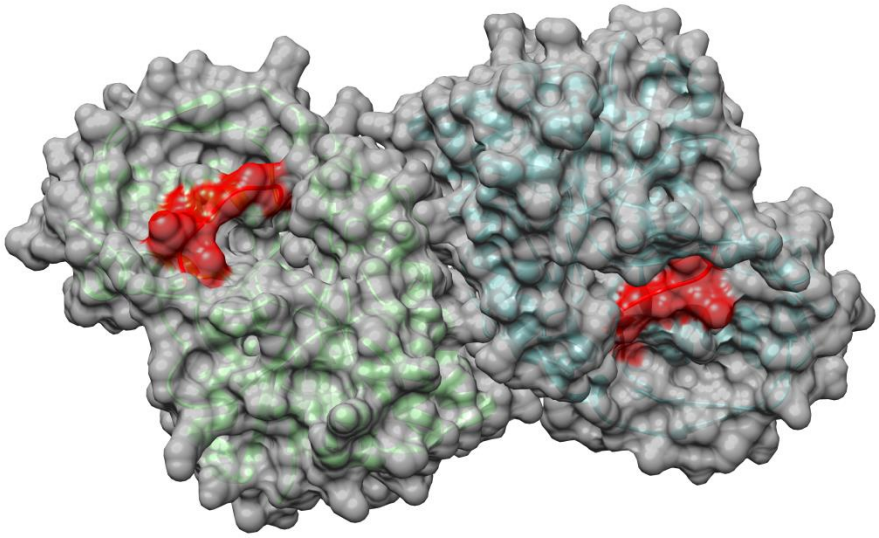

Figure S16 Combined ribbon and surface representation of the TDH dimer, determined in crystallographic model PDB: 5K4U. In one subunit (green ribbon, left) Loop 1 is in the open conformation. In the second subunit (blue ribbon, right) Loop 1 is in the closed conformation. Loop 1 is coloured red in both subunits.