

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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Model	Oligomer	Accessible surface	Buried surface	∆G <sub>diss</sub> *	Stable in	Visual
		area (Ų)	area (Ų)	(kcal.mol⁻	solution?	confirmation
				<sup>1</sup> )		
Model: <u>5L9A</u>	Dimer	25869.5	3161.9	2.5	Yes	$\checkmark$
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						
Model: <u>5LC1</u>	Dimer	24444.8	5980.5	3.8	Yes	$\checkmark$
Space group: P2 <sub>1</sub> 2 <sub>1</sub> 2						
Unit cell parameters:	<b>.</b> .	24270 4	64474	2.6	X	$\checkmark$
a=132.03, b=276.49,	Dimer	24278.4	6117.1	3.6	Yes	v
c=55.74; αβγ=90°						
Molecules in the	Dimer	24600.3	5880.2	3.3	Yes	$\checkmark$
asymmetric unit: 6						
Model: <u>5K4Q</u>	Dimer	24918.1	5470.9	3.3	Yes	$\checkmark$
Space group: P2 <sub>1</sub> 2 <sub>1</sub> 2						
Unit cell parameters:					N.	$\checkmark$
a=132.04, b=276.49,	Dimer	24832.7	5449.2	2.6	Yes	v
c=55.74; αβγ=90°						
Molecules in the	Dimer	24993.7	5428.6	2.3	Yes	$\checkmark$
asymmetric unit: 6						
Model: <u>5K4T</u>	Dimer	25239.1	5028.6	3.2	Yes	$\checkmark$
Space group: P4 <sub>3</sub> 2 <sub>1</sub> 2						
Unit cell parameters:						
ab=91.76, c=93.60;						
αβγ=90°						
Molecules in the						
asymmetric unit: 1						

**Table S1**TDH quaternary structure identified by PISA and confirmed visually. \*  $\Delta G_{diss} =$ dissociation barrier.

Model	Oligomer	Accessible surface	Buried surface	∆G <sub>diss</sub> *	Stable in	Visual
		area (Ų)	area (Ų)	(kcal.mol <sup>-</sup>	solution?	confirmation
				<sup>1</sup> )		
Model: <u>5K4V</u>	Dimer	24519.8	4880.8	4.4	Yes	$\checkmark$
Space group: P2 <sub>1</sub> 2 <sub>1</sub> 2						
Unit cell parameters:						
a=90.40, b=131.53,						
c=55.02; αβγ=90°						
Molecules in the						
asymmetric unit: 2						
Model: <u>5K4U</u>	Dimer	24264.3	7309.4	4.1	Yes	$\checkmark$
Space group: P2 <sub>1</sub> 2 <sub>1</sub> 2						
Unit cell parameters:						
a=133.03,b=273.06,						
c=55.80; αβγ=90°						
Molecules in the						
asymmetric unit: 2						
Model: <u>5K50</u>	Dimer	24167.7	4813.6	4.3	Yes	$\checkmark$
Space group: P2 <sub>1</sub> 2 <sub>1</sub> 2						
Unit cell parameters:		24294.1	4772.0	3.1	Yes	$\checkmark$
a=133.03, b=273.06,	Dimer					
c=55.80; αβγ=90°						
Molecules in the	Dimer	24217.8	4790.1	2.4	Yes	$\checkmark$
asymmetric unit: 6						
						/
Model: <u>5K4W</u>	Dimer	24587.0	5788.1	0.3	Yes	$\checkmark$
Space group: P2 <sub>1</sub> 2 <sub>1</sub> 2						
Unit cell parameters:						
a=83.45, b=136.12,	Tetramer	46831.2	13919.2	-1.6	Uncertain	x (see
c=55.69; $\alpha\beta\gamma$ =90°	retrainer	10031.2	13313.2	1.0	Giftertail	Supplementary
Molecules in the						Figure 12)
asymmetric unit: 2						
Model: <u>5K4Y</u>	Dimer	24779.8	6050.2	4.5	Yes	$\checkmark$
Space group: P2 <sub>1</sub> 2 <sub>1</sub> 2						
Unit cell parameters:		24400 5	6254 4			$\checkmark$
a=133.45, b=278.63,		24400.6	6351.1	4.1	Yes	v
b=56.27; αβγ=90°						

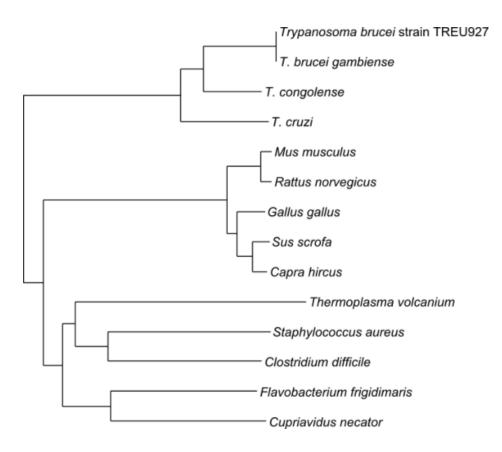
Model	Oligomer	Accessible surface area (Å <sup>2</sup> )	Buried surface area (Ų)	ΔG <sub>diss</sub> * (kcal.mol <sup>-</sup> <sup>1</sup> )	Stable in solution?	Visual confirmation
Molecules in the asymmetric unit: 6		24521.6	6357.1	3.1	Yes	$\checkmark$

Sample Contents	Ve (ml)	Ve/V <sub>0</sub>	Interpolated MW	Multiple of MW	Suggested
			(kDa)		Oligomeric State
TDH (1.8 x10 <sup>-1</sup> mM [6.8mg/ml])	15	1.79	68.8	1.82	Dimer
TDH (1.1 x10 <sup>-1</sup> mM [4mg/ml]) + 10mM NAD <sup>+</sup>	15	1.79	68.8	1.82	Dimer
KBL (4.4 x10 <sup>-2</sup> mM [2mg/ml])	14.4	1.71	87.5	1.91	Dimer
TDH (5.3 x10 <sup>-2</sup> mM [2mg/ml]) + KBL (4.4 x10 <sup>-2</sup> mM [2mg/ml]) -	12.5	4.40		4.97 (TDH); 4.09	Multi-enzyme
first peak	12.5	1.49	187.7	(KBL)	complex
TDH (5.3 x10 <sup>-2</sup> mM [2mg/ml]) + KBL (4.4 x10 <sup>-2</sup> mM [2mg/ml]) -	16.0	4.00	24.0	0.90 (TDH); 0.74	Monomers (TDH
second peak	16.8	1.99	34.0	(KBL)	and/or KBL)

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

Organism	Amino	Quaternary	Wild-	Ligands	PDB ID	Literature
	acids	structure	type/mutant			reference(s)
Trypnanosoma	332	Dimer	Wild-type	-	5L9A	This manuscript,
brucei						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,	5K50	This manuscript,
				L-allo-threonine		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	312	Dimer	Wild-type	NAD; glycerol	2YY7	Febs J 277: 5124-
frigidimaris						5132 (2010)
Thermoplasma	317	Dimer	Wild-type	NAD	3A1N	J Biol Chem
volcanium	317	Dimer	Wild-type	NAD; pyruvate	3A4V	287:12966-12974
	317	Dimer	Y137F mutant	NAD; L-threonine	3A9W	(2012)
	317	Dimer	Y137F mutant	NAD; L-3-	3AJR	-
				Hydroxynorvaline		
Mus musculus	373	Dimer	Wild-type	NAD <sup>+</sup>	4YR9	J Struct Biol
	373	Dimer	Wild-type	-	4YRA	192:510-518 (2015)
	373	Dimer	R180K mutant	NAD	4YRB	1
Cupriavidus	318	Dimer	Wild-type	NAD; L-threonine	3WMX	J Biol Chem
necator	318	Dimer	Wild-type	-	3WMW	289:10445-10454
						(2014)

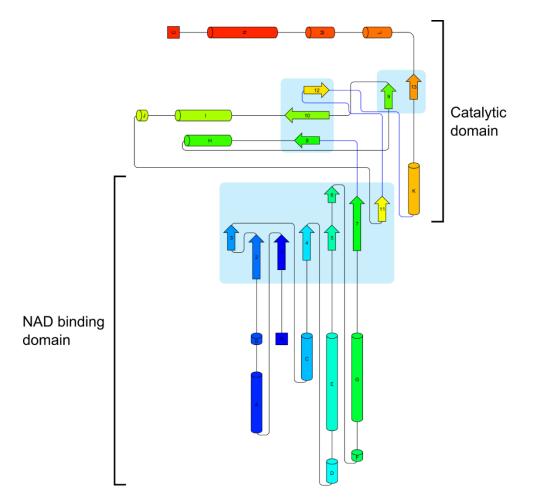
## **Table S3**SDR and GalE-like TDH deposited in the Protein Data Bank.



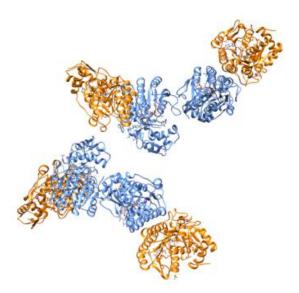
**Figure S1** Phylogenetic Tree of TDH from *Trypanosoma* and other organisms. The lengths of the horizontal lines represent the relative genetic distances between the TDH genes from the named organisms. Genes connected to a common branch share a common genetic lineage. The phylogenetic tree was generated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

Trypanosoma brucei strain TRZU927 T. brucei gambierae T. corpolesae T. corpolesae Varbachorium frigidiraris Cupriavidus necator Mus musculus Staphylococcus sureus C'ottrid'um difficilo Sallus gallus Rottus norvegicus Sus scrofa Copra hircus	HURSEE IN MERSEE IS MERSENSEN INSTADORAL COMMANDER INSTADORAL SSOUVERS IN MERSENSEN INSTADORAL COMMANDER INSTADORAL SSOUVERS MERSENSEN INSTADORAL COMMANDER INSTADORAL SSOUVERS MERSENSEN INSTADORAL COMMANDER INSTADORAL SSOUVERS MERSENSEN INSTADORAL COMMANDER INSTADORAL SSOUVERS MERSENSENSENSENSENSENSENSENSENSENSENSENSENS	GXACGX GXACGX   HTTPUL VTGALGUEGT DUBLALGONF   RMAAPUL VTGALGUEGT DUBLALGONF   RMAAPUL DTGALGUEGT DUBLALGONF   RMAAPUL VTGALGUEGT DUBLALGONF   AMANDUL RTGALGUEGT DUBLALGONF   MAAPUL RTGALGUEGT DUBLALGONF   MAAPUL RTGALGUEGT DUBLALGONF   MAAPUL RTGALGUEGT DUBLALGUEGT   MAAPUL RTGALGUEGT RTGALGUEGT   MAAPUL RTGALGUEGT RTGALGUEGT   MAAPUL RTGALGUEGT RTMARGUEGT   MAAPUL RTGALGUEGT RTMARGUEGT   MAAPUL RTGALGUEGT RTMARGUEGT   MAAPUL RTGALGUEGT RAMAPULAPUL   MAAPUL RTGALGUEGT RAMAPULAPUL   MAAPUL RTGALGUEGT RAMAPULAPUL   MAAPUL RTGALGUEGT RAMAPULAPUL   RAMAPUL RTGALGUEGT RAMAPULAPUL   RAMAPUL RTGALGUEGT RAMAPULAPUL   RAMAPUL RTGALGUEGT RAMAPULAP
Trypanosoma bricei sirsin TR2U927 T. brucei gimisinze T. congolesse T. congolesse T. congolesse Vieta and the sector Cuprisvidus mestor Mus mosculus Shaphylococus mireus Chaptalococus mireus Calls gellus Satus norvegicus Sus mirofa Capra hirous		ALENVIER ALVELANDIN N.S. TALEL ATTSUDGE REPOLANDIN N.S. TATELEL ATTSUDGE REPOLADIN N.S. TATELEL ATTSUDGE REPOLADIN STREFT ATTSUDGE REPOLADIN ALENVIER ALVELANDIN ALENVIER ALVELANDIN ALVELANDIN ALVELANDIN ALE
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Trypanosoma brucel strain TAEU927 T. brucef gemölense T. congolesse T. corgolesse T. corgolesse volcanium P.avobacterium frigioimarie Cupriavidus mecator Mus misculus Staphylococcus surets Clostridium difficile Gallus gallus Rattus norvegicus Sus surofa Capra hircus	EVENVEG-TO ORLANSAEDE LDDINALDUG GHEANDUDG ISEDULGLE U. EVENVEG-TO ORLANSAEDE LDDINSAEDE (SVETEVIDAV HEDDINGT VI. ELEVEND-ER ORLANSAEDE LDESSAEDE (SVETEVIDAV HEDDINGT) ELEVEND-ER ORLANSAEDE LDESAEDE (SVETEVIDAV HEDDINGT) UN FREU-TR OLLANGAEDE LDDESAEDE (SVETEVIDAV HEDDINGT)	TRVAQVN

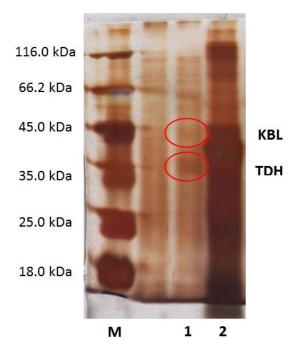
**Figure S2** Primary sequence alignment of TDH from *Trypanosoma* species and other organisms. Each residue is represented by the corresponding single-letter code, and residues are coloured according to 8 default families of amino acids sharing biochemical characteristics. Sequences of non-aligning 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.



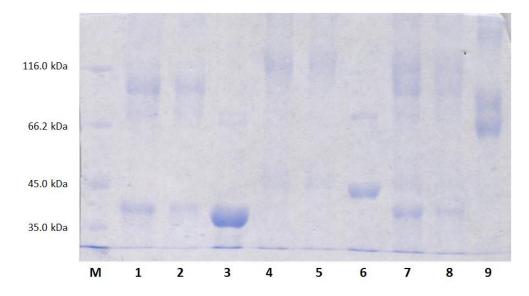
**Figure S3** Protein topology diagram of TDH from *T. brucei*. From the N-terminus (N) to C-terminus (C), alpha helices are lettered from A to Z and beta strands are numbered in ascending order. The secondary structure elements are coloured from blue, starting at the N-terminus, through green, yellow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



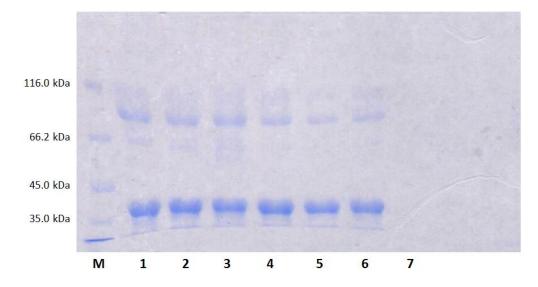
**Figure S4** Visualisation of TDH structure (PDB: <u>5K4W</u>) produced by pdbset, showing all symmetry-related molecules in the crystallographic unit cell. There is one dimer in the asymmetric unit, and four dimers are created by crystallographic symmetry. Subunits within the same dimer are coloured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



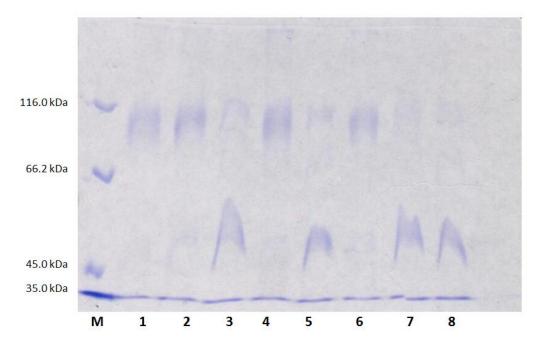
**Figure S5** Silver stain of an SDS-PAGE gel indicating the presence of TDH and KBL in fractions that corresponded to peaks on the chromatogram resulting from the size-exclusion chromatography of a mixture of TDH and KBL (See Supplementary Table 2). M = molecular weight standards; 1 = fraction at 12-14ml 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.



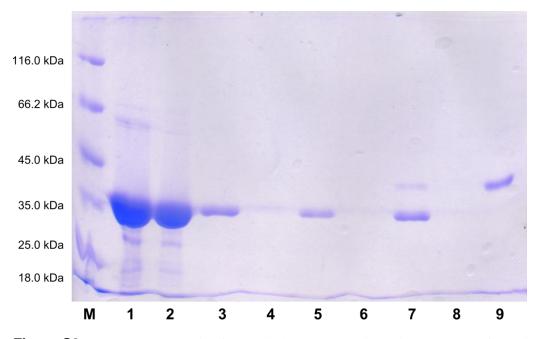
**Figure S6** SDS PAGE analysis of cross-linking experiments with TDH and KBL, on a 9% polyacrylamide gel. M = molecular weight standards;  $1 = \text{TDH} (2.6 \times 10-2 \text{ mM}[1\text{mg/ml}])$ ;  $2 = \text{TDH} (1.3 \times 10^{-2} \text{ mM} [0.5\text{mg/ml}])$ ;  $3 = \text{TDH} (2.6 \times 10^{-2} \text{ mM} [1\text{mg/ml}])$  non-cross-linked control;  $4 = \text{KBL} (2.2 \times 10^{-2} \text{ mM} [1\text{mg/ml}])$ ;  $5 = \text{KBL} (1.1 \times 10^{-2} \text{ mM} [0.5\text{mg/ml}])$ ;  $6 = \text{KBL} (2.2 \times 10^{-2} \text{ mM} [1\text{mg/ml}])$  non-cross-linked control;  $7 = \text{TDH} (2.6 \times 10^{-2} \text{ mM} [1\text{mg/ml}])$  and KBL ( $2.2 \times 10^{-2} \text{ mM} [1\text{mg/ml}]$ );  $8 = \text{TDH} (1.3 \times 10^{-2} \text{ mM} [0.5\text{mg/ml}])$  and KBL ( $1.1 \times 10^{-2} \text{ mM} [0.5\text{mg/ml}]$ ); 9 = BSA control ( $1.5 \times 10^{-2} \text{ mM} [1\text{mg/ml}]$ ). 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.



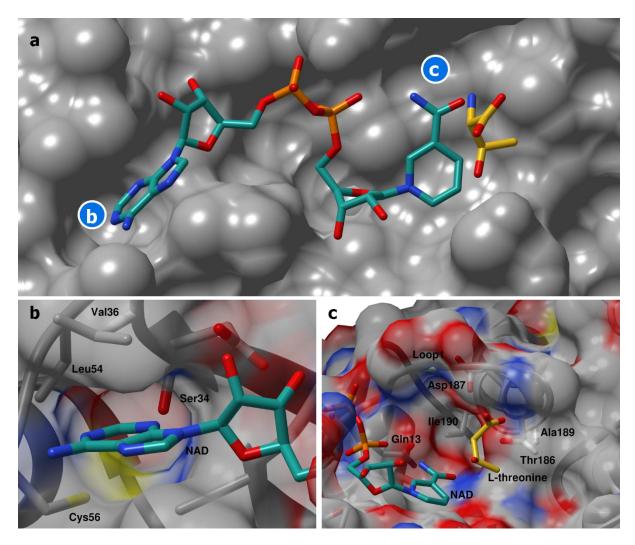
**Figure S7** SDS PAGE analysis of cross-linking experiments with TDH (at 2.6 x  $10^{-2}$  mM [1mg/ml] concentration) on a 9% polyacrylamide gel. M = molecular weight standards; 1 = TDH; 2 = TDH and 1mM NAD<sup>+</sup>; 3 = TDH and 10mM NAD<sup>+</sup>; 4 = TDH, 1mM NAD+ and 30mM L-threonine; 5 = TDH,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



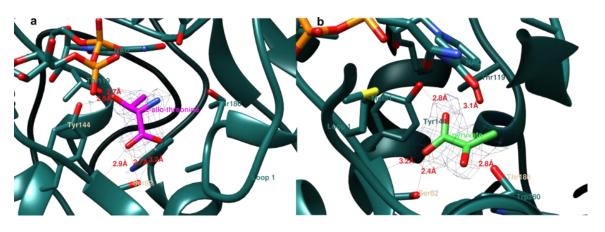
**Figure S8** SDS PAGE analysis of cross-linking experiments with KBL ( $2.2 \times 10^{-2} \text{ mM} [1 \text{ mg/ml}]$ ) on a 9% polyacrylamide gel. M = molecular weight standards; 1 = KBL; 2 = KBL and 1mM PLP; 3 = KBL and 30mM glycine; 4 = KBL and 5mM A-CoA; 5 = KBL, 1mM PLP and 30mM glycine; 6 =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



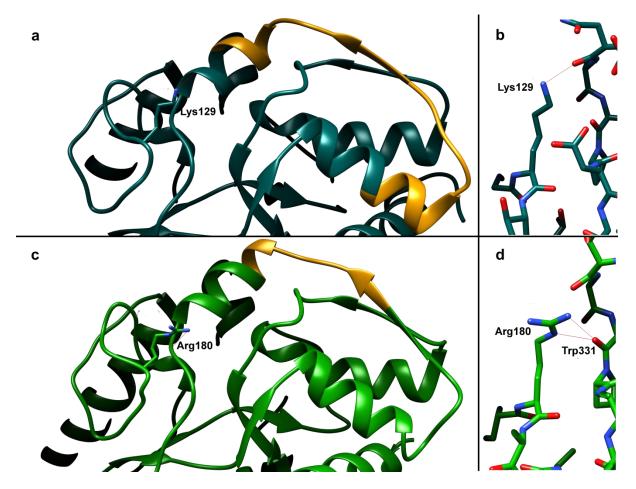
**Figure S9** SDS PAGE analysis of the pull-down assay to investigate complex formation between TDH and KBL. M = molecular weight standards; 1 = original TDH sample; 2 = TDH and thrombin; 3 = TDH with His-tag removed by washing the solution through a Ni-NTA column and benzamidine column attached end-to-end; 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.



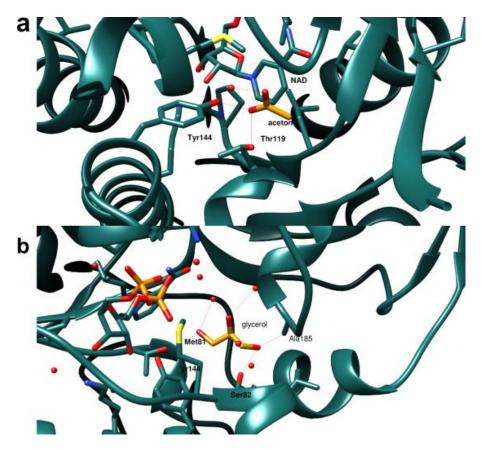
**Figure S10**Additional binding pockets in the NAD binding site. Panel a shows a cross-section of TDH, focusing on the NAD binding site. The foci of panels b (additional pocket adjacent to C2 atom of adenine) and c (additional pocket close to nicotinamide amide) are highlighted in panel A.



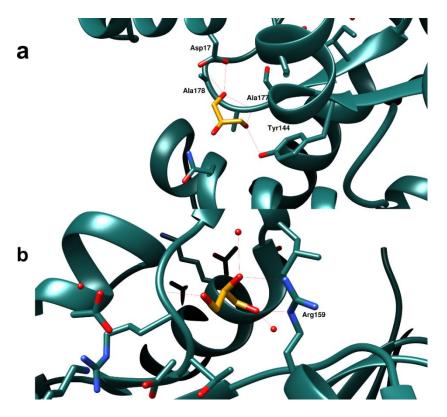
**Figure S11** Binding of L-*allo*-threonine (a, PDB: <u>5K50</u>) and pyruvate (b, PDB: <u>5LC1</u>) to TDH in the L-threonine binding pocket. Hydrogen bonds are indicated by red lines, electron density of bound ligands is represented by a blue mesh.



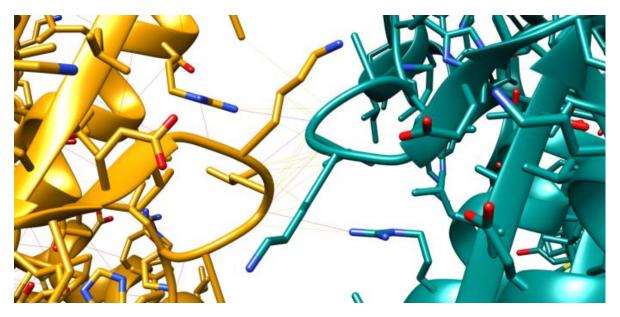
**Figure S12** Equivalent positions of Lys129 and Arg180 in *Tb*TDH (top, PDB: <u>4YR9</u>) and *Mm*TDH (bottom, PDB: <u>5K4W</u>), respectively. Panels a and b correspond to *Tb*TDH and panels c and d correspond to *Mm*TDH. Regions identified as conformationally variable in this study (for TbTDH) and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



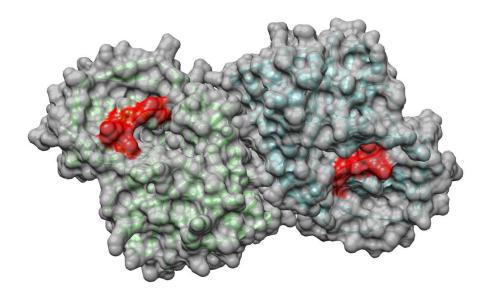
**Figure S13** Binding poses of acetone (a) and glycerol (b) in the L-threonine binding pocket. These positions were observed in more than one monomer and/or structure model.



**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: <u>5K50</u>) 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: <u>5K4U</u>. 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.