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online data

SUPPLEMENTARY ONLINE DATA

Structural and mechanistic insights into type II trypanosomatid tryparedoxin-dependent peroxidases

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Table S1 Data collection and refinement statistics for *Tb*TDPX2

Numbers in parentheses represent the highest resolution bin of width approx. 0.06 Å.

Parameter	Value
Space group	$P2_1$
Cell dimensions	
a	43.3 Å
b	32.7 Å
c	58.3 Å
β	95.9°
X	
Resolution (Å)	58.0–2.1
Observed reflections	37396
Unique reflections	9469
Wilson *B* (Å^2)	27.3
Completeness (%)	96.2 (78.5)
Multiplicity	3.9 (2.7)
R_{merge} (%)	5.9 (26.0)
$\langle I/\sigma(I)\rangle$	25.5 (12.2)
R_{work} (%)	20.5 (23.2)
R_{free} (%)	26.3 (24.9)
r.m.s.d. from ideal values	
Bond lengths (Å)	0.008
Bond angles (°)	1.114
B factors (Å^2)	
Overall	27.7
Main chain	27.2
Side chain	28.1
Waters	31.5
Residues in most favourable regions (%)	92.1
Residues in additional allowed regions (%)	7.9

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The model co-ordinates and structure factors have been deposited with the RSCB PDB (Research Collaboratory for Structural Bioinformatics Protein Data Bank) under the accession code 2VUP.

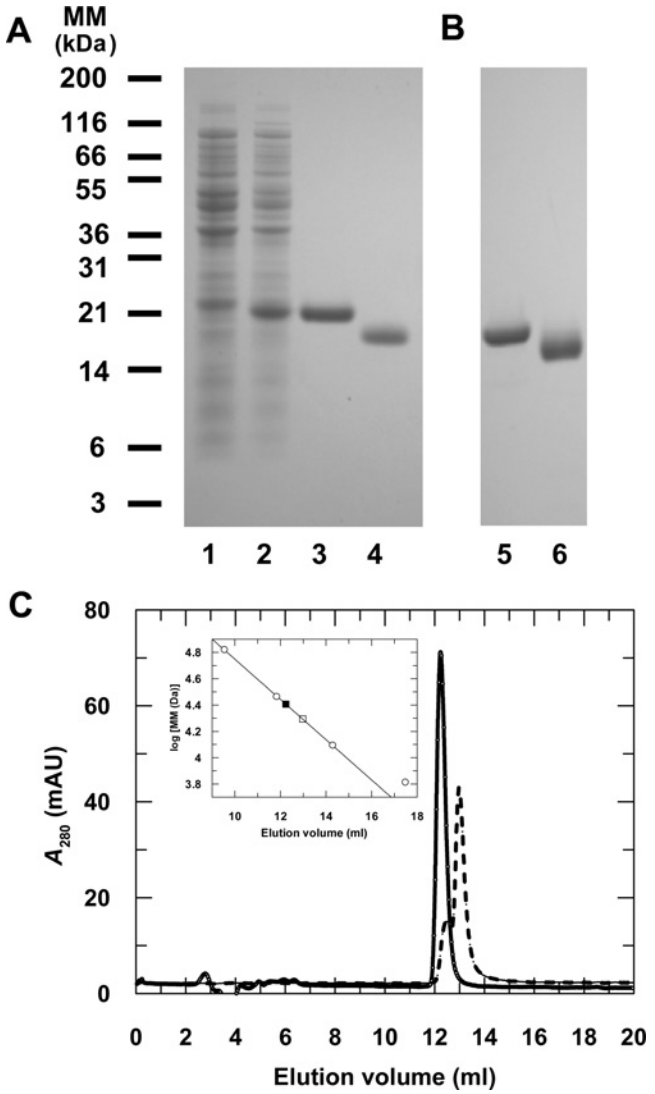

Figure S1 Purification of recombinant *Tb*TDPX2 from *E. coli*

(**A**) SDS/PAGE analysis: lane 1, uninduced fraction of BL21 Star (DE3) pLysS (pET-15b-TbTDPX2); lane 2, 4 h after induction with isopropyl β-D-thiogalactopyranoside; lane 3, 2 μg of purified His_6-tagged protein; lane 4, 2 μg of non-tagged *Tb*TDPX2. Abbreviation: MM, molecular mass. (**B**) SDS/12%-(w/v)-NuPAGE analysis: lane 5, 2 μg of non-tagged reduced *Tb*TDPX2 (50 mM dithiothreitol); lane 6, 2 μg of non-tagged oxidised *Tb*TDPX2 (5-fold molar excess of H_2O_2). The redox state was maintained with 100 mM iodoacetamide. (**C**) Size-exclusion chromatography of reduced and oxidized *Tb*TDPX2 on Superdex 75 10/300 GL. Broken line, *Tb*TDPX2 reduced with 50 mM dithiothreitol; continuous line, *Tb*TDPX2 oxidized with a 5-fold molar excess of H_2O_2. The inset shows a plot of elution volume versus log (molecular mass) in Da {log [MM (Da)]} of low-molecular-mass standards (Sigma–Aldrich; albumin, 66000 Da; carbonic anhydrase, 29000 Da; cytochrome c, 12400 Da; aprotinin, 6500 Da). The predicted molecular mass of the *Tb*TDPX2 monomer is 19168 Da. The elution volume of the reduced protein is represented by □ (molecular mass 19600 Da) and the oxidized protein by ■ (molecular mass 25 400 Da). Abbreviation: mAU, milli-absorbance unit.

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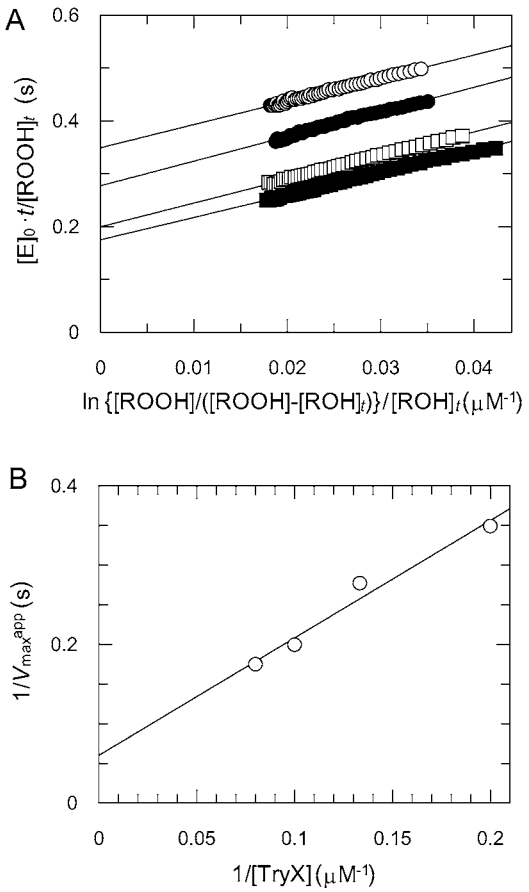

Figure S2 Kinetic properties of *Tb*TDPX2 analysed by the integrated Dalziel rate equation

Kinetic properties of *Tb*TDPX2 were analysed by progress-curve analysis using the integrated Dalziel rate equation for a two-substrate reaction. (**A**) The peroxidase activity of *Tb*TDPX2 was determined with 75 μM cumene hydroperoxide and various concentrations of TryX (○, 5 μM; ●, 7.5 μM; □, 10 μM; ■, 12.5 μM). The slope corresponds to $\varphi 1$, the reciprocal of the rate constant, k_1, for cumene hydroperoxide. $[E]_0$ is initial enzyme concentration, and t is time. (**B**) Secondary plot of the reciprocal apparent V_{max} data, calculated from the intercepts of the first plot, against the reciprocal TryX concentrations. The slope corresponds to $\varphi 2$, the reciprocal of rate constant, k_2, for TryX, and the ordinate intercept corresponds to $\varphi 0$, the reciprocal of the catalytic-centre activity, k_{cat}. Kinetic constants are reported in Table 1 of the main paper.