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Supporting information for article:

Catalytic mechanism and evolutionary characteristics of thioredoxin from *Halobacterium salinarum* NRC-1

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Table S1 Comparison of the structural characteristics of Precambrian TRXs.

L89K mutant LPBCA-TRX (PDB entry: 4ULX) is eliminated from this table.

Protein [§]	LBCA-TRX	AECA-TRX	LACA-TRX	LPBCA-TRX	LGPCA-TRX	LECA-TRX	LAFCA-TRX
PDB code	4BA7	3ZIV	2YNX	2YJ7	2YNI	2YOI	2YPM
Time before present (billion years)	4.2 to 3.5	4.2 to 3.5	4.2 to 3.5	2.5	1.61	1.6	1.37
Resolution (Å)	2.45	2.65	1.75	1.65	1.3	1.3	2.2
Sequence identity for HsTRX-A (%)	39.2	39.2	37.6	39.2	37.3	35	34.3
Cavity (Å ³)/whole volume (Å ³)	11.2%	9.8%	9.9%	9.6%	9.3%	8.8%	8.8%
	(1,603/14,313)	(1,356/13,884)	(1,423/14,337)	(1,359/14,147)	(1,285/13,765)	(1,258/14,222)	(1,191/13,537)
ASA (Å ²)	5,851	5,594	5,777	5,757	5,653	5,926	5,433
(Ala+Gly+Ser+Thr)/(Ile+Leu+Val+Met) [†]	0.6	0.56	0.52	0.58	0.86	0.66	0.85
Asp close proximity to the active site and its pKa	Asp25 (7.49)	Asp24 (6.43)	Asp24 (7.80)	Asp 25 (7.70)	Asp25 (7.20)	Asp25 (7.45)	Asp25 (5.92)
RMSD for HsTRX-A							
Whole C α atoms (Å)	1.09	1.14	0.96	1.09	1.31	1.32	1.48
Main chain atoms of active site (Å)	0.15	0.05	0.09	0.08	0.15	0.07	0.18
Main chain atoms of motif1 (Å)	0.18	0.23	0.18	0.13	0.22	0.15	0.20
Main chain atoms of motif2 (Å)	0.30	0.18	0.12	0.04	0.29	1.32	0.63
Acidic residue content [(Asp+Glu)/(Arg+Lys+His)]							
in the entire structure	1.40 (21/15)	1.20 (18/15)	1.00 (15/15)	1.13 (17/15)	1.38 (18/14)	1.29 (18/14)	1.54 (20/13)
in α helices and β sheets	1.50 (15/10)	1.50 (12/8)	0.91 (10/11)	1.22 (11/9)	1.22 (11/9)	1.17 (14/12)	1.25 (15/12)
at molecular surface (ASA > 0 Å ²)	1.40 (21/15)	1.20 (18/15)	1.00 (15/15)	1.13 (17/15)	1.38 (18/14)	1.29 (18/14)	1.54 (20/13)
Negative charge density at molecular surface (e Å ⁻²)	0.001	0.0005	0	0.0003	0.0009	0.0007	0.0013

§ LBCA, last bacteria common ancestor; AECA, last archaea-eukaryotes common ancestor; LACA, last archaea common ancestor; LPBCA, last common ancestor of the cyanobacterial and deinococcus and thermos groups; LGPCA, last common ancestor of γ -proteobacteria; LECA, last eukaryotic common ancestor; LAFCA, last common ancestor of fungi and animals.

† Ratio of small residues to large aliphatic nonpolar residues.

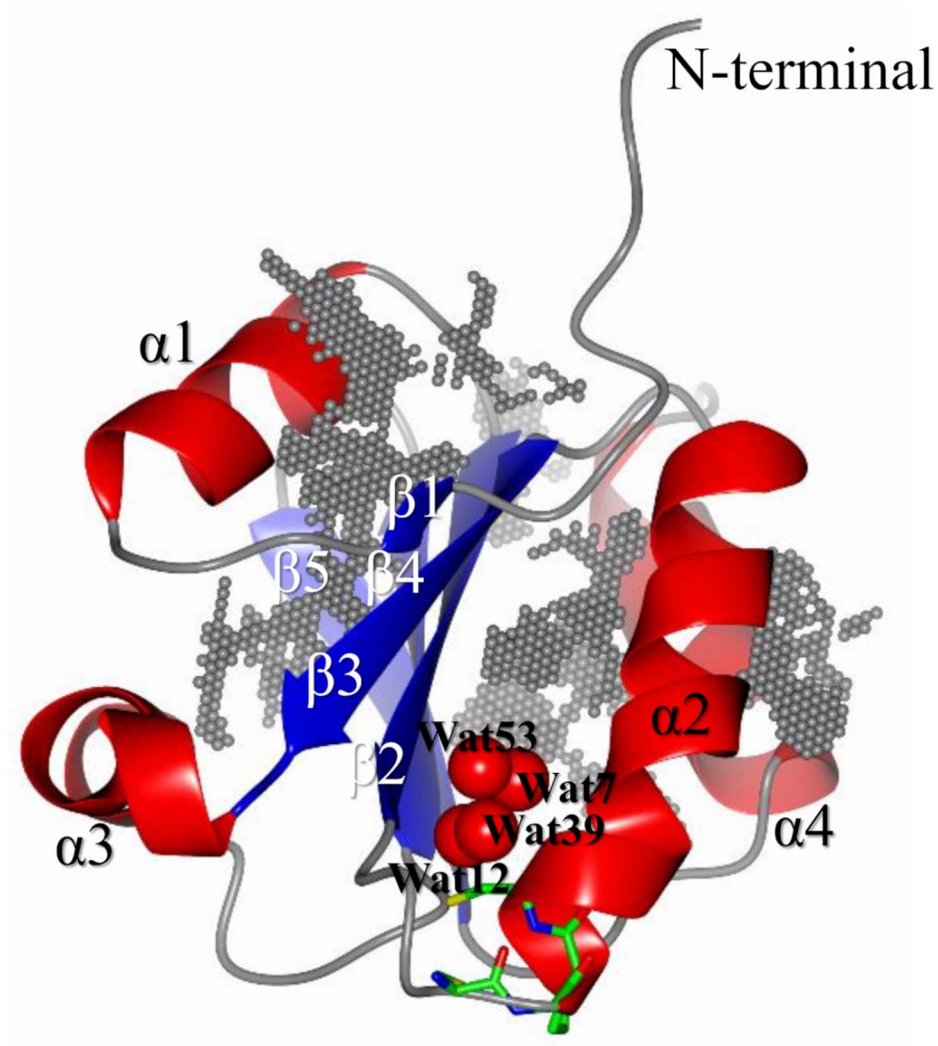


Figure S1 The cavity of WT-HsTRX-A. Spheres colored in grey show the cavity visualized using the web-based *POCASA* program. Spheres colored in red show the water molecules that may contribute to the enzymatic activity. Green sticks show the active site residues.

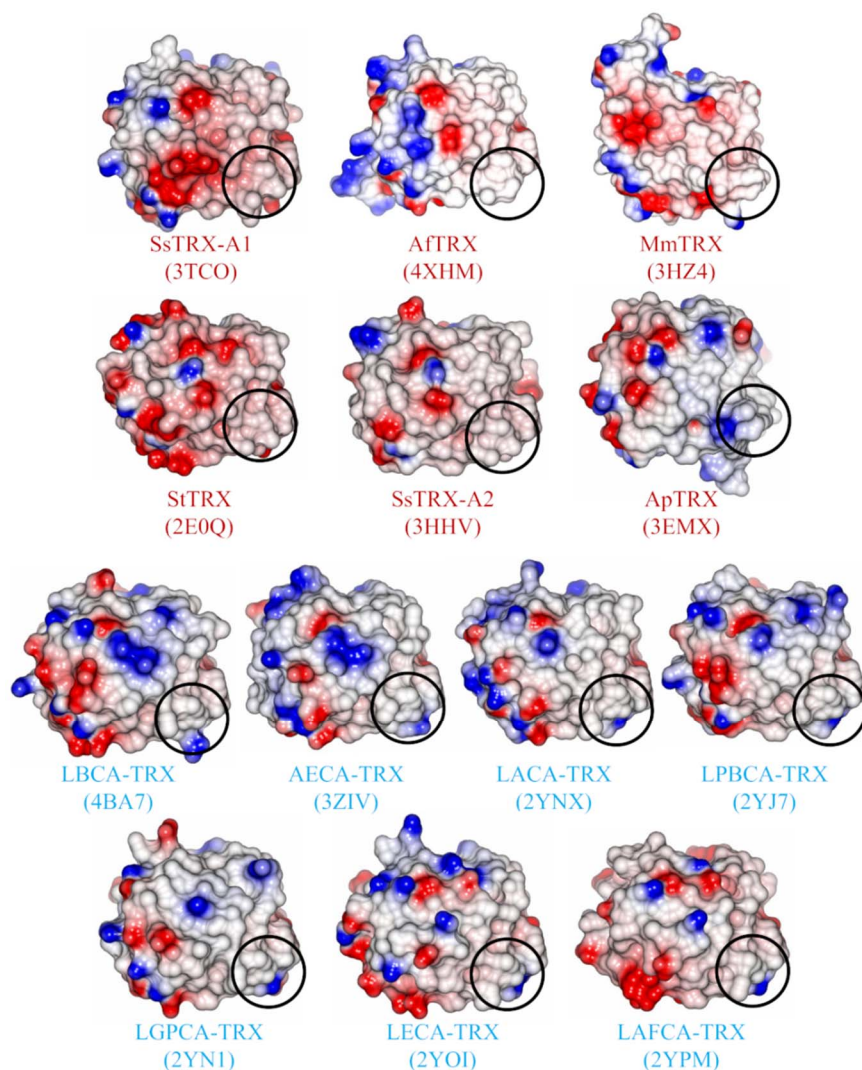


Figure S2 The electrostatic potentials of the molecular surfaces of TRXs homologous to WT-HsTRX-A. The electrostatic surface potentials are contoured from -0.5 V (red) to 0.5 V (blue). Circles show the location of the active site. Colors of PDB entry show the domain of biological source as follows; red, extant archaea; blue, Precambrian ancestor.