SUPPLEMENTAL DATA

Structural snapshots of the yeast alkyl hydroperoxide reductase Ahp1 peroxiredoxin reveal a novel 2-cysteine mechanism of electron transfer to eliminate reactive oxygen species

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Fig. S1. The reaction kinetics of Ahp1 mutants towards substrates t-BOOH and Trx2 were fitted by using the Hill equation. **A.** Ahp1K32R, **B.** Ahp1K32A, **C.** Ahp1K32E towards t-BOOH. **D.** Ahp1K32R, **E.** Ahp1K32A, **F.** Ahp1K32E towards Trx2.

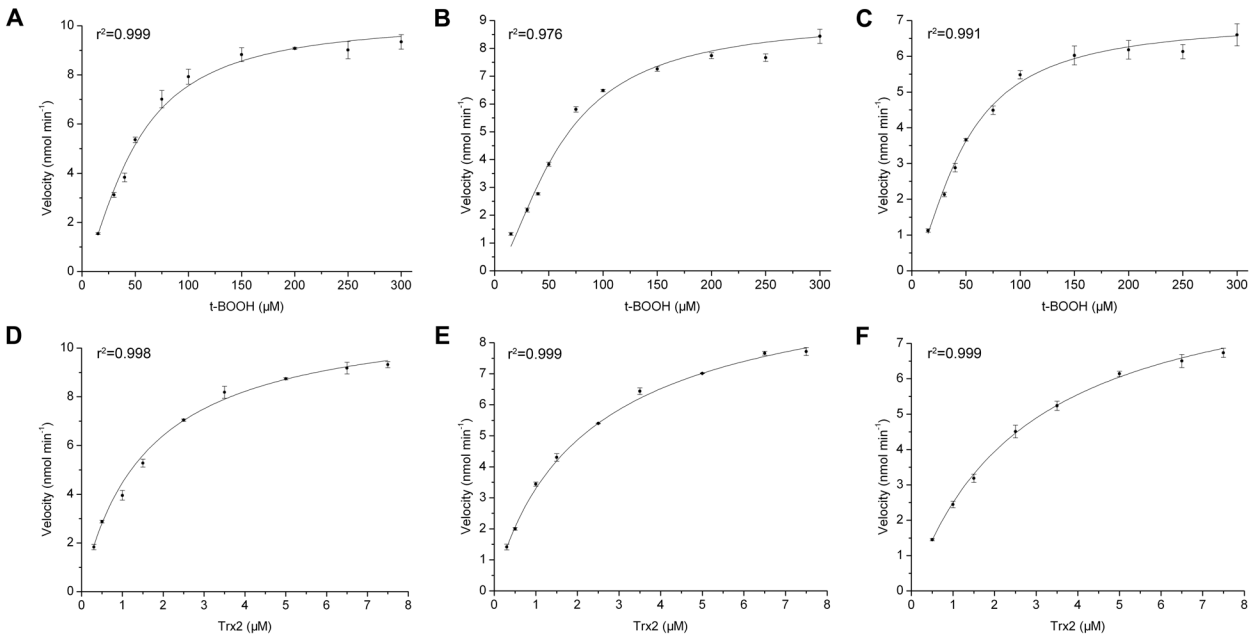

Fig. S2. The 2Fo-Fc electron density maps contoured at 1.0 σ around the two disulfide bonds in oxidized Ahp1. The side chains of Cys31 and Cys62 are shown as sticks and sulfur atoms colored yellow. Residues of subunit B are labeled with a prime.

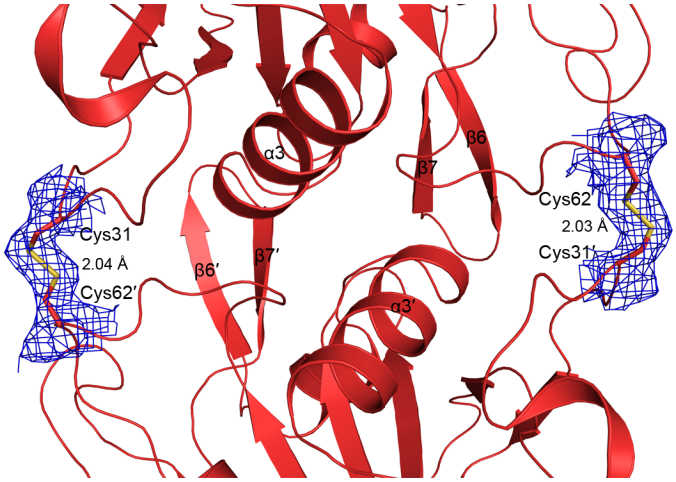

Fig. S3. The 2Fo-Fc electron density map contoured at 1.0 σ around the mixed disulfide bond between Ahp1C62S and Trx2C34S in complex. The side chains of Cys31 are labeled as sticks and sulfur atoms colored yellow. Ahp1 and Trx2 are colored cyan and pink respectively. Residues of Trx2 are labeled with double prime.

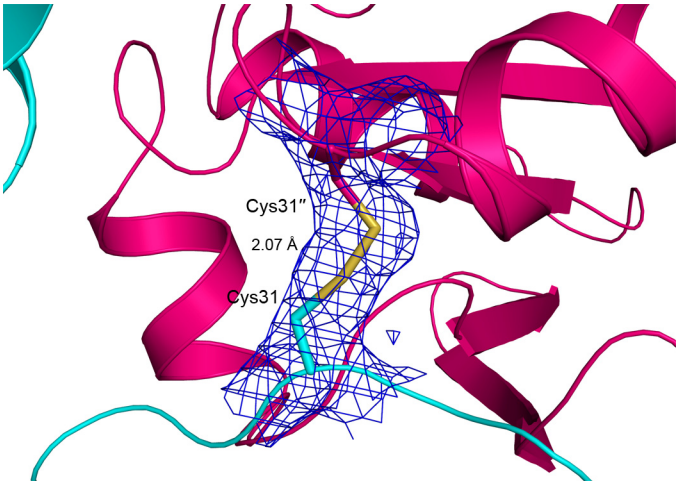

Fig. S4. A docking model of substrate t-BOOH (carbon and oxygen atoms are colored green and red respectively) bound to the active-site pocket of the reduced Ahp1 (the side chains of residues interacted with t-BOOH are colored magenta for subunit A and blue for subunit B). Residues of subunit B are labeled with a prime.

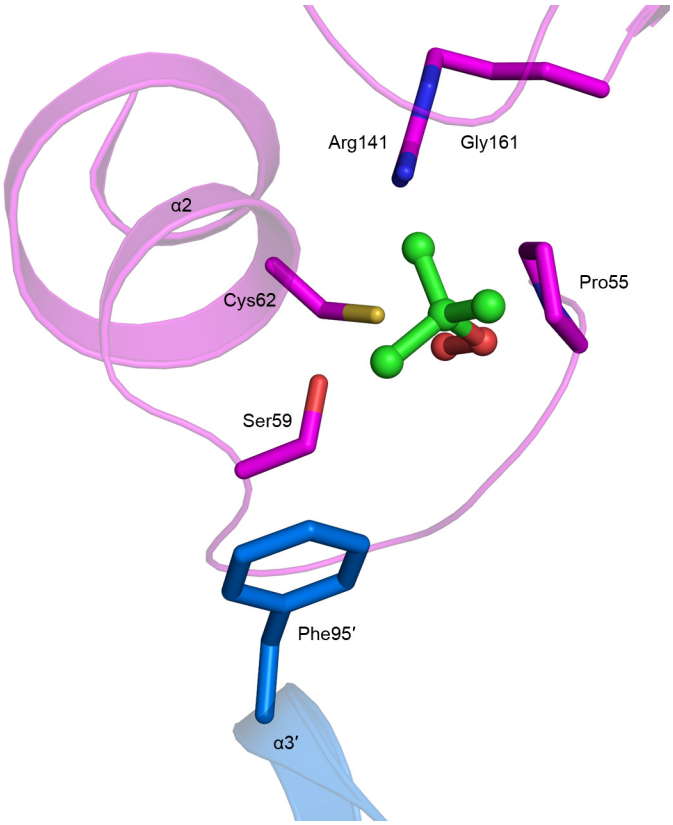

Table S1. Sequences of primers used in this study

Ahp1	Forward primer (5′→3′)	Reverse primer (5′→3′)
WT	CATCATATGTCTGACTTAGTTAACAAGA	ATTGCGGCCGCCTACAAATGAGCCAAGACACT
C31S	ACAGTGAATCTTCTAAGATGCCACA	GTTTGTGGCATCTTAGAAGATTCAC
C62S	CTTTCTCCCCAACCTCTACTGT	TGACAGTAGAGGTTGGGGAGAA
C120S	GACCCAGGCTCTGCTTTCACCA	ATTTGGTGAAAGCAGAGCCTGG
K32R	GAATCTTGTCGGATGCCACAAACAG	TGTGGCATCCGACAAGATTCACTGT
K32A	GAATCTTGTGCGATGCCACAAACAG	TGTGGCATCGCACAAGATTCACTGT
K32E	GAATCTTGTGAGATGCCACAAACAG	TGTGGCATCTCACAAGATTCACTGT

Table S2. The primary sequence of the recombinant Ahp1

MGHHHHHHMSDLVNKKFPAGDYKFQYIAISQSDADSESCKMPQTVEWSKLISENKKVIITGAPA
AFSPTCTVSHIPGYINYLDELVKEKEVDQVIVVTVDNPFANQAWAKSLGVKDTTHIKFASDPGCA
FTKSIGFELAVGDGVYWSGRWAMVVENGIVTYAAKETNPGTDVTVSSVESVLAHL