

**Transition steps in peroxide reduction and a molecular switch for peroxide robustness of prokaryotic peroxiredoxins**

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**Keywords:** Reactive oxygen species, redox homeostasis, oxidative stress, peroxiredoxins, alkyl hydroperoxide reductase, molecular switch

**Supplementary Figure S1.** (A) Comparison of the ten subunits (chains A-J) of decameric oxidized *EcAhpC*<sub>1-186</sub>-YFSKHN. Each subunit is shown with different color and the C<sub>P47</sub> adopts LU conformation in all subunits. The C-terminal tail is not shown for clarity. (B) The dimeric unit (chain A and B: shown in dark and light colors) shows that the C-terminal tail of chain A is stabilized in the oxidized *EcAhpC*<sub>1-186</sub>-YFSKHN structure by the packing interactions with the symmetry related molecule (grey color). (C) The C-terminus of chain H is stabilized by the packing interactions with the symmetry related molecule. Similarly, the C-terminal tail in chain G and J are also stabilized by packing interactions.

**Supplementary Figure S2.** (A) Comparison of the active site conformations between the ten subunits (chains A-J) of decameric reduced *EcAhpC*<sub>1-186</sub>-YFSKHN. Each subunit are shown with different color and the C-terminal tail is not shown for clarity. The C<sub>P47</sub> adopts two different conformation; in one subunit (chain A; red) the C<sub>P47</sub> adopts a loop conformation and chains B-J adopt helical conformation. The influence of crystal packing interactions on the active site conformation for (B) chain A and (C) G is shown as a representative for the rest of the subunits in the decameric reduced *EcAhpC*<sub>1-186</sub>-YFSKHN. No symmetry related molecule is in contact with the C<sub>P47</sub> of the reduced structure.

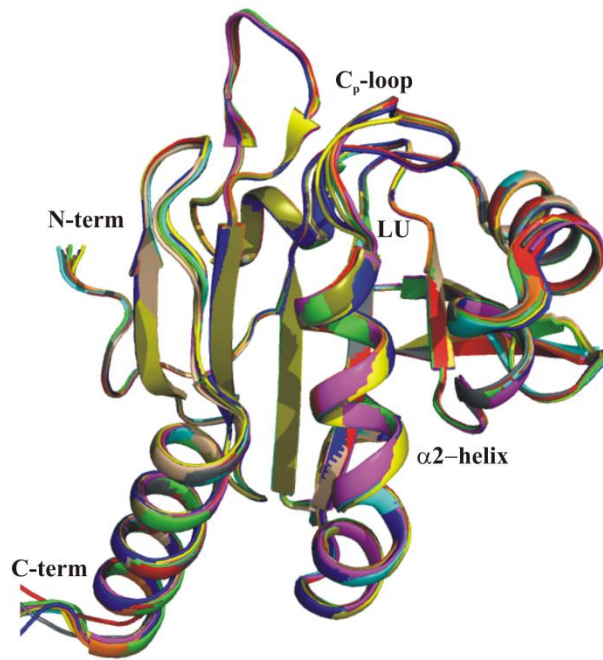
**Supplementary Figure S3.** Non-reducing SDS-PAGE analysis of oxidation of (A) WT *EcAhpC*, (B) *EcAhpC*<sub>1-172</sub> and (C) *EcAhpC*<sub>1187G</sub> by H<sub>2</sub>O<sub>2</sub>. The reduced AhpC proteins were treated with varying H<sub>2</sub>O<sub>2</sub> concentrations (0 to 30 mM) for 5 min. The SDS-PAGE analysis shows that the reduced AhpC proteins without H<sub>2</sub>O<sub>2</sub> run as a major band corresponding to a monomer (*Lane 1*). At 0.1 mM of H<sub>2</sub>O<sub>2</sub> *EcAhpC* becomes oxidized and undergoes the transition from a monomer to

a dimer (*Lane 2*). The monomer band reappears with high concentrations of H<sub>2</sub>O<sub>2</sub> (1 to 30 mM) likely due to over-oxidized *EcAhpC*. The analysis supports that *EcAhpC*<sub>1-172</sub> and *EcAhpC*<sub>1187G</sub> were sensitive to over-oxidation compared to WT *EcAhpC*. *D* indicates the dimeric and *M* the monomeric state.

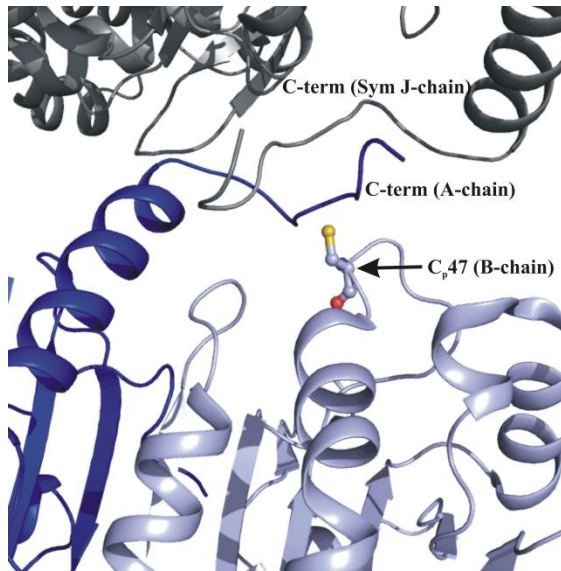
**Supplementary Figure S4.** Comparison of interaction patterns of the reactive C<sub>p47</sub> with the conserved active site residues between the typical FF (left panel; PDB ID: 4MA9) and FF<sub>like</sub> (right panel) conformations. A shift in C<sub>p47</sub>, located at helix  $\alpha$ <sub>2</sub> of the FF<sub>like</sub> active site of reduced *EcAhpC*<sub>1-186</sub>-YFSKHN, alters its interaction pattern with the conserved active site residues.

**Supplementary Figure S5.** Gel filtration elution profile of (A) *EcAhpC*<sub>1-186</sub>-YFSKHN, (B) *EcAhpC*<sub>S86A,T88A</sub>, (C) *EcTrx* and (D) *EcTrxR*. *EcAhpC*<sub>1-186</sub>-YFSKHN, *EcAhpC*<sub>S86A,T88A</sub>, and *EcTrx* were purified by Ni-NTA affinity- and size exclusion chromatography using a Superdex 75 column. In case of *EcTrxR*, a Superdex 200 gel filtration column was used. The volume of the shaded peak area was pooled and 3  $\mu$ l of pure eluted protein were applied on a 17% SDS gel to estimate its purity (*inset*).

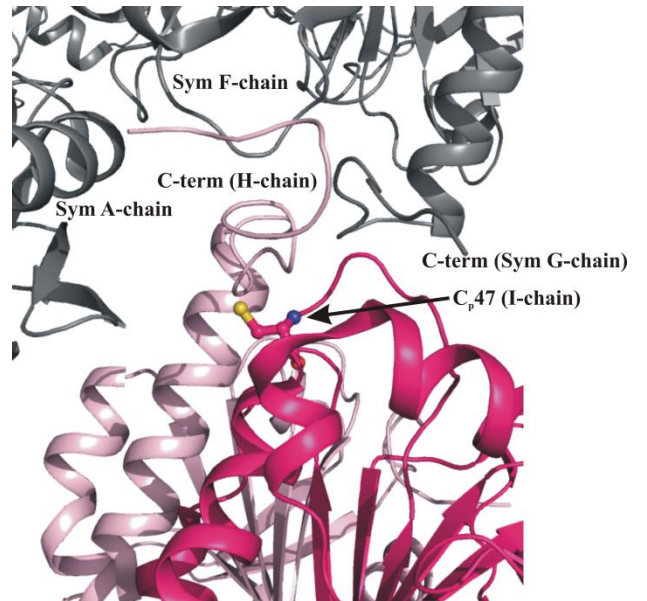
(A)



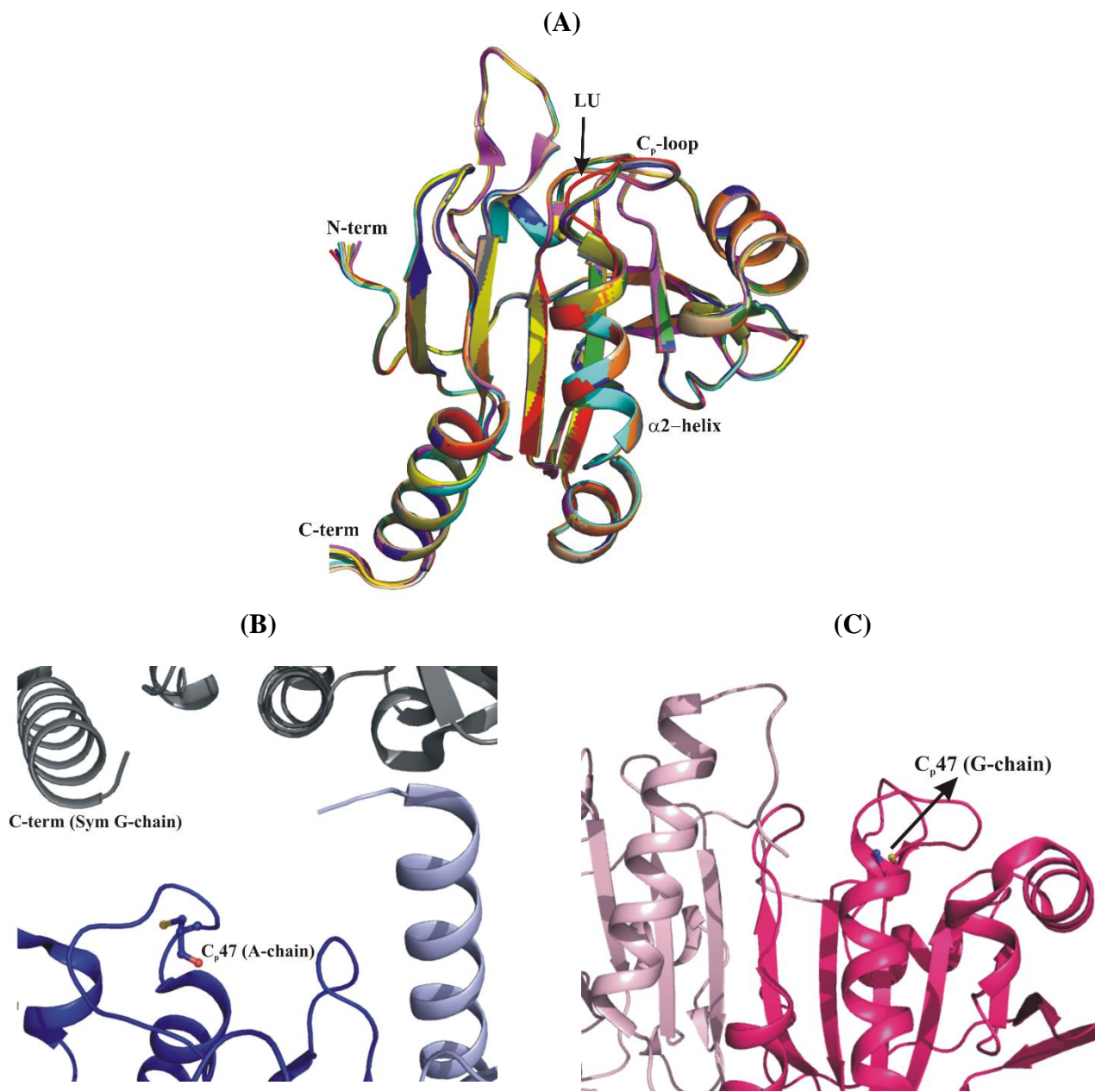
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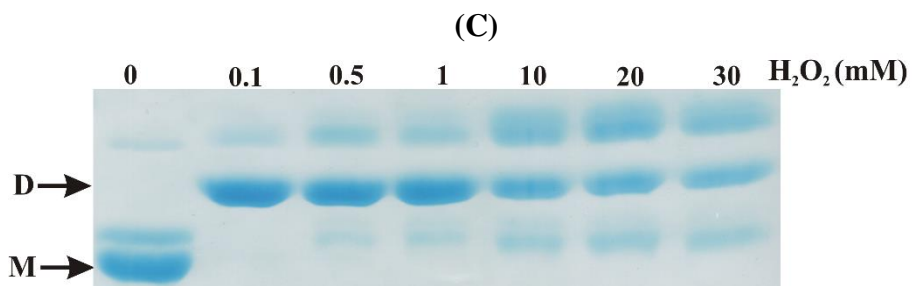
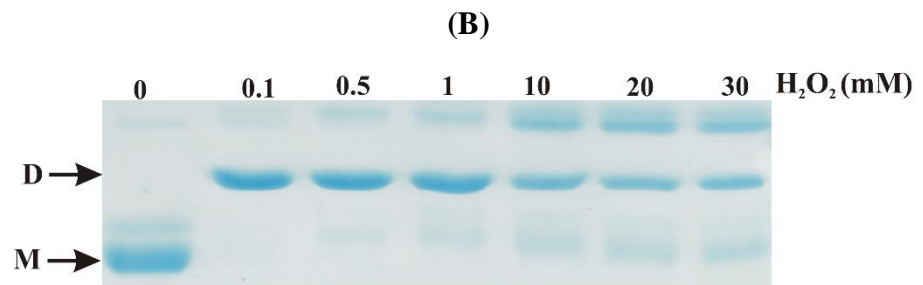
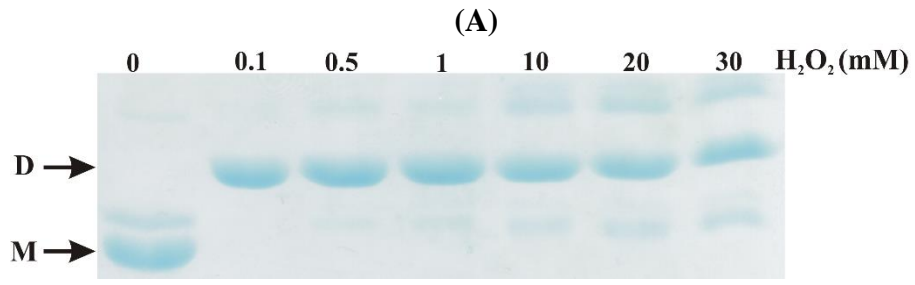
(C)



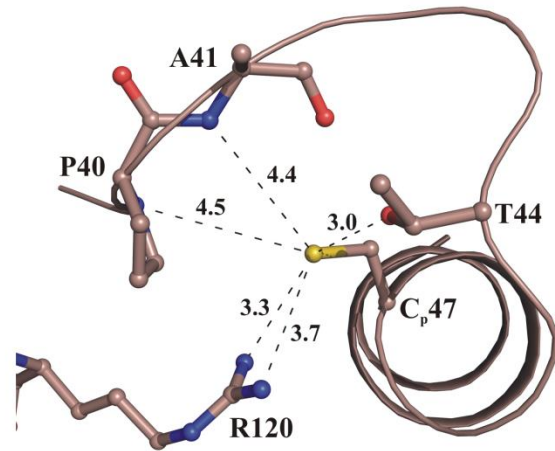
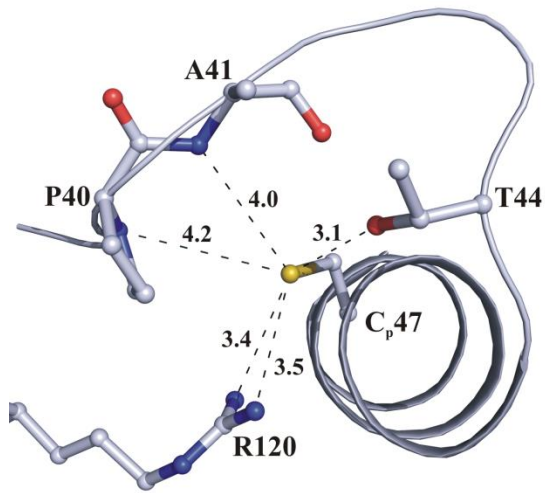
Supplementary Figure S1A-C



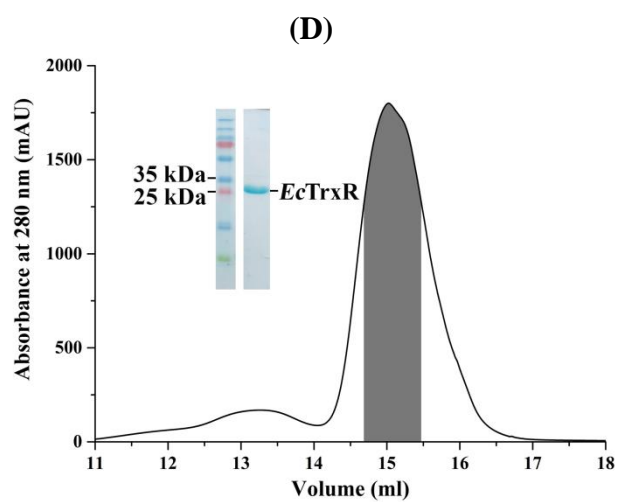
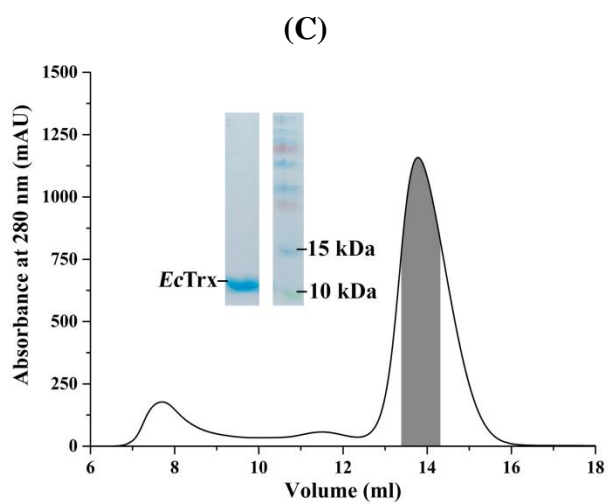
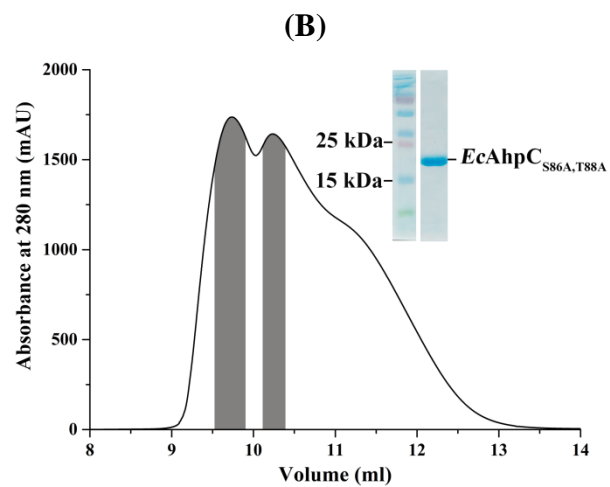
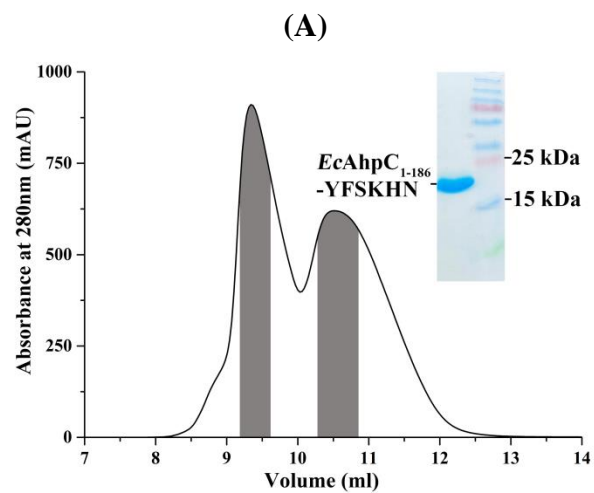
Supplementary Figure S2A-C



Supplementary Figure S3A-C



Supplementary Figure S4



**Supplementary Figure S5A-D**



**Supplementary Table S1:** The backbone dihedral angles for active site residues at different conformations

<b>Conformation</b>	<b>Residue</b>	<b>Phi (°)</b>	<b>Psi (°)</b>
FF	P48	-60.6	-36.5
	C47	-49.2	-53.6
	V46	-71.8	-35.4
	F45	115.8	-61.7
LU	P48	-88.6	48.5
	C47	-55.5	132.7
	V46	-58.1	132.6
	F45	-79.2	-21.3
FF <sub>like</sub>	P48	-61.0	-34.5
	C47	-64.3	-38.9
	V46	-85.9	-30.8
	F45	-86.7	-35.6
LU <sub>like</sub>	P48	-65.5	52.0
	C47	-53.1	150.4
	V46	-67.0	90.1
	F45	-99.9	-15.3