Supplementary information

Peroxidase activity enhancement of horse cytochrome c by dimerization

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Table S1 Formation rate constants of cyt c Compound I and the oxidation rates of cyt c-catalyzed oxidation of guaiacol in the presence of mCPBA

<table>
<thead>
<tr>
<th>Cyt c</th>
<th>Compound I formation rate (ms$^{-1}$) $^a$</th>
<th>Guaiacol oxidation rate $^b$</th>
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<tbody>
<tr>
<td>Monomer</td>
<td>0.0032 ± 0.0002</td>
<td>0.91 ± 0.02</td>
</tr>
<tr>
<td>Dimer</td>
<td>0.019 ± 0.001</td>
<td>5.5 ± 0.1</td>
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$^a$ Pseudo first-order rate constants of Compound I formation were obtained by fitting the absorbance increase at 595 nm to single exponential functions. Reaction conditions: 50 μM protein (heme unit); 2.5 mM mCPBA.

$^b$ Conditions: 2 μM protein (heme unit); 2.5 mM mCPBA; 100 μM guaiacol. The unit is μmol product/(μmol heme·sec).
**Fig. S1** Time-dependent absorbance changes at 595 nm of monomeric (left) and dimeric (right) cyt c by reaction with mCPBA. The pseudo first-order rate constants of Compound I formation are obtained by least-square fitting the data to single exponential functions (solid lines). Conditions: 50 μM cyt c protein (heme unit); 2.5 mM mCPBA; 50 mM potassium phosphate buffer, pH 7.0; 25°C.
**Fig. S2** Elution curves of horse cyt c. (A) Elution curve before separation. (B) Elution curve of monomeric cyt c. (C) Elution curve of dimeric cyt c. Absorbance at 408 nm (red) and 208 nm (black). Conditions: column: Hiload 26/60 Superdex 75; flow rate: 0.8 ml/min; solvent: 50 mM potassium phosphate buffer, pH 7.0; temperature, 4°C.
**Fig. S3** Formation curve of guaiacol oxidation product catalyzed by monomeric cyt c. Absorbance change was monitored at 470 nm. Conditions: 2 μM monomeric horse cyt c; 100 μM guaiacol; 50 mM H₂O₂; 50 mM potassium phosphate buffer, pH 7.0; 25°C. Inset: The first 20-second curve of the product formation. The four phases labeled I–IV are explained in the text.