Electronic Supplementary Information

for

New Class of Artificial Enzyme Composed of Mn-Porphyrin, Imidazole and Cucurbit[10]uril Toward Therapeutic Antioxidant

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**Fig. S1** ESI-MS spectrum of ZndMIm₄P@CB[10].

[ZndMIm₄P@CB[10]]⁺⁺; Anal: 603.92. Found: 603.98.

[ZndMIm₄P@CB[10] • Cl⁻]³⁺; Anal: 816.91. Found: 816.97.

**Fig. S2** ESI-MS spectrum of MndMIm₄P@CB[10].

[MndMIm₄P@CB[10] • 2OH⁻]³⁺; Anal: 813.26. Found: 813.32.

[MndMIm₄P@CB[10] • 2Cl⁻]³⁺; Anal: 825.57. Found: 825.64.
Fig. S3 UV/vis spectrum of 17.8 µM ZndMIm₃P alone (solid line) and 17.8 µM ZndMIm₃P@CB[10] (dotted line) in pure water.
ZndMIm₃P $\lambda_{\text{max}}$: 418 nm, 551 nm, 587 nm.
ZndMIm₃P@CB[10] $\lambda_{\text{max}}$: 419 nm, 551 nm, 587 nm.

Fig. S4 UV/vis spectrum of 9.0 µM MndMIm₃P (solid line) and 9.0 µM MndMIm₃P@CB[10] (dotted line) in 25 mM phosphate buffer (pH 7.4).
MndMIm₃P $\lambda_{\text{max}}$: 443.5 nm, 558.5 nm, 589.5 nm.
MndMIm₃P@CB[10] $\lambda_{\text{max}}$: 447 nm, 553 nm, 586.5 nm.
Fig. S5 DOSY spectrum of ZndMIm$_4$P alone (25°C, 500 MHz, D$_2$O).

Fig. S6 DOSY spectrum of ZndMIm$_4$P@CB[10] (25°C, 500 MHz, D$_2$O).
Fig. S7 Fluorescent spectrum of ZndMIm₄P (blue) and ZndMIm₄P@CB[10] (orange) in pure water measured at the same concentration per ZndMIm₄P (7.5 μM). ZndMIm₄P and ZndMIm₄P@CB[10] were excited at 423.5 nm where absorbance of ZndMIm₄P and ZndMIm₄P@CB[10] are identical.

Fig. S8 DFT-minimized geometry of ZndMIm₄P@CB[10] (B3LYP/6-31G*). (a) side view, (b) top view.
**Fig. S9** Job’s plot for mixtures of ZndMIm₄P@CB[10] and imidazole at a total concentration of 0.5 mM.

**Fig. S10** Plot of the chemical shift (δ₁ of 1.0 mM ZndMIm₄P@CB[10]) as a function of imidazole concentration. The curve fitting analysis using TitrationFit program to determine $K_{a2} = 6.8 \times 10^5$ M⁻².
**Fig. S11** Absorption spectral changes of 20 µM MndMIm₄P@CB[10] in the presence of imidazole in 100 mM phosphate buffer (pH 7.4). Concentration of imidazole ranged from 0 to 140 µM.

**Fig. S12** Job's plot analysis for mixture of MndMIm₄P@CB[10] and imidazole at a total concentration of 50 µM.
Fig. S13 Plot of absorbance at 550 nm of 20 µM MndMImP@CB[10] as a function of imidazole concentration. The curve fitting analysis using TitrationFit program to determine $K_{a2} = 3.0 \times 10^8$ M$^{-2}$. 
Fig. S14 Absorption spectral changes of MndMIm₄P@CB[10] in the presence of various concentrations of imidazole (0 ~ 500 µM). The spectral changes were monitored in water under acidic or basic conditions. (a) 100 mM acetate buffer (pH 4.5). (b) 100 mM phosphate buffer (pH 11). Insets show plots of absorbances of Soret bands as functions of imidazole concentration. The monitoring was performed at the lower MndMIm₄P@CB[10] concentration (3.5 µM) than 20 µM (Fig. S11 and S13) due to unexpected precipitation of MndMIm₄P@CB[10].
Fig. S15 Absorption spectral changes of 25 μM MndMImP alone in 100 mM phosphate buffer (pH 7.4) in the presence of various concentrations of imidazole. (a) From 0 μM to 200 μM. (b) From 25 mM to 250 mM (large excess). Insets show plots of absorbance as functions of [Imidazole].
Fig. S16 Cyclic voltammogram of MndMIm₄P alone (blue), MndMIm₄P@CB[10] (red), mixture of MndMIm₄P and imidazole (six equivalents for MndMIm₄P) (purple), and MndMIm₄P@CB[10] (green). Supporting electrolyte: 10 mM phosphate buffer (pH 7.0) containing 50 mM Na₂SO₄. Scanning rate: (a) 50 mV/s, (b) 100 mV/s.
**Table S1** Redox potential ($E_{1/2}$ for Mn$^{III/II}$ redox couple) versus normal hydrogen electrode (vs NHE).

<table>
<thead>
<tr>
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<th>$E_{1/2}$ for Mn$^{III/II}$ (mV vs NHE)$^a$</th>
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<tbody>
<tr>
<td>MndMIm$_4$P</td>
<td>+ 280</td>
</tr>
<tr>
<td>MndMIm$_4$P@CB[10]</td>
<td>+ 270</td>
</tr>
<tr>
<td>MndMIm$_4$P + Im</td>
<td>+ 280</td>
</tr>
<tr>
<td>MndMIm$_4$P@CB[10];Im</td>
<td>+ 280</td>
</tr>
</tbody>
</table>

$^a$E$_{1/2}$ values (vs Ag/AgCl) obtained from Fig. S16 were converted to those vs NHE by adding 200 mV. For stoichiometric formation of MndMIm$_4$P@CB[10];Im, MndMIm$_4$P@CB[10] was mixed with six equivalents of imidazole (Fig. S11–S13). The same molar ratio was traced for mixture of MndMIm$_4$P and imidazole (MndMIm$_4$P + Im).
Fig. S17 (a): Time course of oxygen production from 1 mM H$_2$O$_2$ (final concentration) catalyzed by MndMIm$_4$P@CB[10];Im (six equivalents of imidazole to MndMIm$_4$P@CB[10]) in 100 mM phosphate buffer (pH 7.4) at 25°C. (b): Plot of observed rate constant ($k_{obs}$) as a function of the concentration of MndMIm$_4$P@CB[10];Im (per MndMIm$_4$P@CB[10]).
Fig. S18 (a) Time course of oxygen production from 1 mM H$_2$O$_2$ (final concentration) catalyzed by MndMIm$_4$P in 100 mM phosphate buffer (pH 7.4) at 25°C. (b) Plot of observed rate constant ($k_{obs}$) as a function of the MndMIm$_4$P concentration.
Fig. S19 (a) Time course of oxygen production from 1 mM H$_2$O$_2$ (final concentration) catalyzed by MndMIm$_4$P@CB[10] in 100 mM phosphate buffer (pH 7.4) at 25°C. (b) Plot of observed rate constant ($k_{obs}$) as a function of the MndMIm$_4$P@CB[10] concentration.
Fig. S20 (a) Time course of oxygen production from 1 mM H$_2$O$_2$ (final concentration) catalyzed by MndMIm$_4$P in the presence of six equivalents of imidazole (Im) in 100 mM phosphate buffer (pH 7.4) at 25°C. (b) Plot of observed rate constant ($k_{obs}$) as a function of the MndMIm$_4$P+ Im concentration (per MndMIm$_4$P).
Fig. S21 (a) Time course of O$_2$ production from 10 mM H$_2$O$_2$ (final concentration) catalyzed by MndMIm$_4$P@CB[10];Im (six equivalents of imidazole to MndMIm$_4$P@CB[10]) in 50 mM phosphate buffer (pH 7.4) at 25°C. Yellow: 5.0 μM. Red: 7.5 μM. Green: 10 μM. (b) Plot of observed rate constant ($k_{\text{obs}}$) as a function of MndMIm$_4$P@CB[10];Im concentration (per MndMIm$_4$P).
Fig. S22 Lineweaver-Burk plot for (a) MndMIm₄P alone, (b) MndMIm₄P@CB[10], (c) MndMIm₄P + Im and (d) MndMIm₄P@CB[10]·Im. Turnover number ($k_{\text{cat}}$) and Michaelis constant ($K_M$) were determined by intercepts of the Lineweaver-Burk plot ($1/V_0$ axis for $k_{\text{cat}}$ and $1/[H_2O_2]$ axis for $K_M$, respectively). The $k_{\text{cat}}$ and $K_M$ values were determined as averages for independent three runs.
Fig. S23 Time course of absorbance at 660 nm (oxidized ABTS) in the presence of 0.2 mM H$_2$O$_2$ (final concentration), 0.5 mM ABTS (final concentration) and 15 µM (final concentration) test samples in 50 mM phosphate buffer (pH 7.4) at 25°C.

Fig. S24 Effect of 10 µM (final concentration) MndMIm$_4$P or MndMIm$_4$P@CB[10];Im (per MndMIm$_4$P@CB[10]) on viability of HeLa cells. The cell viability was measured by Alamar blue assay. N.T: Non-treatment. No significant difference was observed in the cell viability among the three lanes.
Table S2 Literature benchmarks for catalase activity and peroxidase activity of Mn-Salen derivatives.

<table>
<thead>
<tr>
<th>Mn-Salen derivative with distal imidazole</th>
<th>Catalase activity (µM O₂/min)</th>
<th>Peroxidase activity (µM ABTS/min)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>83⁠&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Mn-Salen derivative with distal pyridine</td>
<td>281&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1</td>
</tr>
<tr>
<td>EUK-114</td>
<td>70 ± 8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.8 ± 0.2&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2</td>
</tr>
<tr>
<td>EUK-134</td>
<td>243 ± 18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>37.8 ± 9.6&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2</td>
</tr>
<tr>
<td>EUK-123</td>
<td>112 ± 14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19.9 ± 0.5&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2</td>
</tr>
</tbody>
</table>

<sup>a</sup>Catalase activity was measured in 50 mM phosphate buffer (pH 7.4) by Clark-type oxygen electrode. <sup>b</sup>Oxygen production from 10 mM H₂O₂ (final concentration) catalyzed by 10 µM Mn-Salen derivatives was monitored at 25±0.2°C. <sup>c</sup>Catalase activity was measured in sodium phosphate buffer (pH 8.1) by Clark-type oxygen electrode. <sup>d</sup>Oxygen production from 10 mM H₂O₂ (final concentration) catalyzed by 10 µM Mn-Salen derivatives was monitored at 27±0.2°C.
Table S3 SOD activity ($k_{SOD}$) and ONOO$^-$-reducing activity ($k_{red}$).

<table>
<thead>
<tr>
<th></th>
<th>SOD activity ($k_{SOD}$) ($\times 10^7\text{M}^{-1}\text{s}^{-1}$)</th>
<th>ONOO$^-$-reducing activity ($k_{red}$) ($\times 10^6\text{M}^{-1}\text{s}^{-1}$)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mn-SOD (human)</td>
<td>~ 200</td>
<td>~</td>
<td>3</td>
</tr>
<tr>
<td>Peroxiredoxin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glutathione peroxidase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MndMIm$_4$P</td>
<td>4.3 ± 0.3</td>
<td>4.2 ± 0.4</td>
<td>this work</td>
</tr>
<tr>
<td>MndMIm$_4$P@CB[10]</td>
<td>5.3 ± 0.7</td>
<td>5.5 ± 0.5</td>
<td>this work</td>
</tr>
<tr>
<td>MndMIm$_4$P + Im</td>
<td>3.6 ± 1.1</td>
<td>5.2 ± 0.2</td>
<td>this work</td>
</tr>
<tr>
<td>MndMIm$_4$P@CB[10]:Im</td>
<td>5.0 ± 0.5</td>
<td>7.6 ± 0.8</td>
<td>this work</td>
</tr>
<tr>
<td>MnM4Py$_4$P</td>
<td>2.1 ± 0.1</td>
<td>2.0 ± 0.2</td>
<td>this work</td>
</tr>
</tbody>
</table>

$^a$SOD activity was determined by Stopped-flow method according to the previous procedure. Briefly, time decay of superoxide at 245 nm was spectrophotometrically monitored in HEPES buffer (pH 8.1) at 21°C. For each concentration of sample, observed rate constant ($k_{obs}$) was calculated. $k_{SOD}$ was determined from the slope of the plot of $k_{obs}$ as a function of the sample concentration. SOD activity of Mn(III)-5,10,15,20-tetrakis(N-methylpyridinium-4-yl)porphyrin (MnM4Py$_4$P) (control experiment) is consistent with that of the previous report. ONOO$^-$-reducing activity was determined by the similar procedure to that for SOD activity. Time decay of ONOO$^-$ at 302 nm was spectrophotometrically monitored in the presence of 2 mM ascorbic acid (Asc) in 50 mM phosphate buffer (pH 7.4) at 25°C. The subsequent procedure is same as that for SOD activity. The catalytic rate constant ($k_{cat}$) of ca. $10^7\text{M}^{-1}\text{s}^{-1}$ for MnM4Py$_4$P was obtained as previously reported. For stoichiometric formation of MndMIm$_4$P@CB[10]:Im, MndMIm$_4$P@CB[10] was mixed with six equivalents of imidazole to MndMIm$_4$P@CB[10] (Fig. S11–S13). The same molar ratio was traced for mixture of MndMIm$_4$P and imidazole (MndMIm$_4$P + Im).
References


