Supplementary Information for:

PEGylation of an artificial $\text{O}_2$ and CO receptor: synthesis, characterisation and pharmacokinetic study

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Table S1  Extinction coefficients of CO-coordinated hemoCD and PEG(mw)-hemoCDs at 422 nm in 0.05 M phosphate buffer at pH 7.0 and 25 °C.a

<table>
<thead>
<tr>
<th></th>
<th>$10^{-5} \varepsilon_{422} / \text{M}^{-1} \text{cm}^{-1}$</th>
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<tbody>
<tr>
<td>hemoCD</td>
<td>3.71b</td>
</tr>
<tr>
<td>PEG750-hemoCD</td>
<td>3.77</td>
</tr>
<tr>
<td>PEG5k-hemoCD</td>
<td>3.53</td>
</tr>
<tr>
<td>PEG10k-hemoCD</td>
<td>3.51</td>
</tr>
<tr>
<td>PEG20k-hemoCD</td>
<td>3.60</td>
</tr>
</tbody>
</table>

CO-coordinated PEG(mw)-hemoCDs were quantitatively formed by the additions of excess Na$_2$S$_2$O$_4$ to the solutions of the ferric-forms of PEG(mw)-hemoCDs under CO atmospheres. bRef 35.

Fig. S1  MALDI-TOF MS spectra of HOOC(CH$_2$)$_3$(CO)O-PEG750-OCH$_3$ (a, positive mode), P-PEG750 (b, negative mode) and Fe$^{III}$P-PEG750 (c, negative mode) with a subsequent addition of a mixture of α-cyano-4-hydroxycinnamic acid and 2,5-dihydroxybenzoic acid matrices.
**Fig. S2** MALDI-TOF MS spectra of HOOC(CH$_2$)$_3$(CO)O-PEG10k-O(CO)(CH$_2$)$_3$COOH (a, positive mode), P-PEG10k (b, negative mode) and Fe$^{III}$P-PEG10k (c, negative mode) with a subsequent addition of a mixture of $\alpha$-cyano-4-hydroxycinnamic and 2,5-dihydroxybenzoic acid matrices.

**Fig. S3** MALDI-TOF MS spectra of HOOC(CH$_2$)$_3$(CO)O-PEG20k-O(CO)(CH$_2$)$_3$COOH (a, positive mode), P-PEG20k (b, negative mode) and Fe$^{III}$P-PEG20k (c, negative mode) with a subsequent addition of a mixture of $\alpha$-cyano-4-hydroxycinnamic and 2,5-dihydroxybenzoic acid matrices.
Fig. S4  UV-Vis spectral changes of $\text{Fe}^{\text{III}}\text{P}$-PEG750 (1 x $10^{-5}$ M) upon addition of Py3CD in 0.05 M phosphate buffer at pH 7.0 and 25 °C. Inset shows plots of the changes in absorbances of $\text{Fe}^{\text{III}}\text{P}$-PEG750 versus [Py3CD]. The solid lines indicate theoretical curves for the 1:1 complexation to give the binding constant ($K$).

Fig. S5  UV-Vis spectral changes of $\text{Fe}^{\text{III}}\text{P}$-PEG10k (1 x $10^{-5}$ M in the porphyrin concentration) upon addition of Py3CD in 0.05 M phosphate buffer at pH 7.0 and 25 °C. Inset shows plots of the changes in absorbances of $\text{Fe}^{\text{III}}\text{P}$-PEG10k versus [Py3CD]. The solid lines indicate theoretical curves for the 1:1 complexation to give the binding constant ($K$).
**Fig. S6** UV-Vis spectral changes of Fe$^{III}$P-PEG20k (1 x 10$^{-5}$ M in the porphyrin concentration) upon addition of Py3CD in 0.05 M phosphate buffer at pH 7.0 and 25 °C. Inset shows plots of the changes in absorbances of Fe$^{III}$P-PEG20k versus [Py3CD]. The solid lines indicate theoretical curves for the 1:1 complexation to give the binding constant ($K$).
Calorimetric titrations of Fe\(^{III}\)-PEG(mw)s with 25 aliquots (10 μL each) of Py3CD in 0.05 M phosphate buffer at pH 7.0 and 298.15 K. The initial concentrations of Fe\(^{III}\)-PEG(mw) in a cell and Py3CD in a syringe are given in the respective figures.

**Fig. S7** Calorimetric titrations of Fe\(^{III}\)-PEG(mw)s with 25 aliquots (10 μL each) of Py3CD in 0.05 M phosphate buffer at pH 7.0 and 298.15 K. The initial concentrations of Fe\(^{III}\)-PEG(mw) in a cell and Py3CD in a syringe are given in the respective figures.
**Fig. S8**  Autoxidation reaction of the O$_2$ adduct of PEG750-hemoCD (5.0 x 10$^{-6}$ M) under air in 0.05 M phosphate buffer at 25 °C. Scans were made at 1.0 hour intervals. Inset: First-order plot based on the absorption change at 422 nm.

**Fig. S9**  Autoxidation reaction of the O$_2$ adduct of PEG5k-hemoCD (5.0 x 10$^{-6}$ M) under air in 0.05 M phosphate buffer at 25 °C. Scans were made at 1.0 hour intervals. Inset: First-order plot based on the absorption change at 422 nm.
**Fig. S10** Autoxidation reaction of the O₂ adduct of PEG10k-hemoCD (5.0 x 10⁻⁶ M) under air in 0.05 M phosphate buffer at 25 °C. Scans were made at 1.0 hour intervals. Inset: First-order plot based on the absorption change at 422 nm.

**Fig. S11** Autoxidation reaction of the O₂ adduct of PEG20k-hemoCD (5.0 x 10⁻⁶ M) under air in 0.05 M phosphate buffer at 25 °C. Scans were made at 1.0 hour intervals. Inset: First-order plot based on the absorption change at 422 nm.
**Fig. S12**  Autoxidation reaction of the O$_2$ adduct of PEG10k-hemoCD (5.0 x 10$^{-6}$ M) in the presence of catalase (100 units) under air in 0.05 M phosphate buffer at 25 °C. Scans were made at 1.0 hour intervals. Inset: First-order plot based on the absorption change at 422 nm.

$k_{\text{obs}} = 0.027 \pm 0.006 \text{ h}^{-1}$

$t_{1/2} = 26.4 \pm 5.9 \text{ h}$
**Fig. S13**  UV-Vis spectral changes of PEG750-hemoCD (5.0 x 10^{-6} M) as a function of the O_2 partial pressure \( P^{O_2} \) in N_2 in 0.05 M phosphate buffer at pH 7.0 and 25 °C (a), plot of \( P^{O_2} / \Delta A_{433 \text{ nm}} \) versus \( P^{O_2} \) for determining \( P_{1/2}^{O_2} \) (b) and the titration curve for O_2 binding to PEG750-hemoCD (c).
Fig. S14  UV-Vis spectral changes of PEG5k-hemoCD (5.0 x 10^{-6} M) as a function of the O_2 partial pressure (P^{O_2}) in N_2 in 0.05 M phosphate buffer at pH 7.0 and 25 °C (a); plot of P^{O_2} / ΔA_{433 nm} versus P^{O_2} for determining P_{1/2}^{O_2} (b) and the titration curve for O_2 binding to PEG5k-hemoCD (c).
Fig. S15  UV-Vis spectral changes of PEG10k-hemoCD (5.0 x 10^{-6} M) as a function of the O_2 partial pressure (P^{O_2}) in N_2 in 0.05 M phosphate buffer at pH 7.0 and 25 °C (a), plot of P^{O_2} / \Delta A_{433\ nm} versus P^{O_2} for determining P_{1/2}^{O_2} (b) and the titration curve for O_2 binding to PEG10k-hemoCD (c).
Fig. S16  UV-Vis spectral changes of PEG20k-hemoCD (5.0 x 10^{-6} M) as a function of the O\textsubscript{2} partial pressure ($P^{O_2}$) in N\textsubscript{2} in 0.05 M phosphate buffer at pH 7.0 and 25 °C (a), plot of $P^{O_2} / \Delta A_{433 \text{ nm}}$ versus $P^{O_2}$ for determining $P_{1/2}^{O_2}$ (b) and the titration curve for O\textsubscript{2} binding to PEG20k-hemoCD (c).
Fig. S17  The amount of urinary excreted PEG(mw)-hemoCD at 6 h after the infusion of oxy-PEG(mw)-hemoCD (5 x 10^{-4} M solution in PBS was infused to the femoral vein of a rat at the rate of 1.0 mL/h for 2 h). The $P$ values obtained from student’s T-test indicate the probability that the observed differences between the groups has occurred by chance.