Electronic Supplementary Material (ESI) for RSC Advances.

A polyacrylamide gel containing an engineered hexameric hemoprotein as a cross-linking unit toward redox-responsive materials

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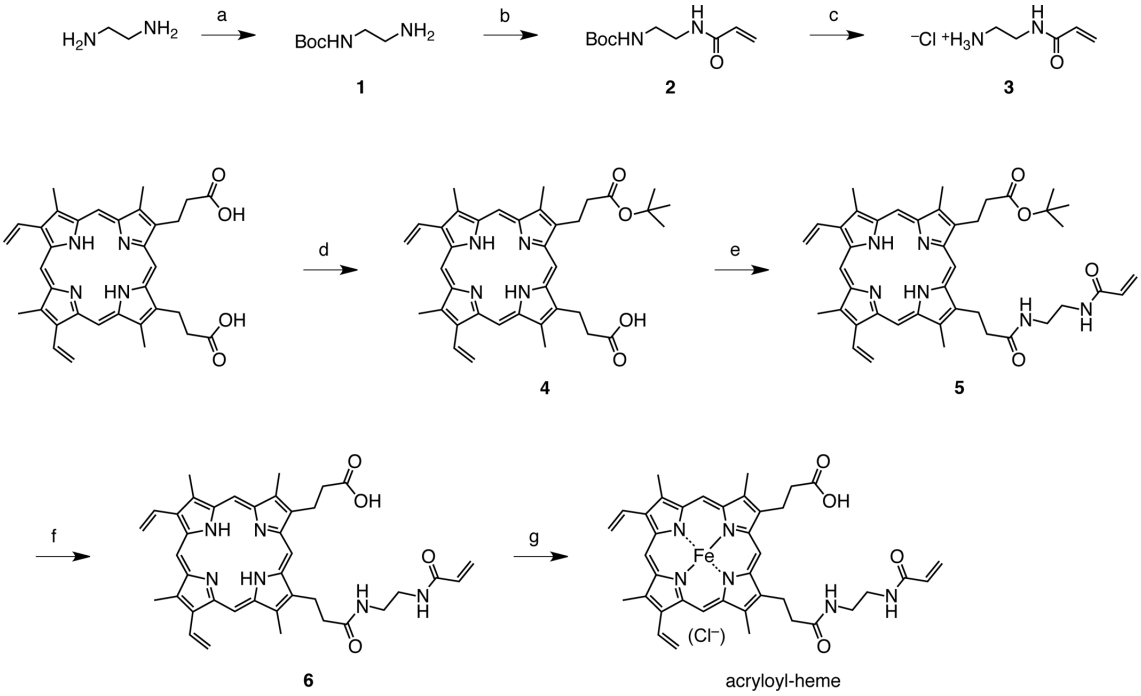
Scheme S1 Synthesis of acryloyl-heme



Compound **4** consists of two regioisomers due to the substitution position of the *tert*-butoxy group at the two heme-propionate side chains of protoporphyrin IX. Each compound, **5**, **6** and acryloyl-heme, also forms a similar mixture of two regioisomers. a) $(Boc)_2O$, b) acryloyl chloride, c) HCl, d) $(Boc)_2O$, *tert*-BuOH, e) **3**, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, 1-hydroxybenzotriazole, f) trifluoroacetic acid, formic acid, g) $FeCl_2$

Compounds **1** and **4** were synthesized according to the previous report.[S1]

Detailed synthetic procedures for compounds **3**, and **4**-**6** and acryloyl-heme are described below.

Synthesis of compound 2

Compound **1** (1.00 g, 6.24 mmol), triethylamine (2.08 g, 20.6 mmol, 3.3 eq.), and chloroform (200 mL) were added to a two-necked flask. Acryloyl chloride (677 mg, 7.49 mmol, 1.2 eq.) dissolved in 70 mL of chloroform was added dropwise over 3 h under ice bath conditions followed by stirring for 2 h. The solvent was then removed, redissolved in 100 mL of chloroform, and washed three times with 50 mL brine. After drying over sodium sulfate and filtration, the solvent was evaporated. Recrystallization from hexane/chloroform yielded compound **2** as a white solid (773 mg, 3.58 mmol, 57%).

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) = 1.47 (s, 9H), 3.35 (m, 2H), 3.47 (m, 2H), 4.91 (brs, 1H), 5.67 (d, 1H, J = 10.4 Hz), 6.11 (dd, 1H, J = 10.4, 17.2 Hz), 6.29 (d, 1H, J = 17.2 Hz), 6.39 (brs, 1H).

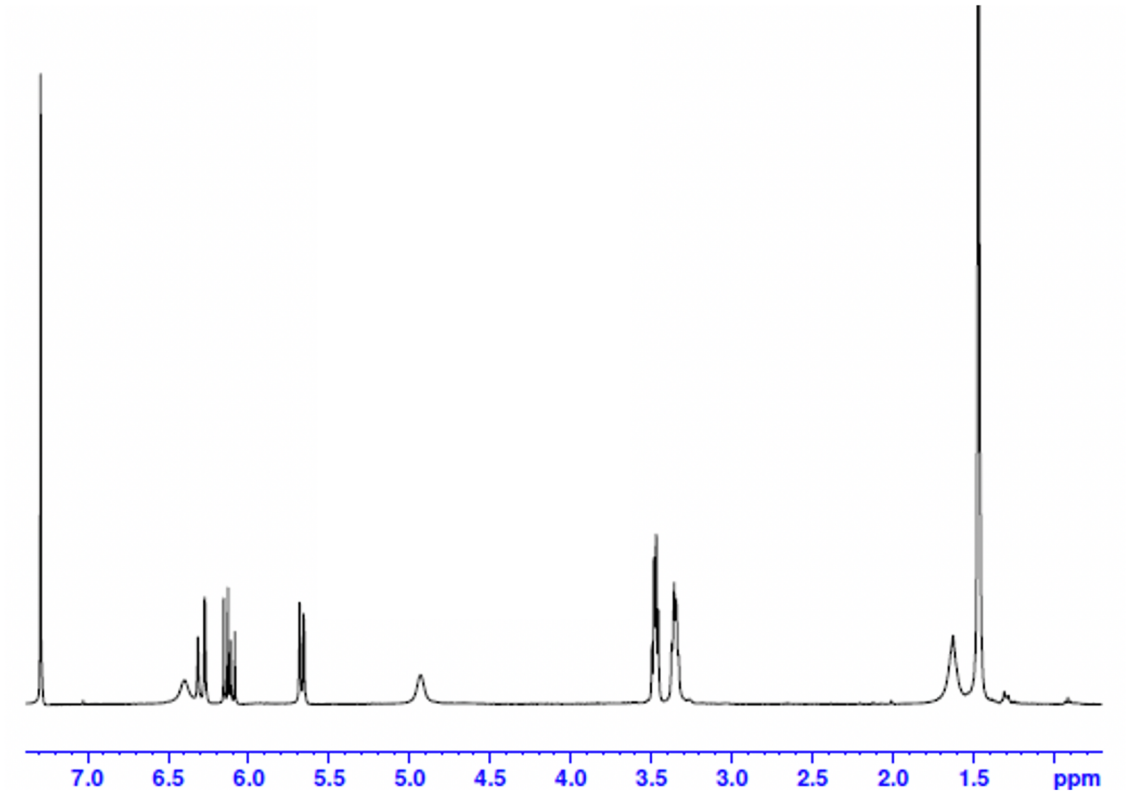


Fig. S1 ^{1}H NMR(400 MHz, $CDCl_3$) spectrum of compound **2.**

Synthesis of compound 3

Compound **2** (50.0 mg, 233 μmol) was dissolved in 10 mL of chloroform. Approximately 4 M HCl/dioxane solution (4.2 mL) was added and stirred at room temperature for 2 h, then the solvent was evaporated. Methanol (20 mL) was added and evaporated twice. After overnight vacuum drying, compound **3** was obtained quantitatively (35.5 mg, 233 μmol).

^{1}H NMR(400 MHz, DMSO-d_6) δ (ppm) = 2.89 (m, 2H), 3.38 (m, 2H), 6.39 (brs, 1H), 5.63 (dd, 1H, J = 2.4, 10.0 Hz), 6.12 (dd, 1H, J = 10.0, 17.2 Hz), 6.23 (dd, 1H, J = 2.4, 17.2Hz).

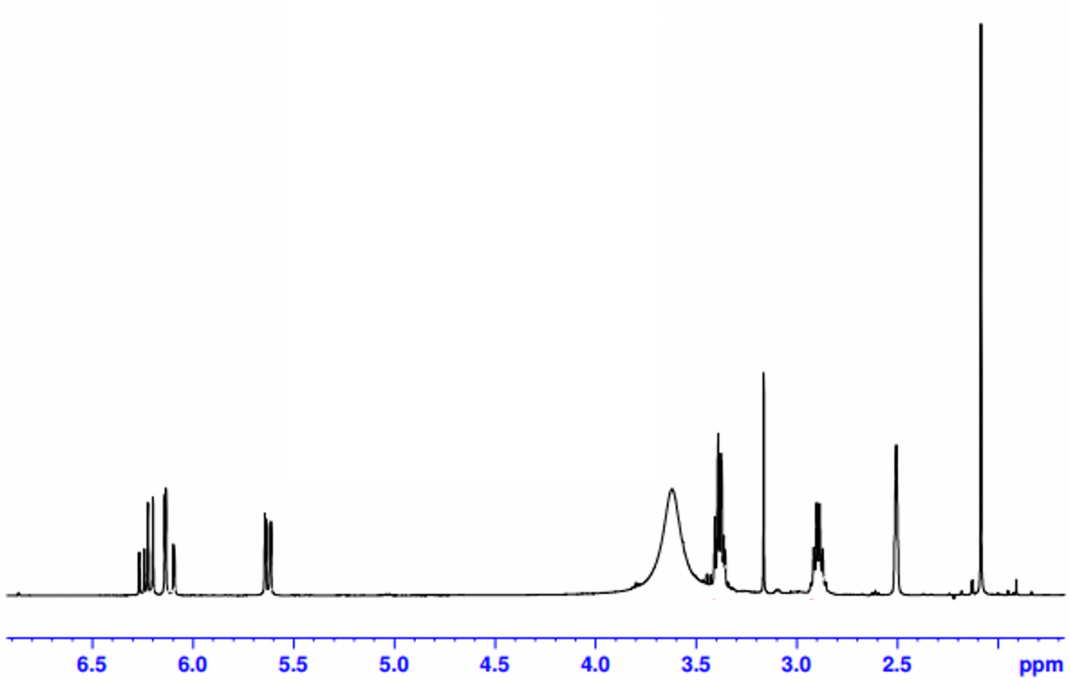


Fig. S2 ^{1}H NMR(400 MHz, DMSO-d_6) spectrum of compound **3.**

Synthesis of compound 5

Compound **4** (50 mg, 83 µmol), EDC (62 mg, 332 µmol, 4eq.), HOBt (43 mg, 334 µmol, 4 eq.), and chloroform (30 mL) were added to a two-necked flask and stirred at room temperature for 1 h. In another flask, compound **3** (59 eq., 757 µmol) was added to methanol (15 mL) and triethylamine (100 mg) and stirred at room temperature for 2 h. The reaction mixtures were combined and stirred overnight at room temperature. After the completion of the reaction, the solvent was evaporated, and the residue was taken up in 50 mL of chloroform and washed three times with 5% citric acid. After evaporation, the residue was purified by silica gel chromatography using chloroform/methanol = 0% to 10% (v/v) as an eluent. Recrystallization from hexane/THF yielded compound **5** as a red solid (35.5 mg, 59 µmol, 71%).

ESI-TOF-MS (positive mode, MeOH) *m/z* 715.40 $(M + H)^+$, calcd for $C_{43}H_{50}N_6O_4$ 715.40

^{1}H NMR(400 MHz, DMSO-d_6) δ (ppm) = –3.92 (s, 2H) 1.25 (s, 9H), 3.03 (m, 4H), 3.22 (m, 4H), 3.68 (m, 12H), 4,34 (m, 4H), 5.37 (m, 1H), 5.91 (m, 2H), 6.22, (m, 2H) 6.45, (m, 2H), 7.85 (brs, 1H), 7.97 (brs, 1H), 8.457-8.55 (m, 2H), 10.213-10.241 (m, 4H).

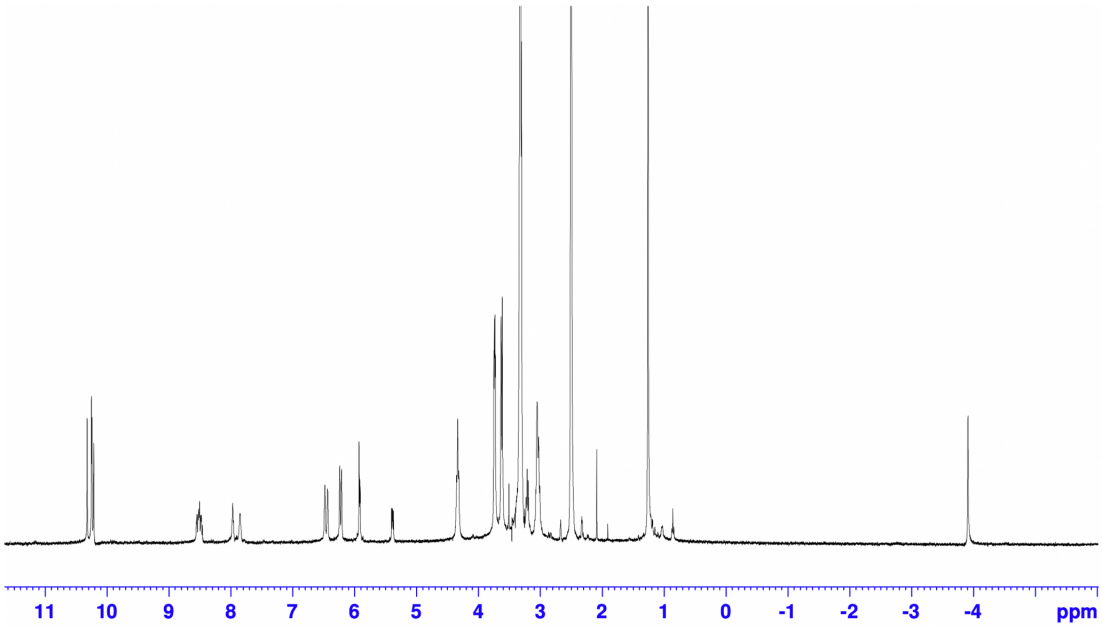


Fig. S3 ^{1}H NMR(400 MHz, DMSO-d_6) spectrum of compound **5**.

Synthesis of compound 6

Compound **5** (10.7 mg, 14.0 μmol) was added to a two-necked flask and purged with nitrogen. Anhydrous dichloromethane (5 mL), formic acid (0.3 mL), and TFA (0.7 mL) were added and stirred at room temperature. After stirring for 2.5 h, the solvent was removed. Precipitation from hexane/THF yielded compound **6** as a red solid (6.4 mg, 60%, 9.71 μmol).

ESI-TOF-MS (positive mode, MeOH) *m/z* 681.32 $(M + Na)^+$, calcd for $C_{39}H_{42}N_6O_4$ 681.31

^{1}H NMR(400 MHz, DMSO-d_6) δ (ppm) = –3.79 (s, 2H), 3.03 (m, 4H), 3.19 (m, 4H), 3,64 (dd, J = 8.8, 4.4 Hz, 6H), 3.78 (m, 6H), 4.36 (brs, 4H), 5.40 (brs, 1H), 5.93 (m, 2H), 6.24 (m, 2H), 6.48 (m, 2H), 7.91 (m, 2H), 8.56 (m, 2H), 10.31 (m, 4H).

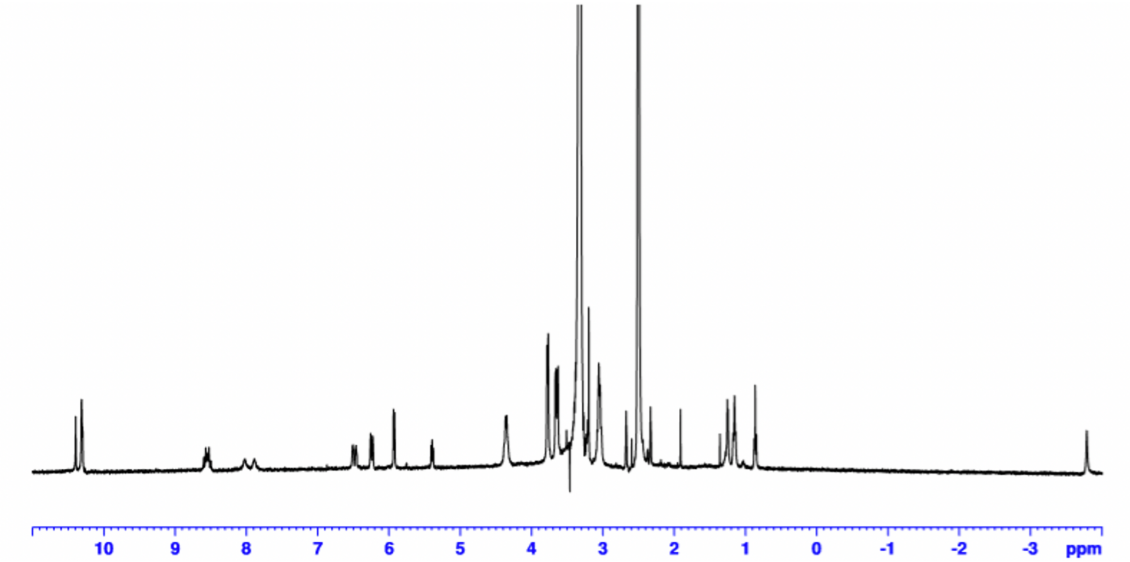


Fig. S4 ^{1}H NMR(400 MHz, DMSO-d_6) spectrum of compound **6.**

Synthesis of acryloyl-heme

Compound **6** (5.5 mg, 8.4 μmol) and ferric chloride tetrahydrate (27.2 mg, 168 μmol, 20 eq.), and sodium bicarbonate (23.2 mg) were added to a two-necked flask under a nitrogen atmosphere. Chloroform/methanol = 10:1 (v/v) (15 mL), which was bubbled with nitrogen, was added, and the mixture was refluxed at 60 °C for 5.5 h. After evaporation of the solvent, a mixture of chloroform/methanol (50 mL) was added, and the organic layer was washed five times with 0.05 M HCl. After drying over sodium sulfate and evaporation, the residue was recrystallized from hexane/THF, and acryloyl-heme was obtained as a red solid (3.0 mg, 48%, 4.2 μmol).

ESI-TOF -MS (positive mode, MeOH) *m/z* 712.25 $(M – Cl)^+$, calcd. for $C_{39}H_{40}FeN_6O_4$ 712.25

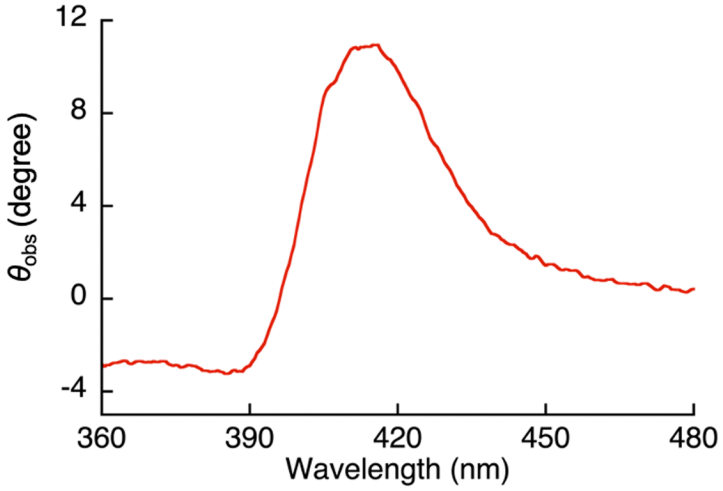
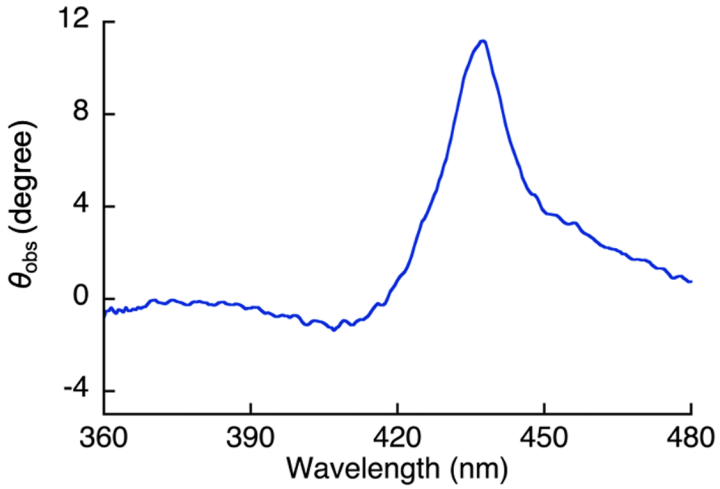


Fig. S5 CD spectrum of the Fe(III) state of HTHP in 100 mM potassium buffer, pH7.0.



Fig, S6 CD spectrum of the Fe(II) state of HTHP in 100 mM potassium buffer, pH7.0.

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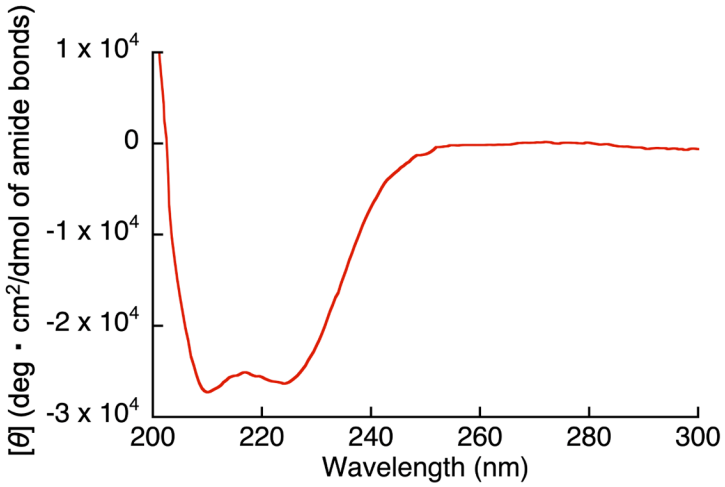

Fig. S7. Far-UV CD spectrum of rHTHP in 100 mM potassium buffer, pH7.0.

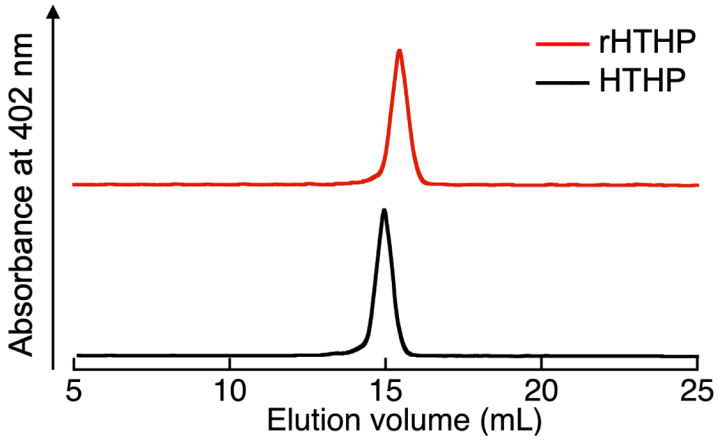

Fig. S8 Size exclusion chromatography traces of HTHP and rHTHP. Eluent: 100 mM potassium buffer, pH 7.0, temperature: 4 ºC, column: Superdex^{TM} 200 increase 10/300 GL (GE healthcare).

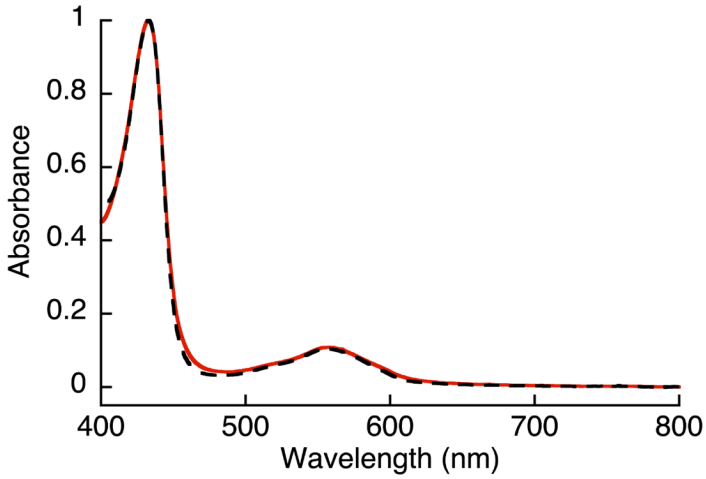

Fig. S9 UV-vis absorption spectra of a solution obtained upon the addition of dithionite into the rHTHP-PAAm gel containing apoMb (red solid line) and the Fe(II) state of Mb in a buffer solution (black broken line).

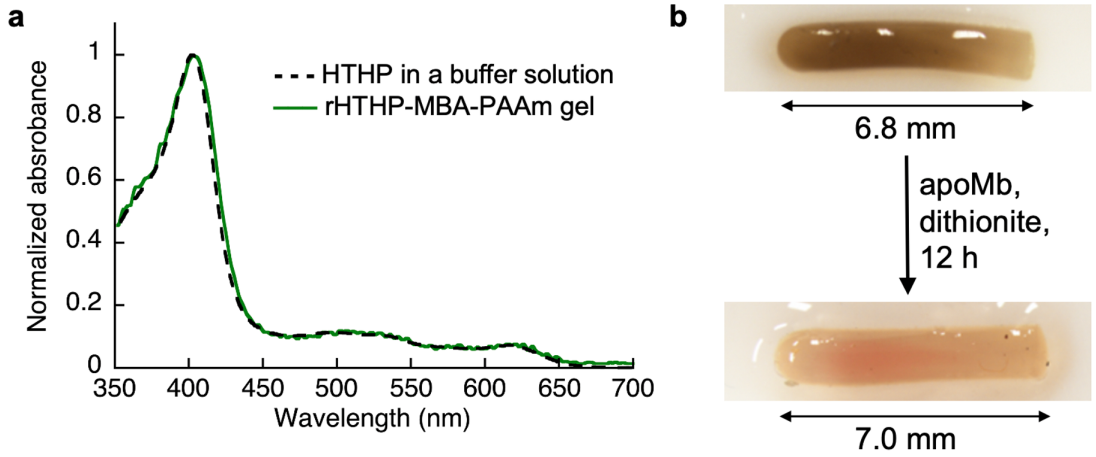

Fig. S10 (a) UV-vis absorption spectra of the Fe(III) states of rHTHP-MBA-PAAm gel and HTHP in a buffer solution. (b) Photographs of the Fe(III) state of rHTHP-MBA-PAAm gel (top) and the gel after soaking into a solution containing apoMb and dithionite for 12 h (bottom). In contrast to the gel-sol transition of the rHTHP-PAAm gel, the gel state was maintained by the reduction in the presence of apoMb due to a covalent cross linkage but the gel was slightly swollen.

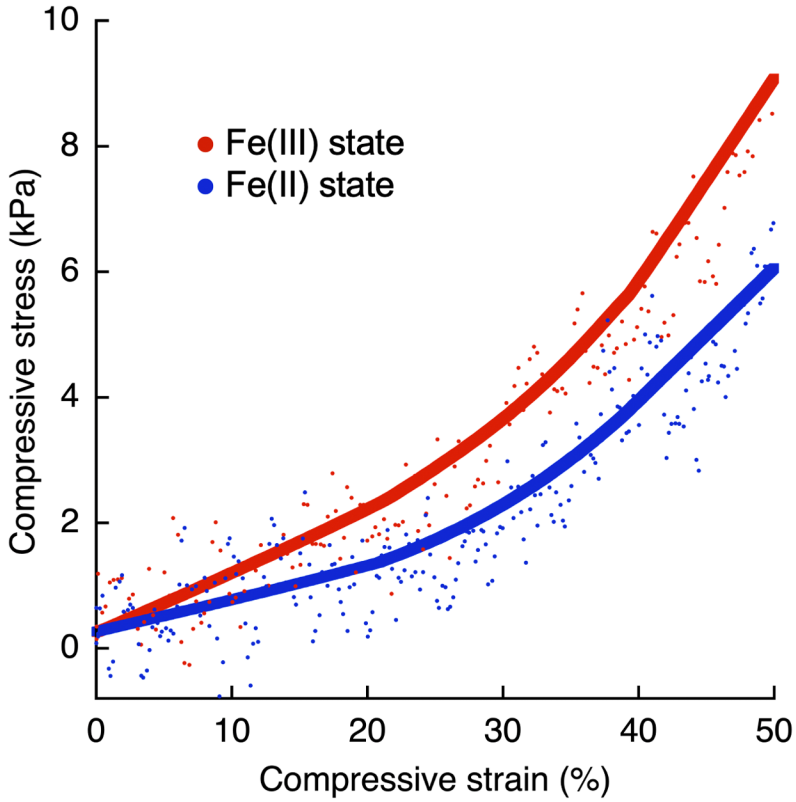

Fig. S11 Stress-strain curves obtained by compression tests of Fe(III) state (red) and Fe(II) state (blue) of rHTHP-MBA-PAAm gel, respectively. The solid lines show weighted averages of raw values shown as dots.

Reference

S1. H. Kitagishi, K. Oohora, H. Yamaguchi, H. Sato, T. Matsuo, A. Harada and T. Hayashi, *J. Am. Chem. Soc.*, 2007, **129**, 10326–10327.