

Electronic supplementary information

Hemin-micelles immobilized in alginate hydrogels as artificial enzymes with peroxidase-like activity and substrate selectivity

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1. Preparations of block copolymers

1.1. Preparation of the macroinitiator PEG-Br

The macroinitiator PEG₁₁₄-Br was synthesized according to ref. 1. The typical procedure to prepare PEG₁₁₄-Br was introduced as shown in Fig. S1a. PEG_{5k} (5.0 g, 1 mmol) was dissolved in 60 mL toluene in a 100 mL three-neck flask. After azeotropic distillation of about 10 mL toluene at reduced pressure to remove traces of water, trimethylamine (210 μ L, 1.5 mmol) was added and the solution mixture was cooled down to 0 °C. Then 2-bromoisobutyryl bromide (185 μ L, 1.5 mmol) was added over 1 hour, and the reaction mixture was stirred overnight at room temperature in N₂ atmosphere. Most of the toluene was removed by rotary evaporation prior to precipitation into a 10-fold excess of cold ether. The crude polymer was dried under vacuum, then dissolved in water at pH 8-9, and then extracted with CH₂Cl₂. The organic layer was collected and dried over MgSO₄, and the purified PEG₁₁₄-Br was isolated after the evaporation of the solvents under vacuum. PEG₁₁₄-Br was characterized by ¹H NMR (Fig. S2).

1.2. Preparation of the RAFT agent PEG-CTA

The preparation was shown in Fig. S1b. PEG₁₁₄-Br (2.0 g, 0.4 mmol) was dissolved in 20 mL anhydrous tetrahydrofuran (THF) in a 50 mL flask. Potassium ethylxanthate (715 mg, 4 mmol) was added to the solution mixture, and the reaction mixture was stirred for 48 hours at room temperature in N₂ atmosphere.² Most of the THF was removed by rotary evaporation, and the dried solid was dissolved in 50 mL CH₂Cl₂. The solution mixture was washed by 50 mL deionized water for three times to remove unreacted potassium ethylxanthate. The organic layer was collected and dried over MgSO₄, and the purified PEG₁₁₄-CTA was isolated after the evaporation of the solvents under vacuum. PEG₁₁₄-CTA was characterized by ¹H NMR (Fig. S3).

1.3. Preparation of the block copolymer PEG₁₁₄-*b*-PVIm₁₅

The preparation was shown in Fig. S1c. PEG₁₁₄-CTA (1.62 g, 0.3 mmol), AIBN (4.93 mg, 0.03 mmol) and 1-Vinylimidazole (2.70 mL, 30 mmol) were added into the reaction flask and then 10 mL of DMF was added. After three freeze-degas-thaw cycles, polymerization was conducted at 110 °C for 48 h. Most of the DMF was removed by rotary evaporation, and the mixture was diluted with CH₂Cl₂, and then precipitated into excess diethyl ether. The precipitate was dried under vacuum. PEG-*b*-PVIm was characterized by ¹H NMR (Fig. S4), the block number of PVIm was calculated to be 15. The PDI of the polymer was about 1.07 (GPC using DMF as eluent).

2. Supplementary figures

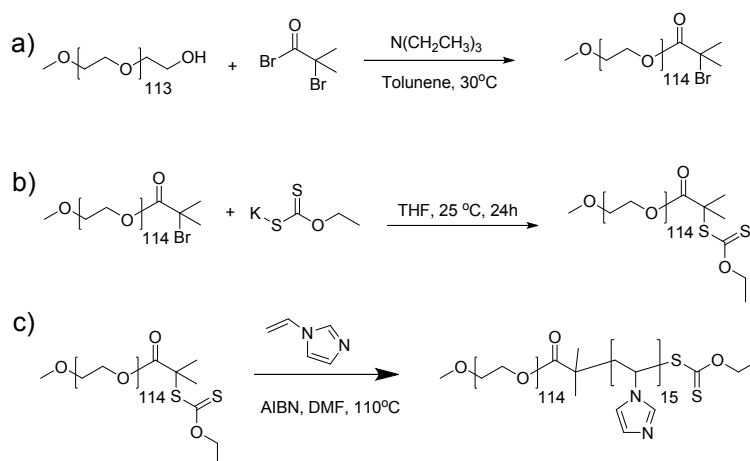


Fig. S1 Synthetic routes of (a) PEG-Br, (b) PEG-CTA and (c) PEG-*b*-PVIm.

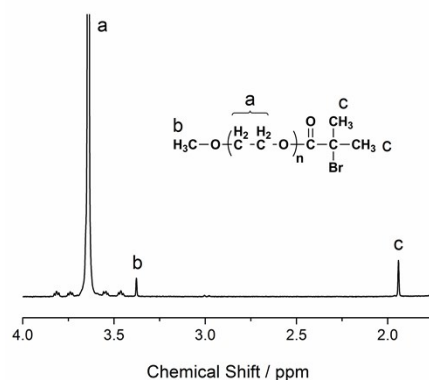


Fig.S2 ¹H NMR spectra of PEG-Br in CDCl₃.

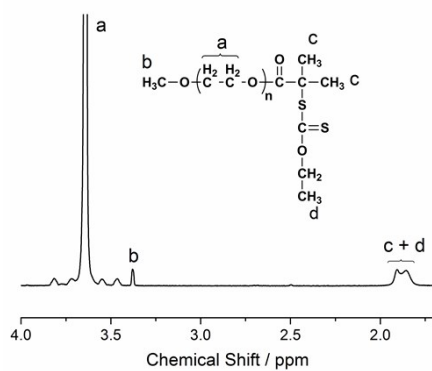


Fig. S3 ^1H NMR spectra of PEG-CTA in CDCl_3 .

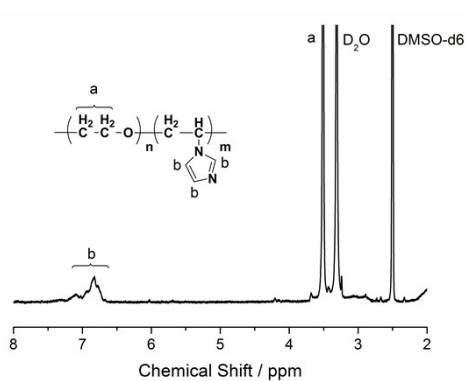


Fig. S4 ^1H NMR spectra of PEG-*b*-PVIm in DMSO-d_6 .

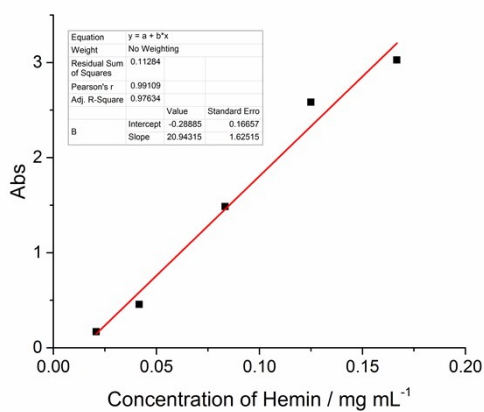


Fig. S5 The standard curve of PEG-*b*-PVIm/Hemin complex micelles.

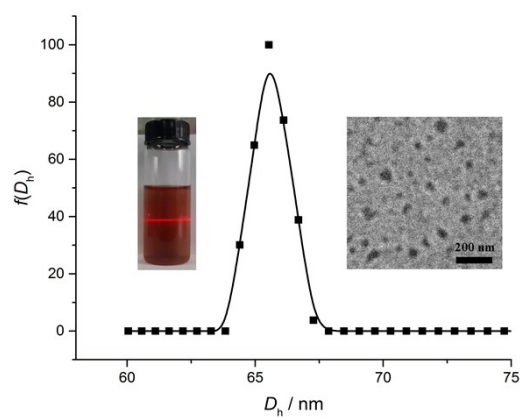


Fig. S6 Hydrodynamic diameter distribution of the hemin micelle in aqueous solution. Inset: the photograph (left) and TEM image (right, scale bar: 100 nm) of the micelle. Laser light (inset, left) was obtained by using a simple laser generator (650 nm, 5 mW).

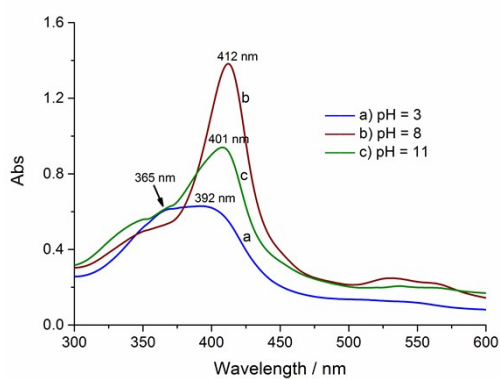


Fig. S7 The UV-vis spectra of the hemin micelles at different pHs.

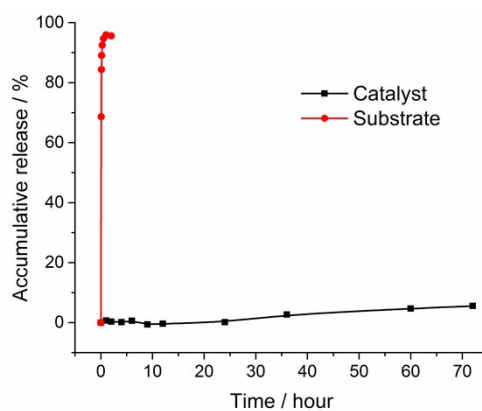


Fig. S8 Accumulative releases of the HM (catalyst) and Orange II (substrate). The release was performed at 25 °C.

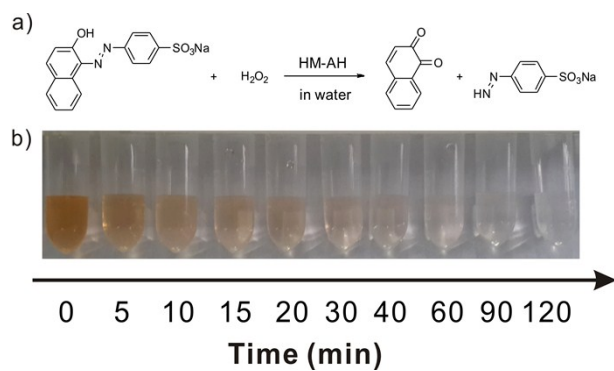


Fig. S9 (a) The peroxidized model reaction catalyzed by HM-AH in water. (b) Color changes of the substrates orange II in water along with the catalytic reactions.

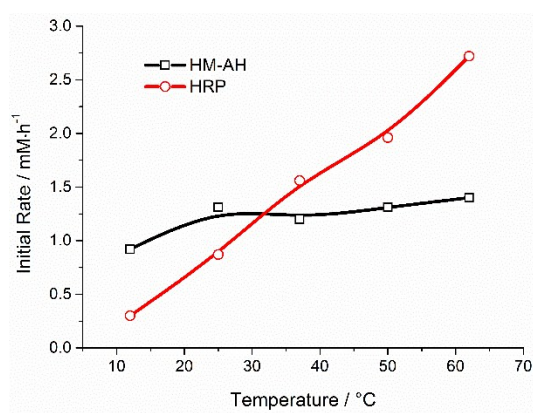


Fig. S10 Initial rates of the catalytic reactions catalyzed by HM-AH and HRP at different temperatures.

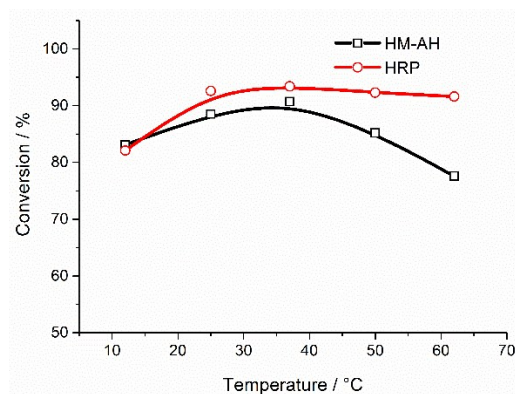


Fig. S11 Conversions of the catalytic reactions catalyzed by HM-AH and HRP at different temperatures.

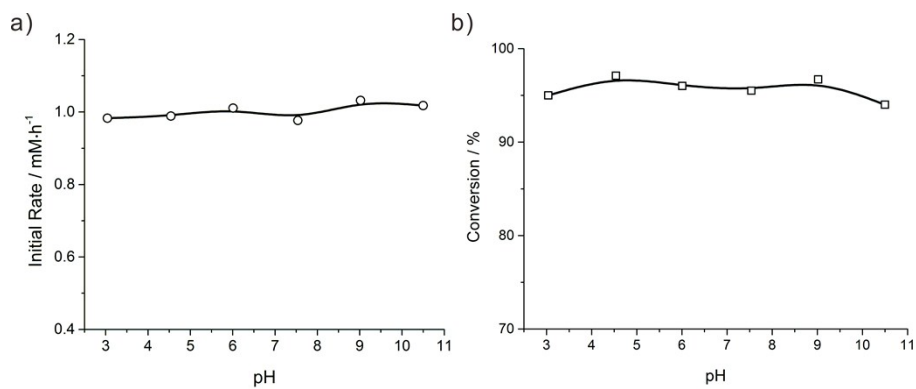


Fig S12. Initial rates (a) and conversions (b) of the catalytic reactions catalyzed by HM-AH at different pHs. 10 mM phosphate buffer was used to prepare the substrate solutions, and monovalent strong base or acid was used to adjust the pH.

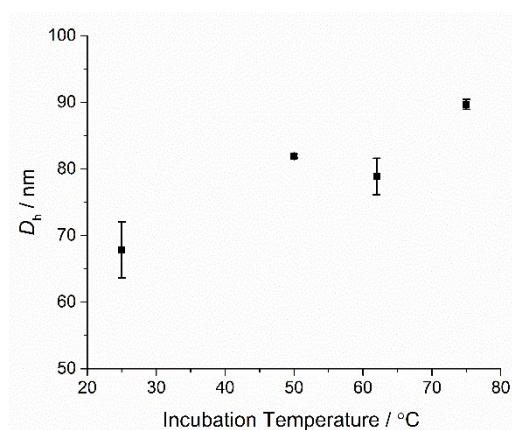


Fig. S13 The hydrodynamic diameters of hemin-micelles after incubation in aqueous media at different temperatures for one hour. The dynamic light scattering (DLS) was performed at 25 °C.

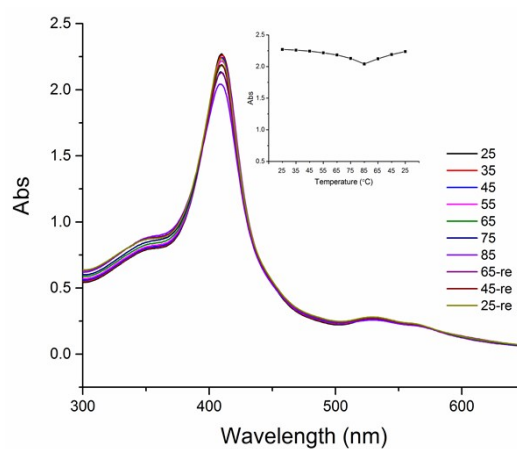


Fig. S14 UV-vis spectra of hemin-micelles during the heating-cooling process.



Fig. S15 (a) The peroxidized model reaction catalyzed by HM-AH in toluene. (b) Color changes of the substrates pyrogallol in toluene along with the catalytic reactions. (c) The optical image of HM-AH in toluene during the catalytic reaction.

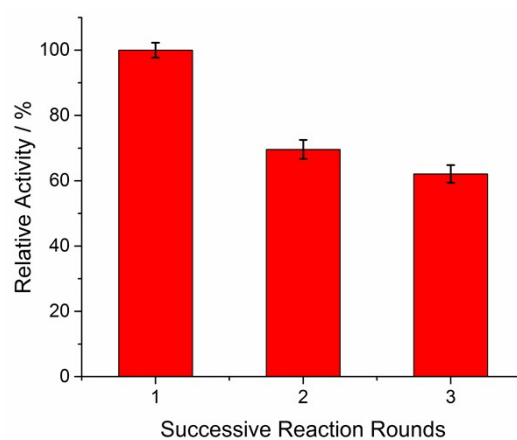


Fig. S16 The relative activity of the fresh and the recycled samples of orange II reaction in water with 15 minutes total conversion as the comparing index.

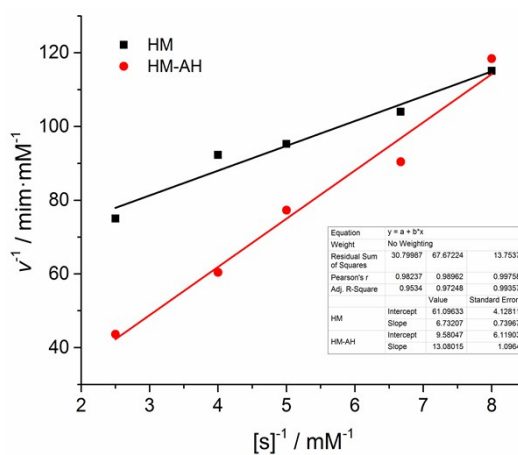


Fig. S17 The fitted curves of Lineweaver-Burk equation of HM and HM-AH at the fixed concentration of H_2O_2 versus varying concentration of Orange II.

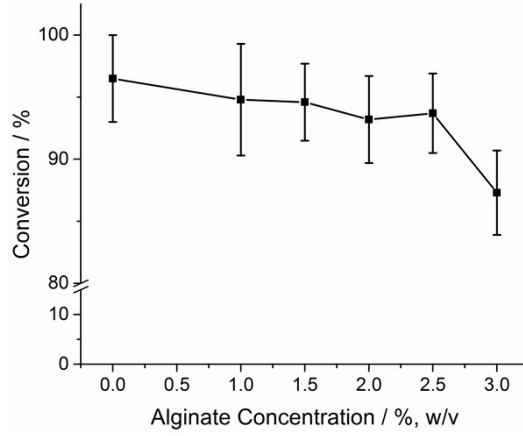


Fig. S18 Conversions of catalytic reactions by HM-AHs with different alginate concentrations.

3. Supplementary tables

Table S1 The comparison of the two block copolymers PEG-*b*-P4VP and PEG-*b*-PVI_m.

	M _n	PDI ^a	M _n (PEG)/M _n
PEG ₄₅ - <i>b</i> -P4VP ₁₄₅	17,225	1.07	0.12
PEG ₁₁₄ - <i>b</i> -PVI _m ₁₅	6,410	1.07	0.78

^a PDIs of block copolymers were obtained by GPC.

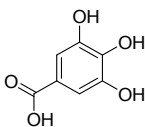
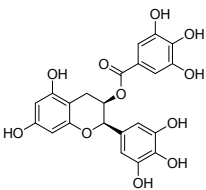
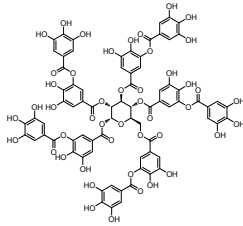
Table S2 Summary of diffusion models based on obstruction theory⁴

model expression	references
$\frac{D_g}{D_0} = \exp\left[-\frac{(r_s + r_f)}{r_f}\right]\sqrt{\varphi}^a$	Ogston ⁵
$\frac{D_g}{D_0} = \exp(-0.84\alpha^{1.09})^b$	Johansson et al. ⁶
$\frac{D_g}{D_0} = \left(1 + \frac{2}{3}\alpha\right)^{-1b}$	Tsai et al. ⁷
$\frac{D_g}{D_0} = \exp\left[-\pi\left(\frac{r_s + r_f}{k_s\varphi^{1/2} + r_f}\right)^2\right]^c$	Amsden ⁸

^a D_g , diffusion coefficient of the solute in a gel; D_0 , diffusion coefficient of the solute in the liquid; r_s , the radius of the solute; r_f , the radius of the hydrogel fiber; φ , the volume fraction of polymer in the

gel. ^b $\alpha = \varphi\left(\frac{r_s + r_f}{r_f}\right)^2$. ^c k_s , constant for a given polymer-solvent system.

Table S3. Structures and property data of three polyphenols with different sizes.

	GA	EGCG	TA
Full Name	gallic acid	(-)-epigallocatechin gallate	tannic acid
Chemical Formula			
M_n	170.12	458.37	1701.20
Polar Surface Area ^a (Å ²)	98	197	778

^a Data cited from ChemSpider. The property data were generated using the ACD/Labs Percepta Platform- PhysChem Module.

References

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