Supporting Information

Encapsulation of enzyme-immobilized smart polymer-membrane in metal-organic framework for enhancement in catalytic performance

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Contents

Experimental section

Chemicals

N-3-Aminoproyl-morpholine, *N,N*-diethylethylenediamine, 1-2-aminoethyl-pyrrolidine, 2-mercaptoethanol, 2-methylimidazole (2-MIM), zinc acetate dihydrate, 2,2'-azobisisobutyronitrile (AIBN), and 3,3',5,5'-tetramethylbenzidine (TMB) was purchased from Beijing Innochem Technology Co., Ltd. (Beijing, China). Acryloyl chloride, *N*-3-aminopropyl-diethanolamine, *N*-isopropyl acrylamide (N), glucose oxidase (GOx) and glutathione reduced (GSH) were supplied by Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). 2,2-Dipyridyl disulfide was obtained from Afaesa Chemical CO., Ltd. (Beijing, China). Maleic anhydride (M) was bought from Guangfu reagent (Tianjin, China). Styrene (S) was obtained from Shanghai Chemical Plant (Shanghai, China). Benzyl benzodithioate (BBDT) and fluorescein isothiocyanate (FITC) were purchased from Sigma-Aldrich (St. Louis, USA). 1,4-Dioxane, *N,N*-dimethylformamide (DMF), tetrahydrofuran (THF), isopropanol, ethyl ether and other reagents were bought from Tianjin Concord Technology Co., Ltd. (Tianjin, China). Horseradish peroxidase (HRP) was gotten from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Acetic acid (HAc) and sodium hydroxide (NaOH) were provided by Beijing Chemical Works (Beijing, China). Trypsin (TRY) and D-glucose were purchased from Xinjingke Biotechnologies Co., Ltd. (Beijing, China). Milli-Q Ultrapure water purification system (Millipore, Bedford, MA, USA) was used to prepare deionized water.

Instruments

The ^{1}H nuclear magnetic resonance (^{1}H NMR) spectra of liposome were recorded on Bruker Avance III 400 spectrometer (Bruker Corporation, Billerica, MA, USA) using chloroform as the solvent. The ultraviolet-visible (UV-*vis*) absorption spectra were measured using a TU-1900 UV-*vis* double-beam spectrometer (Purkinje General, China). A 1.0 mL capacity cuvette with a 1.0 cm path length was used for measuring the UV-*vis* absorbance at 650 nm. Fourier transform infrared (FTIR) was performed in a Bruker Tensor-27 spectrophotometer (Bruker, Germany) at wave-number range of 4000-400 cm^{-1} for characterization of the ZIF-8, GOx-HRP@PSMN@ZIF-8 and GOx-HRP@PSMN@HZIF-8. Circular dichroism (CD) spectra were measured by a CD spectroscopy (J-1700, Japan). Transmission electron microscopy (TEM) images were obtained using a transmission electron microscope (JEM-2010, Japan electron optics laboratory, Japan) at a voltage of 200 kV. The surface morphology of GOx-HRP@PSMN@HZIF-8 was observed by JSM-7900F field emission scanning electron microscopy (SEM) (JEOL, Japan). The size of PSMN membrane (about 200.0 nm) was carried out with a dynamic light scattering (DLS) analyzer (Zetasizer Nano ZS ZEN3600, British). Element analysis was finished using an elemental analyzer (Thermo Scientific^{TM}, Italy). Confocal laser scanning microscopy (CLSM, FV1000-IX81, Olympus, Japan) was used to determine the FITC-enzymes@L and FITC-enzymes@PSMN@HZIF-8. Thermo-gravimetric analysis (TGA) was performed in air with temperature increasing at 10 °C min^{-1} and a temperature range from 20 °C to 900 °C using a synchronous thermal analyzer (STA 449 F3

Jupiter, Nestal, Germany). The samples were dried in vacuum at 50 °C for 12.0 h before TGA analysis. Powder x-ray diffraction (PXRD) patterns of ZIF-8, GOx-HRP@PSMN@ZIF-8 and GOx-HRP@PSMN@HZIF-8 at 2θ were obtained by using PANalytical Empyrean diffractometer (Empyrean, PANalytical B.V., Netherlands) at room temperature.

Fabrication of GOx-HRP@PSMN@ZIF-8

Briefly, 0.5 mL of GOx-HRP@PSMN mixed with 1.5 mL sodium acetate buffer (40.0 mM, pH 5.0). The mixture was mixed at room temperature for 10.0 min. Afterwards, 1.1 mL of 2-MIM (115.0 mg/1.1 mL) was quickly added into the mixture, and the reaction reacted at room temperature for 10.0 min. Next, 0.5 mL of Zn^{2+} (11.88 mg/mL) was added into the mixture, which was incubated at 37.0 °C for 30.0 min. The product GOx-HRP@PSMN@ZIF-8 was collected by centrifugation at 10, 000 rpm for 10.0 min and washed three times by deionized water. Finally, 1.5 mL of sodium acetate buffer (40.0 mM, pH 5.0) was added, the ultrasonic suspension was stored at 4 °C for later analysis.

Preparation of GOx-HRP@PSMN@LZIF-8

Typically, 12.0 μg of L was added into 1.5 mL sodium acetate buffer (40.0 mM, pH 5.0). Then, 0.5 mL of GOx-HRP@PSMN was added and encapsulated by L. Afterwards, 1.1 mL of 2-MIM (115.0 mg/1.1 mL) was quickly added and the mixture was replaced at room temperature for 10.0 min. Next, 0.5 mL Zn^{2+} (11.88 mg/mL) was added into the mixture, and incubated at 37.0 °C for 30.0 min. Following ZIF-8 growing on the surface of GOx-HRP@PSMN@L, the product GOx-HRP@PSMN@LZIF-8 was collected by centrifugation at 10, 000 rpm for 10.0 min and washed three times by deionized water. Finally, 1.5 mL of sodium acetate buffer (40.0 mM, pH 5.0) was added, the ultrasonic suspension was stored at 4 °C for later analysis**.**

Construction of GOx-HRP@PSMN@HZIF-8

Briefly, 12.0 μg of L and 0.5 mL of GOx-HRP@PSMN were mixed with 1.5 mL sodium acetate buffer (40.0 mM, pH 5.0) at room temperature for 10.0 min under stirring. Then, 11.1 mL of 2-MIM (115.0 mg/1.1 mL) was quickly added and the mixture was placed at room temperature for 10.0 min, followed by addition of 0.5 mL of Zn^{2+} (11.88 mg/mL), the mixture was incubated at 37.0 °C for 30.0 min. Following ZIF-8 growing on the surface of GOx-HRP@PSMN@L, the product GOx-HRP@PSMN@LZIF-8 was obtained. Next, 2.0 μg of GSH was added. After L degraded at room temperature for 5.0 min, the GOx-HRP@PSMN@HZIF-8 was prepared. The product was collected by centrifugation at 10, 000 rpm for 10.0 min and washed three times by deionized water. Finally, 1.5 mL of sodium acetate buffer (40.0 mM, pH 5.0) was added, the ultrasonic suspension was stored at 4 °C for later analysis.

Preparation of FITC-enzymes@L and FITC-enzymes@PSMN@HZIF-8

Briefly, 1.0 mL enzymes (1.25 mg/mL) and 0.25 mL FITC (0.16 mg/mL) were mixed with 1.0 mL sodium acetate buffer (40.0 mM, pH 9.0). The mixture was placed in a microwave oven and reacted for 15.0 min. After the derivatization, the mixture was on dialysis for 12.0 h. Next,

the FITC-enzymes@LZIF-8 and FITC-enzymes@PSMN@HZIF-8 composites were prepared following the above procedures.

Steady-state kinetic studies

The maximum enzymatic reaction rate (V_{max}) and the kinetic Michaelis-Menten constant (K_m) were computed in order to analyze the parameters of the designed GOx-HRP@PSMN@HZIF-8 in a cascade reaction. Under various circumstances, the capacity to control enzymatic efficiency was assessed. After plotting the initial reaction rate against the substrate concentration, the K_m and V_{max} values were derived from the Michaelis-Menten (Equation 1):

$$[S] / V = K_m / V_{max} + [S] / V_{max} \quad (1)$$

Where, V_{max} is the maximum enzymatic reaction rate, [S] is the initial substrate concentration, and K_m is the Michaelis-Menten constant.

Measurement of the enzyme-loading amount

Bradford proteins assay was used to evaluate the loaded enzymes in GOx-HRP@ZIF-8, GOx-HRP@PSMN@ZIF and GOx-HRP@PSMN@HZIF-8 by comparing the concentration of the enzymes in the supernatant before and after immobilization. Typically, 0.5 mL of enzyme solution and 1.5 mL of deionized water were added to a tube. Following that 1.0 mL of Coomassie Brilliant Blue G-250 reagent was added. The solution was collected and detected the UV-*vis* absorbance of ox-TMB at 650 nm using spectra max. Based on the standard calibration curve, the enzymes (GOx-HRP) loading amount was determined.

Detection of glucose and recovery of the proposed method

Rat serum samples were obtained from Beijing Vital River Laboratory Animal Technology Co. Ltd. (Beijing, China). The entire experiments using rat serum samples was carried out in compliance with the policies for caring and using of laboratory animals from the Chinese regulations (GB/T 27416-2014). The interference-proteins were eliminated from the rat serum samples. Simply, 30.0 µL of the fresh rat serum samples were diluted by 30.0 µL of ethanol and incubated at 25 °C for 10.0 min. In order to prepare the samples for further analysis centrifuged it at 10,000 rpm for 10.0 min. The supernatant was collected and kept at 4 °C for further analysis. For detection of serum glucose, 10.0 µL of rat serums, 50.0 µL of GOx-HRP@PSMN@HZIF-8 composites, 30.0 µL of TMB (25.0 mM) and 170.0 µL of sodium acetate buffer (40.0 mM, pH 5.0) were mixed, which were incubated at 37 °C for 20.0 min. Then, the UV-*vis* absorbance of ox-TMB at 650 nm was recorded.

For measurement the recovery of the proposed method, 10.0 µL of rat serums, 50.0 µL of GOx-HRP@PSMN@HZIF-8-TMB composites, 30.0 µL of TMB (25.0 mM), 50.0 µL glucose (1.0-6.0 mM) and 120.0 µL of sodium acetate buffer (40.0 mM, pH 5.0) were mixed, which were incubated at 37 °C for 20.0 min. Then, the UV-*vis* absorbance of ox-TMB at 650 nm was recorded.

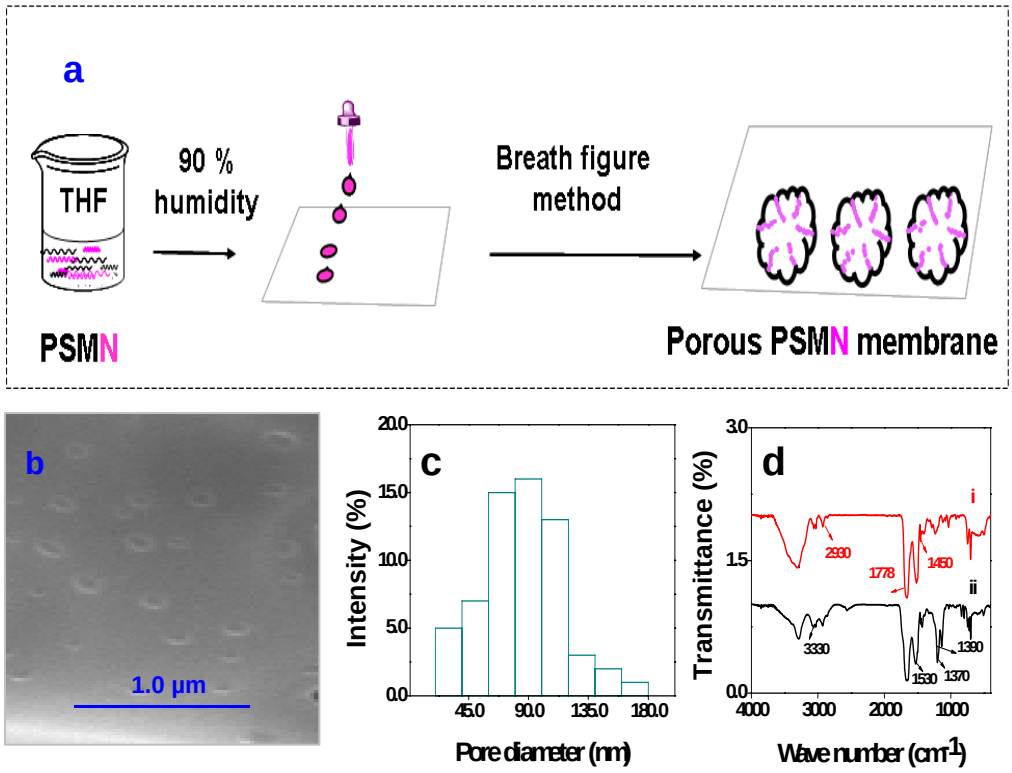

Fig. S1 (a) Fabrication process of porous PSMN membrane; (b) SEM image and pore size (c) of the resultant porous PSMN membrane; (d) FT-IR of PSM (i) and PSMN (ii) with absorption peaks at 1454 cm^{-1} and 2925 cm^{-1} represent C=C and $-CH_2$ in the benzene ring, the peaks at 1778 cm^{-1}, 3330 cm^{-1}, 1530 cm^{-1}, 1390 cm^{-1} and 1370 cm^{-1} represent the N-H stretching, C-N-H bending and isopropyl groups of N.

Table S1 Element contents of different membranes

Membranes	Carbon (%)	Nitrogen (%)	Hydrogen (%)
PSM	60.99	< 0.30	5.71
PSMN	66.63	8.91	8.84

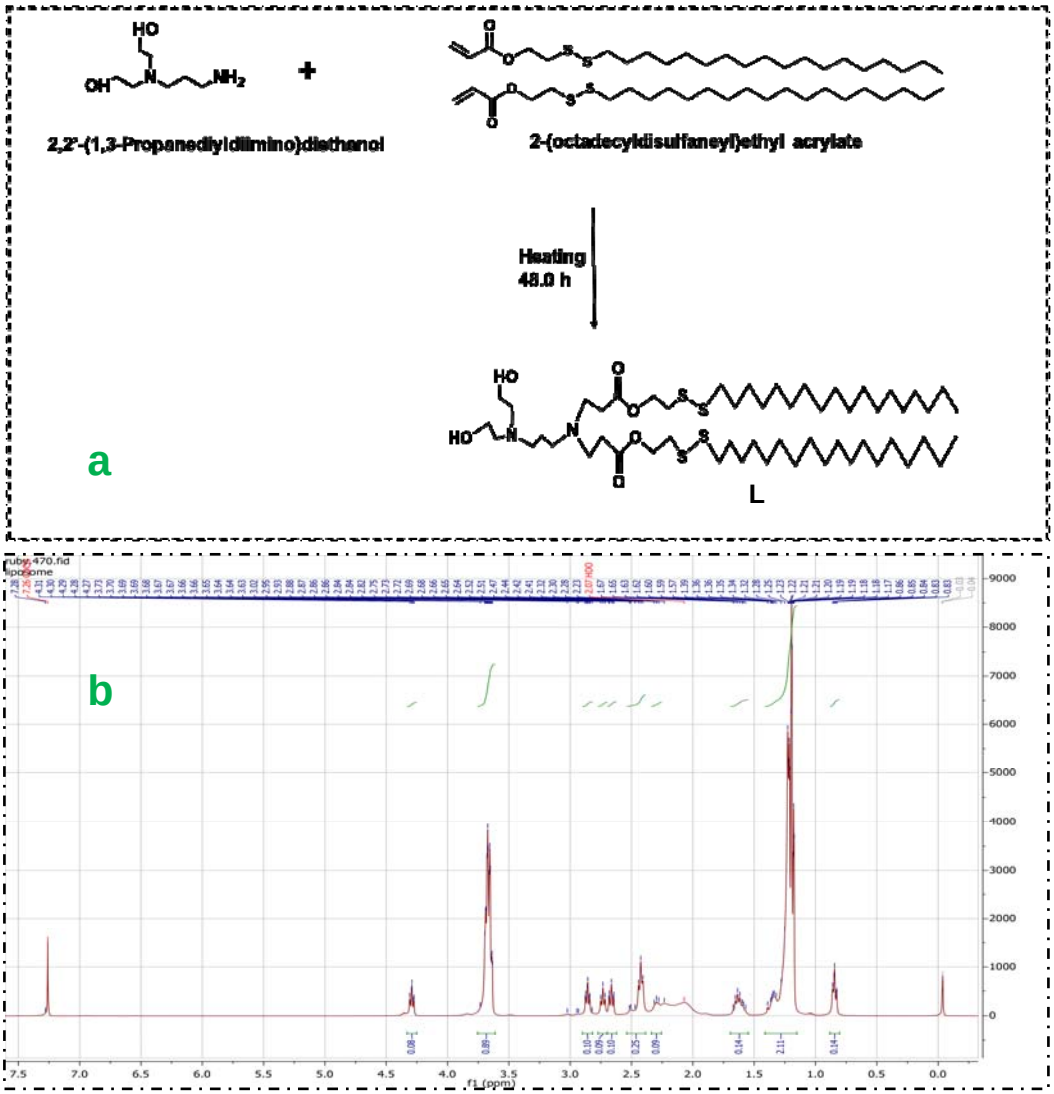

Fig. S2 (A) Synthesis process of L; (B) ^{1}H NMR spectrum of L.

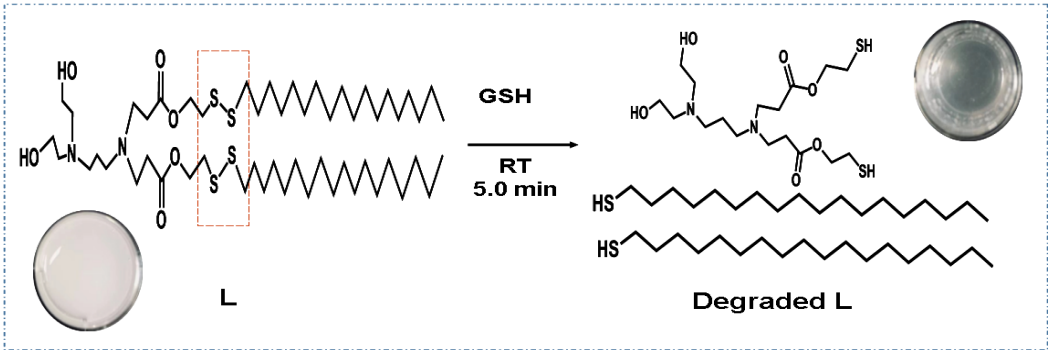

Fig. S3 Illustration of L degradation by GSH. Inset photos: GOx-HRP@PSMN@LZIF-8 system (left) and GOx-HRP@PSMN@HZIF-8 system (right) after removal of L *via* addition of GSH.

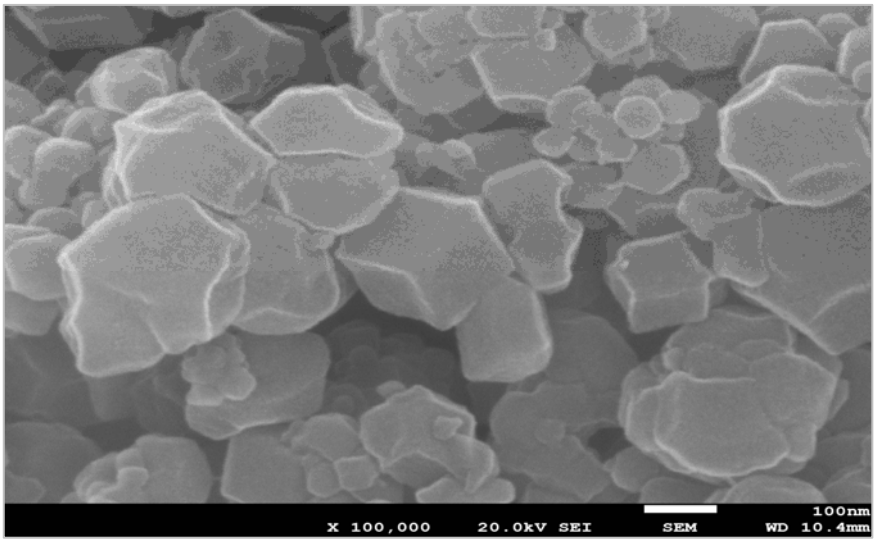

Fig. S4 SEM image of GOx-HRP@PSMN@HZIF-8.

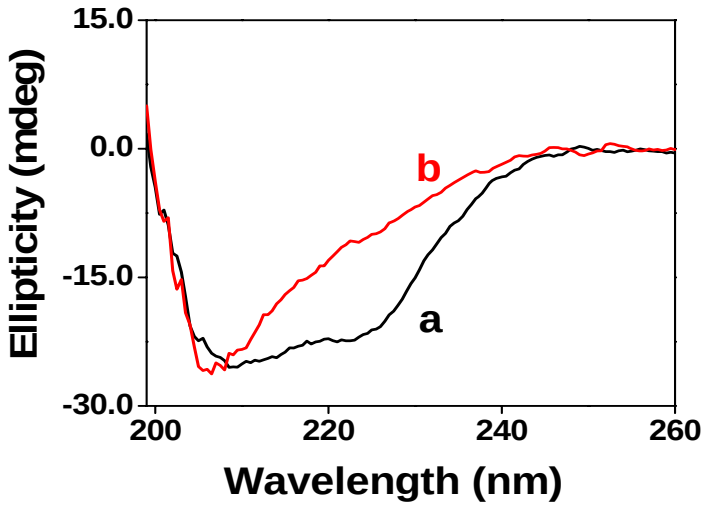

Fig. S5 CD spectra of (a) free HRP (6.0 μg/mL) and (b) HRP obtained *via* the HRP@PSMN@HZIF-8 composites collapsed by adding acetic acid.

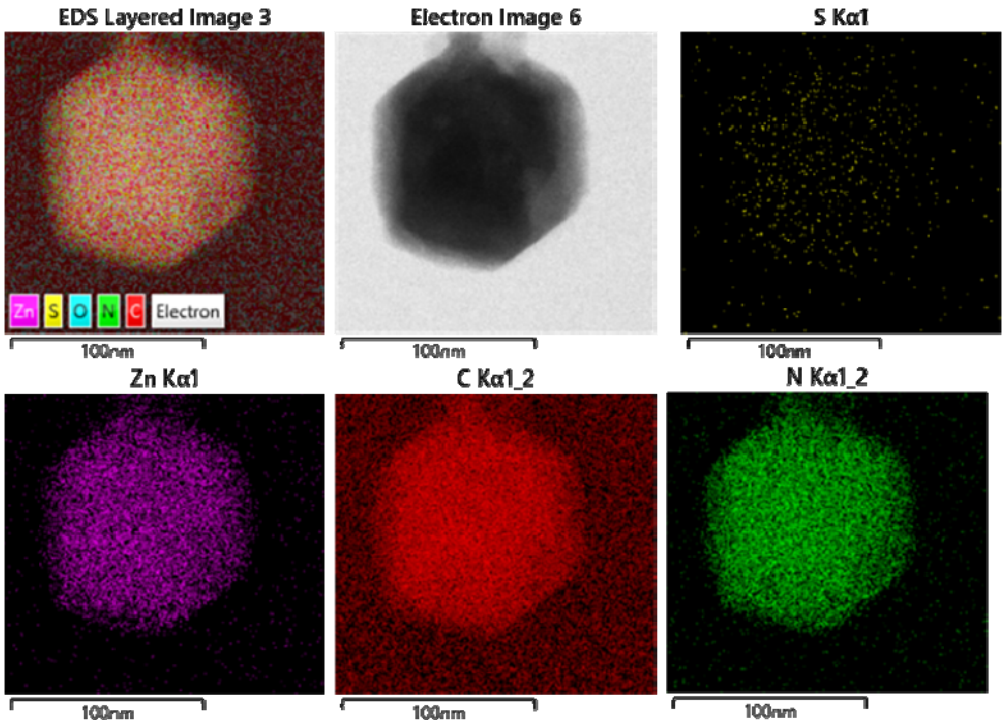

Fig. S6 EDS mapping of GOx-HRP@PSMN@ZIF-8.

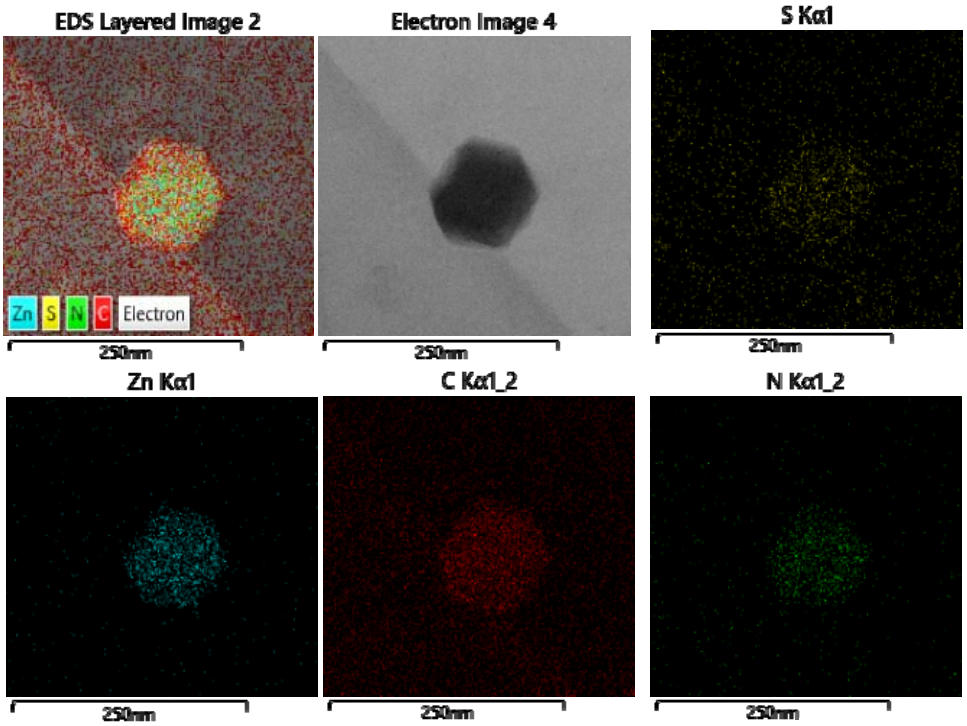

Fig. S7 EDS mapping of GOx-HRP@PSMN@LZIF-8.

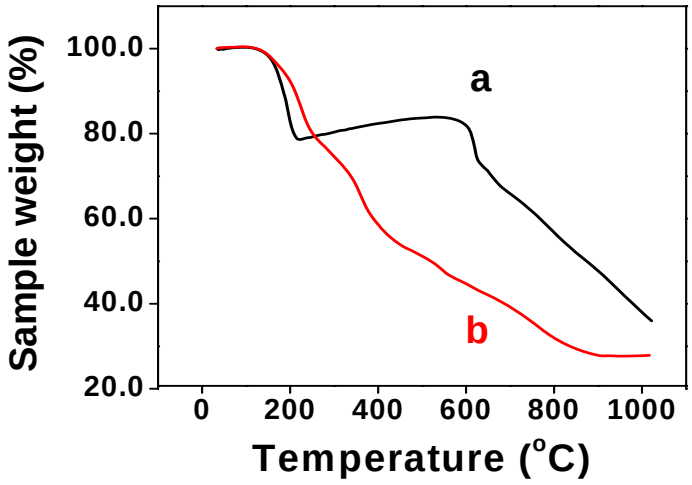

Fig. S8 TGA curves of (a) ZIF-8 and (b) GOx-HRP@PSMN@HZIF-8.

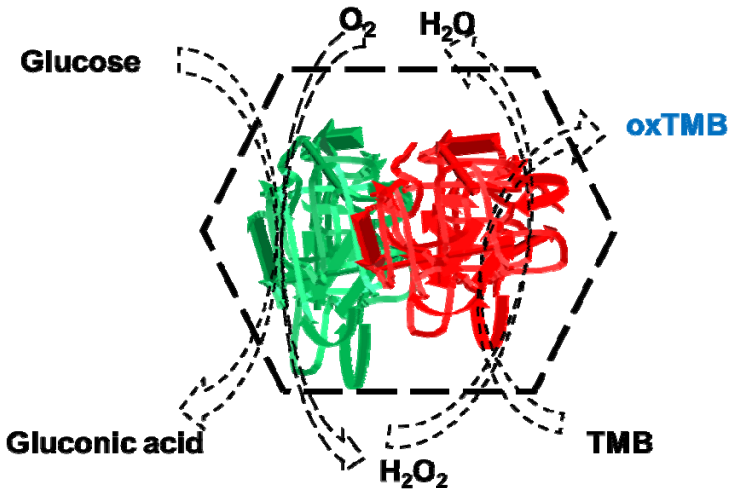

Fig. S9 Schematic representation of the cascade catalytic reaction.

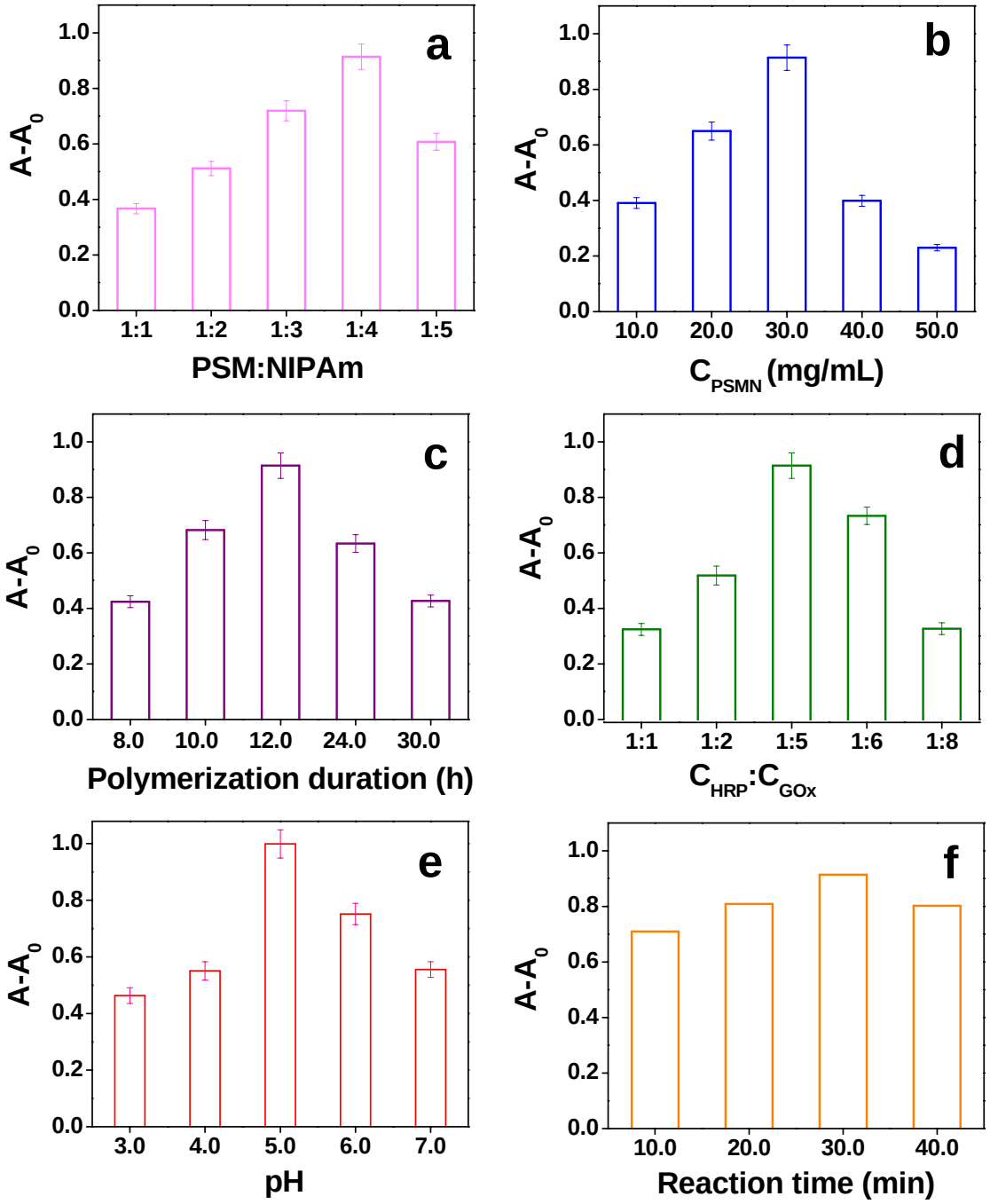

Fig. S10 Effect of (a) concentration ratio of PSMN to NIPAm; (b) concentration of PSMN; (c) polymerization durations; (d) concentration ratio of HRP: GOx; (e) pH and (f) reaction time on the catalytic activity of GOx-HRP@PSMN@HZIF-8. A and A_0 referred as the UV-*vis* absorption of ox-TMB at 650 nm in GOx-HRP@PSMN@HZIF-8-TMB system in the presence and absence of glucose at 37 °C, respectively.

Table S2 The calculated immobilizing contents of different nano-composites

Nano-composites	Immobilizing content (wt %)*
GOx-HRP@PSMN@LZIF-8	70.5
GOx-HRP@PSMN@ZIF-8	86.6
GOx-HRP@PSMN@HZIF-8	92.9

The immobilizing content was evaluated by examining the content of enzymes in the supernatant before and after immobilization using a standard Bradford assay.

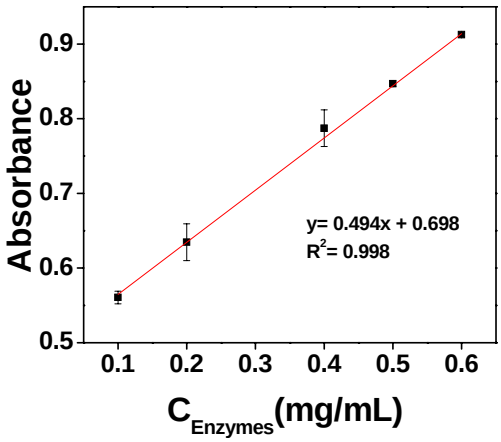

Fig. S11 Linear relationship of free enzymes (GOx-HRP).

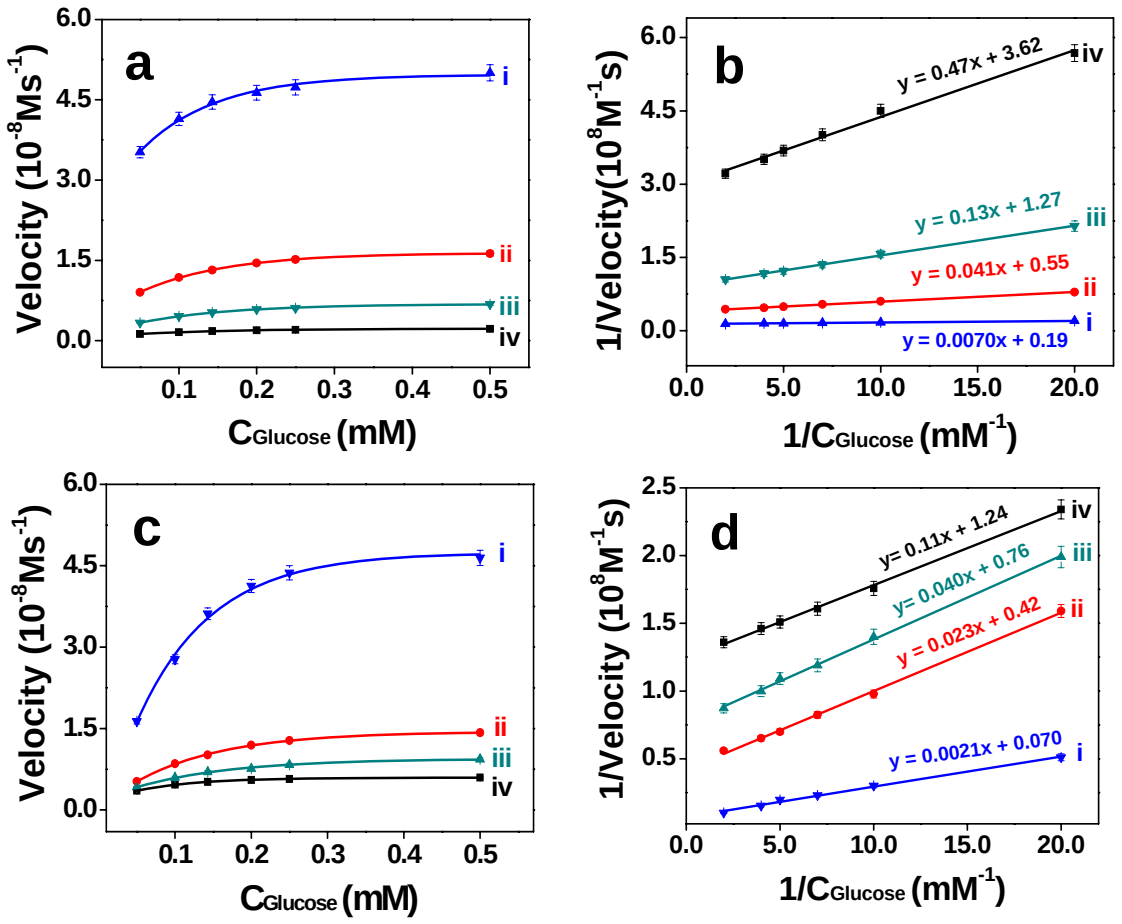

Fig. S12 Kinetics steady-state of (i) GOx-HRP@PSMN@HZIF-8, (ii) GOx-HRP@PSMN@ZIF-8, (iii) GOx-HRP and (iv) GOx-HRP@PSMN@LZIF-8 system at (a, b) 25 °C and (c, d) 37 °C respectively.

Table S3 Comparison free GOx-HRP with different GOx-HRP@MOFs composites for sensing glucose

GOx-HRP@MOFs composites	Chromogenic substrates	Samples	V_{max} enhanceed (folds)	Ref.
GOx-HRP@ZIF-8	ABTS	Standard glucose	2.0	X. L. Wu, *et. al.* *Chem. Commun.* 2015, 51, 13408
GOx-HRP@ZIF-8	Amplex Red	Standard glucose	7.5	W. H. Chen, *et. al.* *Nat. Cat.* 2018, 1, 689
GOx-HRP@ZIF-8	TMB	Standard glucose	3.2	G. Chen, *et. al.* *Angew. Chem. Int. Ed.* 2019, 58, 1463
GOx-HRP@ZIF-8	TMB	Cells	3.0	J. Bai. *et. al.* *ACS Biomater. Sci. Eng.* 2019, 5, 6207
GOx-HRP-A@ZIF-8	TMB	Standard glucose	0.8	G. Chen, *et. al.* *Angew. Chem. Int. Ed.* 2020, 132, 2889
GOx@ZIF-8@HRP@ZIF-8	OPD	Standard glucose	5.8	T. Man, *et. al.* *Nat Commun.* 2022, 13, 305
GOx-HRP@PSMN@HZIF-8	TMB	Rat serums	10.8	**This work**

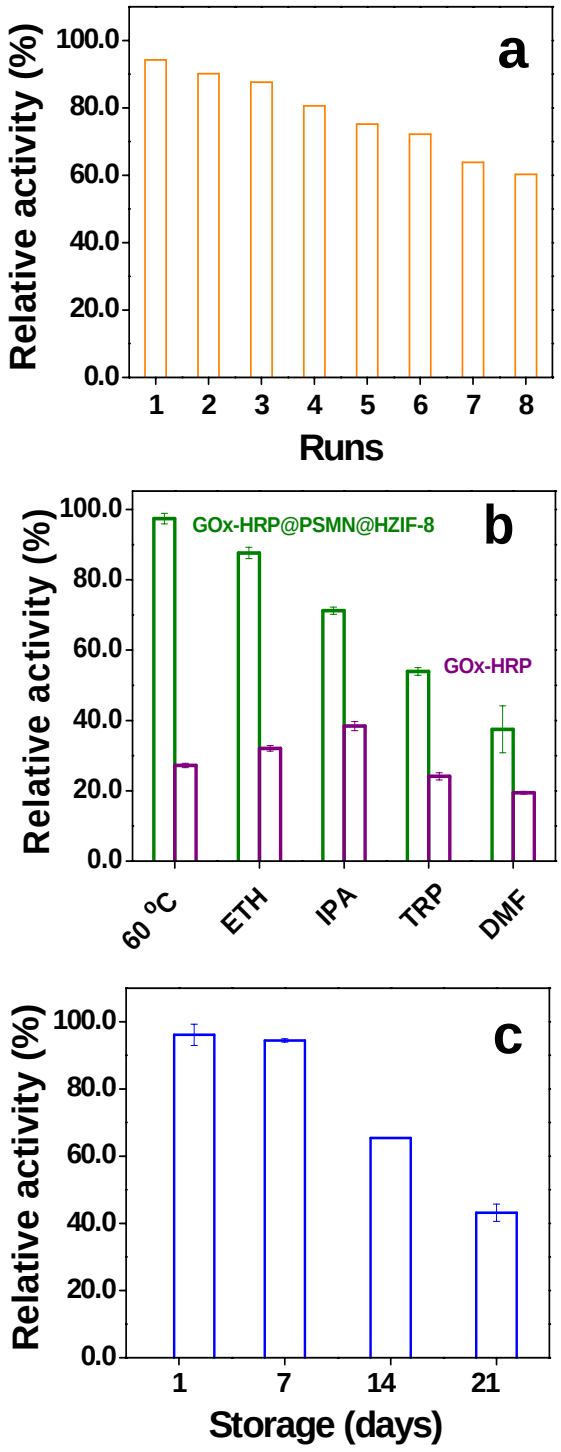

Fig. S13 (a) Recyclability of GOx-HRP@PSMN@HZIF-8. (b) Stabilities of GOx-HRP@PSMN@HZIF-8 and free GOx-HRP. (b) Reusability of GOx-HRP@PSMN@HZIF-8.

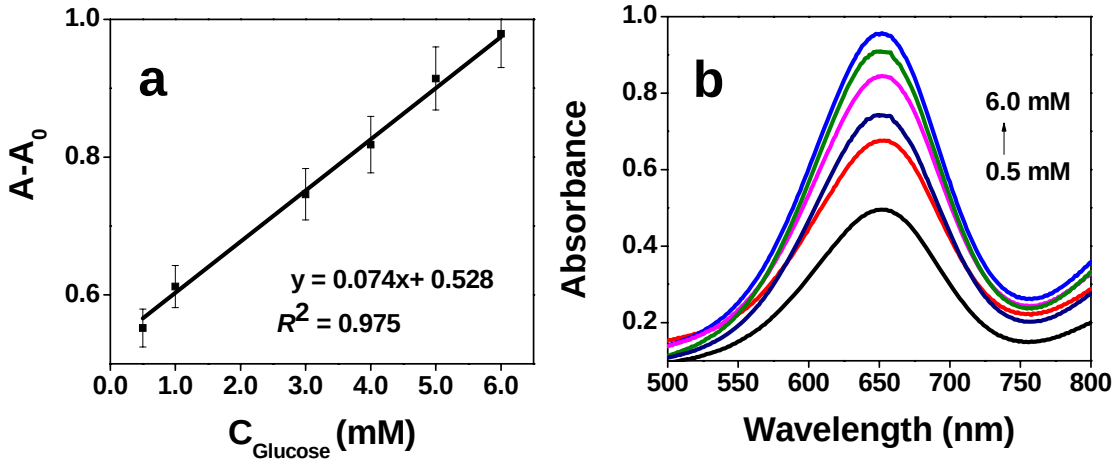

Fig. S14 (a) Linear relationship between the UV-*vis* absorbance of oxTMB and $C_{Glucose}$; (b) UV-*vis* absorbance of oxTMB variation with $C_{Glucose}$.

Table S4 Comparison with linear range and LOD for sensing glucose

Sensing materials	Linear range (mM)	LOD (mM)	Ref.
GOx@ZIF-8 nanoflowers	0.01-0.30	0.3	H. J. Chun, *et. al.* *BioChip J* 2014, 8, 218
SWCNHs-COOH nanohorns	0.10-2.0	0.1	S. Zhu, *et. al.* *Analyst* 2015, 140, 6398
GOx@ZIF-8 composites	1.0-10.0	0.05	R. Singh, *et. al.* *J. Mater. Chem. C* 2021, 9, 7677
GOx/FIC/ PLL@ SPCE strips	2.8-27.5	2.3	M. J. Lin, *et. al.* *Sensors* 2019, 19, 1448
GOx-HRP@PSMN@HZIF-8 composites	0.10-6.0	0.03	**This work**

Table S5 Recovery of proposed method*

Rat serums	Found (mM)	Added (mM)	Detected (mM)	Recovery ± RSD (%)
1	0.43	1.0	1.54	108.22 ± 0.10
		3.0	3.44	100.37 ± 0.21
		5.0	5.35	98.54 ± 1.26
2	0.63	1.0	1.52	93.55 ± 0.64
		3.0	3.77	103.96 ± 0.45
		5.0	5.92	105.32 ± 0.82
3	0.58	1.0	1.66	105.48 ± 0.19
		3.0	3.70	103.58 ± 0.56
		5.0	5.65	101.01 ± 0.21

* Blank controlled rat serums diluted 10-fold were used for recovery study (n=3).