

Supporting Information

Design of enzyme@metal organic framework composites with thermo-responsivity for colourimetric detection of glucose

Muhammad Ali Tajwar ^{a,b}, Cheng Cheng ^{a,c}, Li Qi* ^{a,b}

^a Beijing National Laboratory for Molecular Sciences; Key Laboratory of Analytical Chemistry for Living Bio-systems, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100190, P. R. China

^b University of Chinese Academy of Sciences (UCAS), Beijing 100049, P. R. China

^c College of Chemistry and Environmental Science, Hebei University, Baoding 071002, P.R. China

***Corresponding author: Li Qi**

E-mail: qili@iccas.ac.cn

Experimental Section

Materials and Chemicals

UiO-66-NH₂ (U) was purchased from Beijing Krre Technology Co., Ltd. (Beijing, China). *N*-isopropyl acrylamide (N) monomer, glucose oxidase (GOx), horseradish peroxidase (HRP) and Cosmic brilliant blue (G-250) were obtained from Aladdin Chemistry Company (Shanghai, China). Glucose and fluorescein isothiocyanate (FITC) were bought from Beijing Xinjingke Biotech. Ltd. (Beijing, China). 2-Vinyl-4,4-dimethylazlactone (VDMA) monomer acquired from Beijing Institute of Coolight Fine Chemicals (Beijing, China). (Shanghai, China). Dimethylformamide (DMF), 1,4-dioxane and anhydrous ethanol were provided by Tianjin Concord Technology Co., Ltd. (Tianjin, China). Phosphate buffered saline (PBS) was gotten from ThermoFisher Biochemical Products (Beijing, China). 2,2'-azobisisobutyronitrile (AIBN), 3,3',5,5'-tetramethylbenzidine (TMB) and other chemicals were gotten from Beijing Innochem Technology Co. Ltd. (Beijing, China). All chemicals used in this work were of analytical grade. Deionized water from Milli-Q Ultrapure Water Purification System (18.2 M Ω.cm, Millipore, Bedford, MA, USA) was used throughout the experiment. The enzymes in GOx-HRP@U@PN were labeled by FITC in assistance of microwave.

Instruments

Ultraviolet-visible (UV-vis) absorption spectra were recorded 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.

Powder x-ray diffraction (PXRD) patterns of U@PN and U at 2θ were obtained using Epyrean Rayons-X Malvern Panalytica Company.

Transmission electron microscopy (TEM) images were acquired using a transmission electron microscope (JEM-2010, Japan electron optics laboratory, Japan) at a voltage of 200 kV.

Fourier transform infrared (FTIR) spectra of GOx-HRP@U@PN, U@PN and U were measured by a Bruker Tensor 27 spectrophotometer in the wavenumber range from 4000 cm⁻¹ to 400 cm⁻¹ under ambient conditions.

Thermo-gravimetric analysis (TGA) was applied for composing determination of GOx-HRP@U@PN, U@PN and U with a synchronous thermal analyzer (STA 449 F3 Jupiter, Nestal, Germany) in a temperature range from 35 to 900 °C at a heating rate of 10 °C/min in air.

The size changes of U@PN were measured with a dynamic light scattering Zetasizer (DLS) analyser (Zetasizer Nano ZS ZEN3600, British).

Confocal laser scanning microscopy (CLSM, FV1000-IX81, Olympus, Japan) was used to prove the presence of FITC labeled GOx-HRP@U@PN.

Steady-state kinetic studies

The steady-state kinetic studies of the test enzyme-systems were carried out in a reaction system having 3.0 mL PBS buffer solution (pH 5.5), that containing 50.0 μL TMB (25.0 mM), 40.0 μL GOx-HRP@U@PN or GOx@U@PN mixed HRP@U@PN or free GOx-HRP, and 60.0 μL glucose with different concentrations. The solution mixture was incubated at 25 °C and 37 °C for 20.0 min, respectively, and then measured the UV-vis absorbance at 650 nm for detection of oxTMB.

To calculate the steady-state enzymatic kinetic parameters of GOx-HRP@U@PN or GOx@U@PN mixed HRP@U@PN or free GOx-HRP, various concentrations of glucose were prepared in PBS buffer solution (pH 5.5). Double reciprocal Michaelis-Menten curves were plotted and fitted to Lineweaver-Burk equation [1]:

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

Where V is the initial velocity, K_m is the Michaelis-Menten constant, $[S]$ is the concentration of the substrate, and V_{max} is the maximal reaction velocity.

Recovery of the proposed method

The rat serums were obtained from the Beijing Vital River Laboratory Animal Technology Co., Ltd. Animal center (Beijing, China). All experiments concerning with rats were complied with the guide for caring and using of laboratory animals from the Association for Assessment and Accreditation of Laboratory Animal Care.

The rat serum samples were pre-treated to eliminate the interferences-proteins. Simply, 100.0 μL of the fresh rat serum samples was diluted by 100.0 μL of ethanol, which was incubated at 25 °C for 10.0 min. Consequently, the samples were centrifuged at 10,000 rpm for 10.0 min and the supernatant was collected and stored at 4 °C for further analysis.

The proposed colorimetric method system was applied to analyze the glucose in three rat serums. The original concentration of glucose in the rat serums, the amount of glucose added and original one were tested with the proposed protocol.

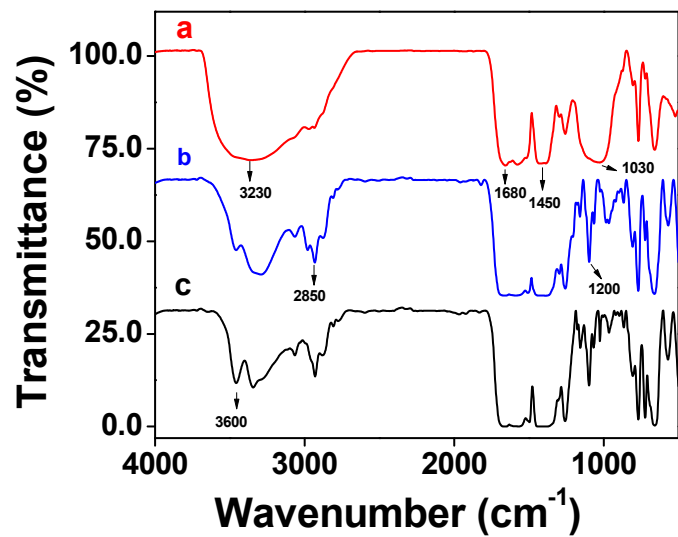


Fig. S1 FTIR spectra of (a) GOx-HRP@U@PN (b) U@PN and (c) U.

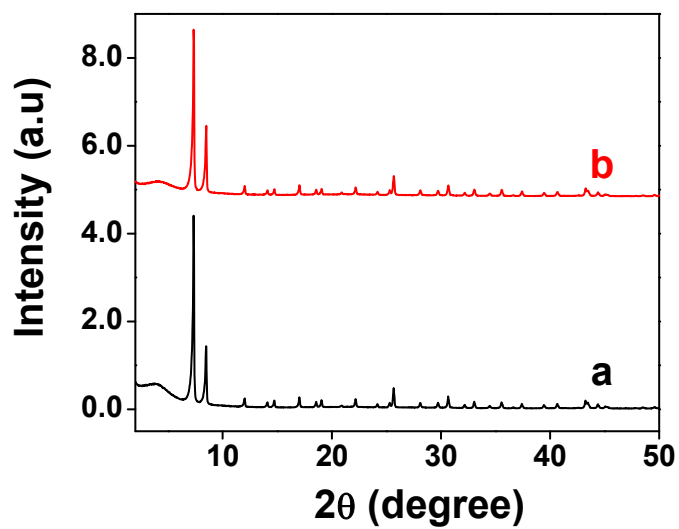


Fig. S2 PXRD patterns of U (a) and U@PN (b)

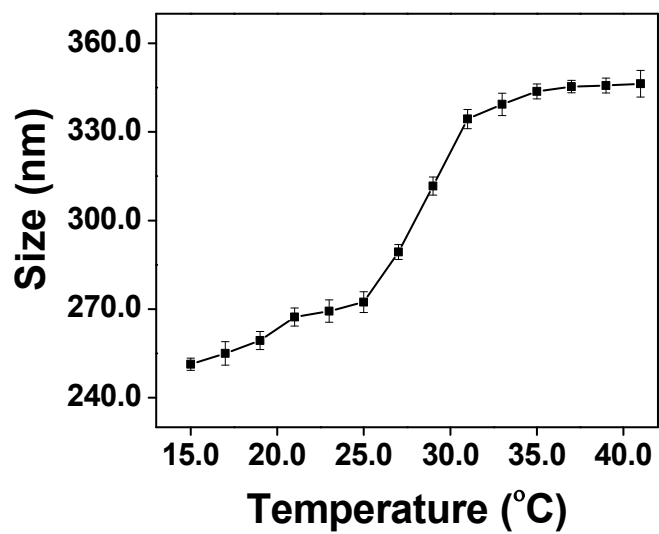


Fig. S3 The size changes of U@PN with variation of temperature.

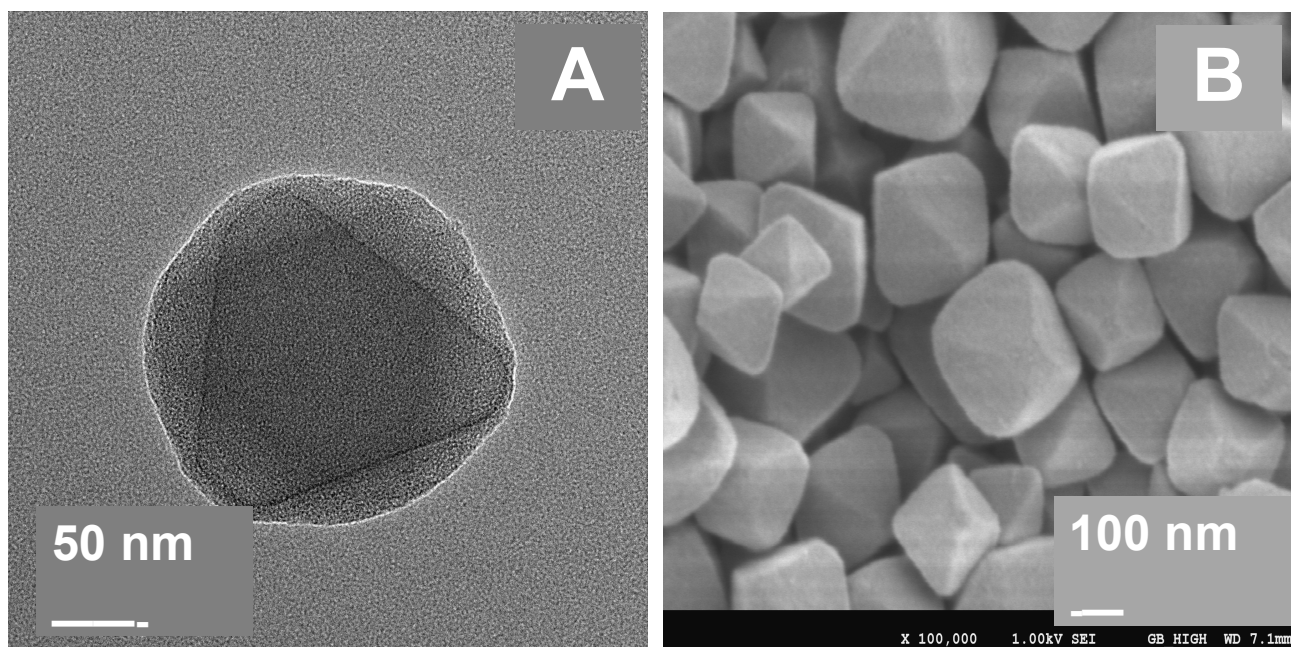


Fig. S4 (A) TEM and (B) SEM images of U.

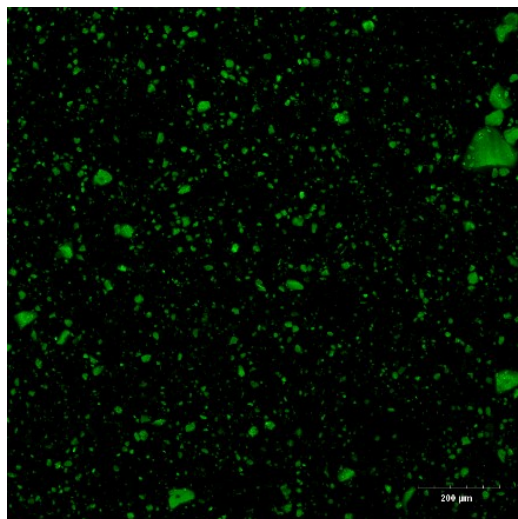


Fig. S5 CLSM image of the FITC labeled enzymes on U@PN.

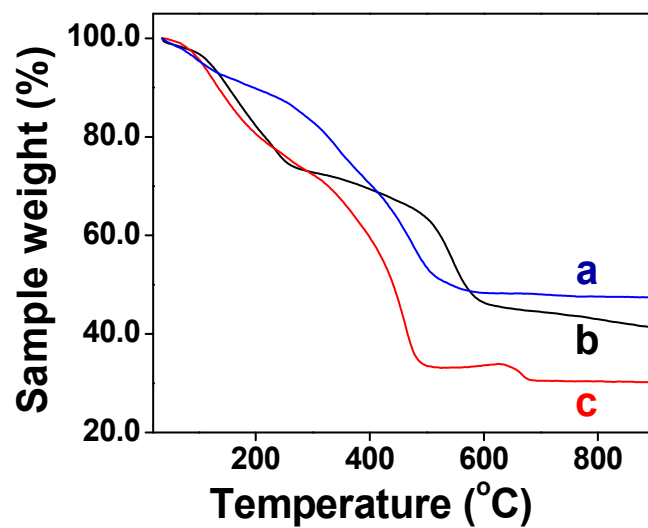


Fig. S6 TGA curves of (a) U, (b) U@PN and (c) GOx-HRP@U@PN.

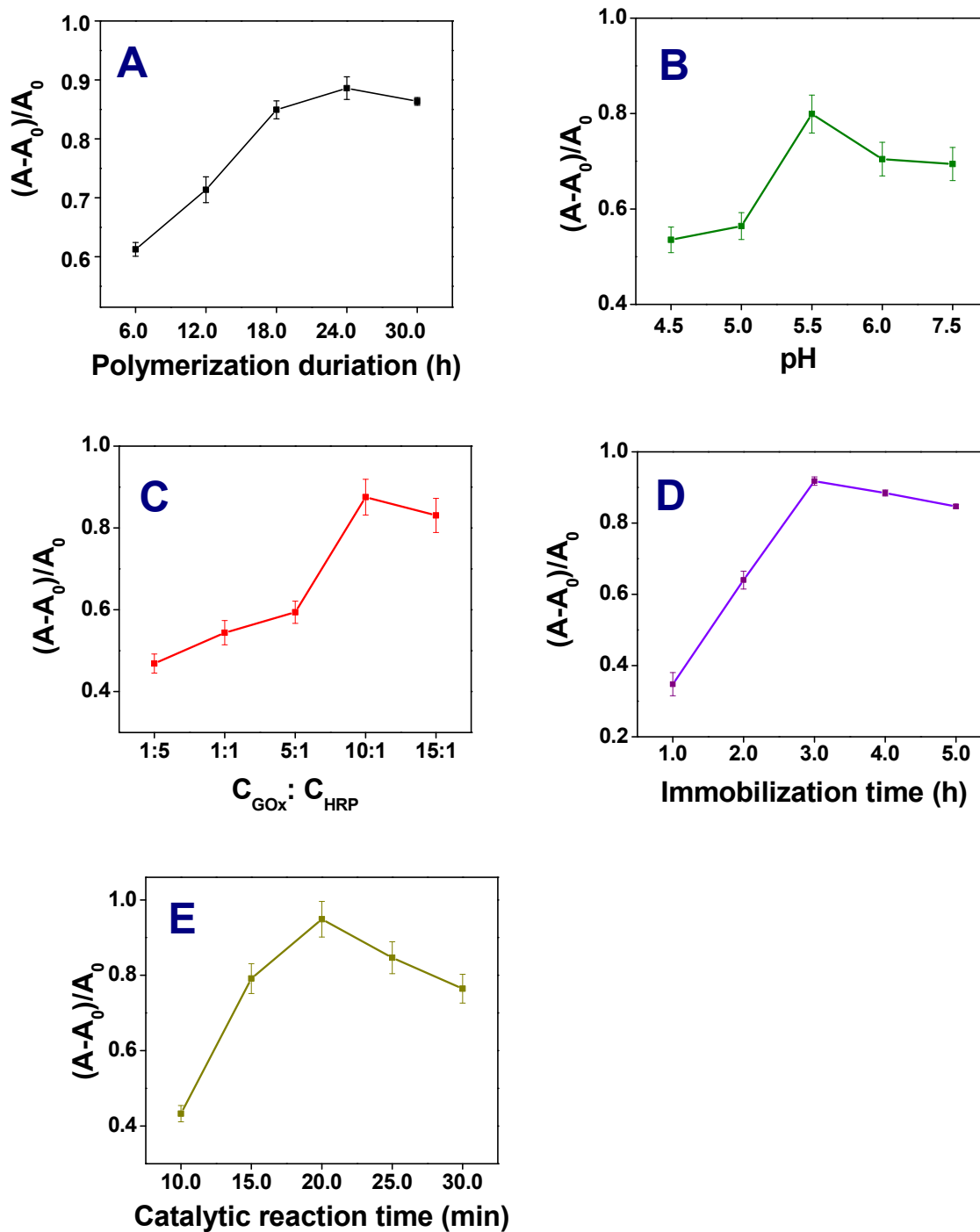


Fig. S7 Effect of (A) polymerization duration; (B) buffer pH values; (C) concentration ratio of GOx to HRP; (D) enzymes immobilization time and (E) catalytic reaction time on the catalytic performance of GOx-HRP@U@PN composites-TMB system. A_0 and A represented the UV-vis absorption of the test system at 25 °C and 37 °C, respectively.

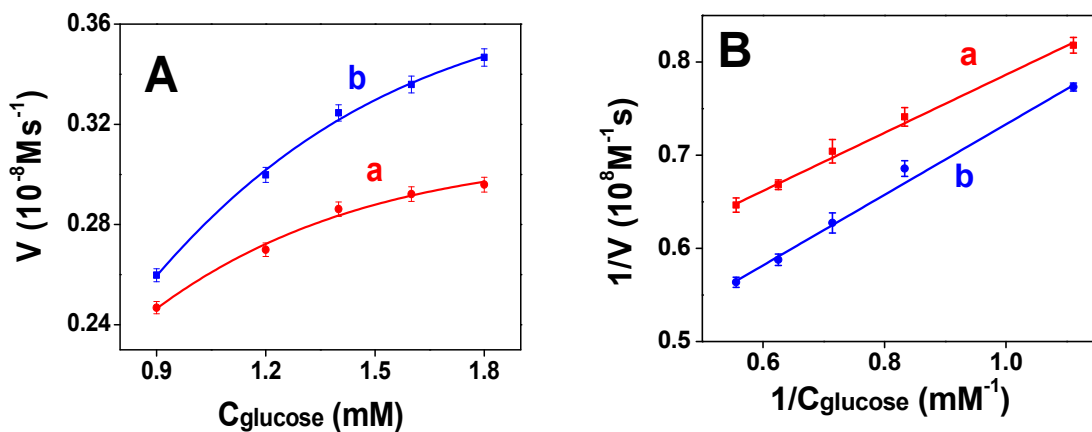


Fig. S8 The relationship between glucose concentration and initial reaction velocity in free GOx and HRP system: (A) direct plots and (B) Lineweaver-Burk double reciprocal plots at (b) 37 °C and (a) 25 °C, respectively

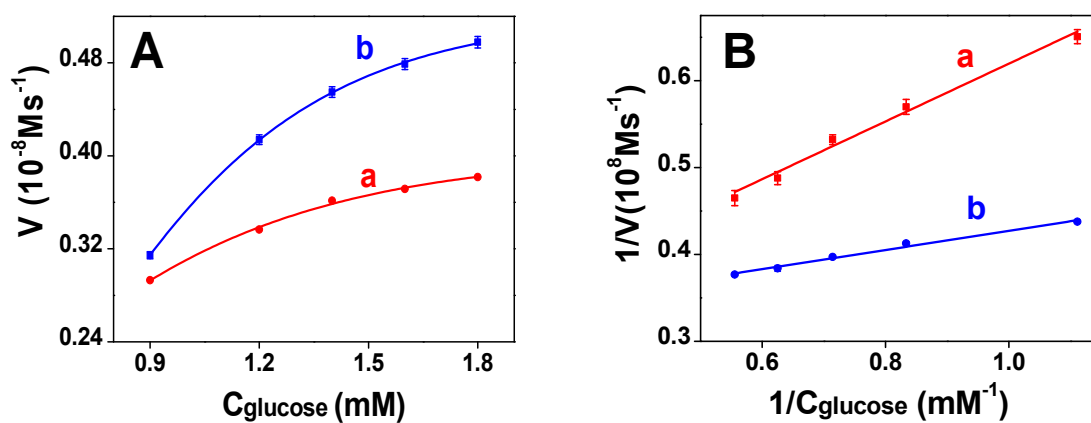


Fig. S9 The relationship between glucose concentration and initial reaction velocity in GOx@U@PN mixed HRP@U@PN system: (A) direct plots and (B) Lineweaver-Burk double reciprocal plots at 37 °C (b) and 25 °C (a), respectively

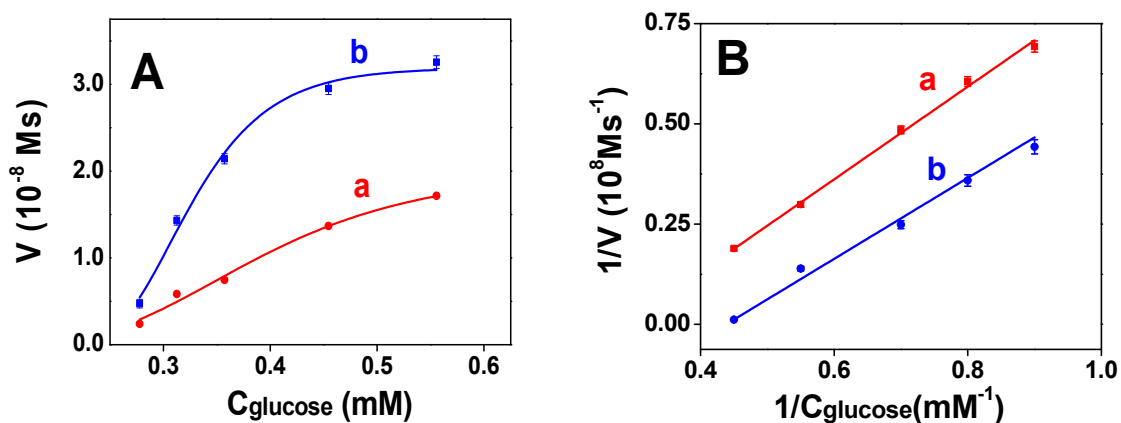


Fig. S10 The relationship between glucose concentration and initial reaction velocity in GOx-HRP@U@PN system: (A) direct plots and (B) Lineweaver-Burk double reciprocal plots at 37 °C (b) and 25 °C (a), respectively.

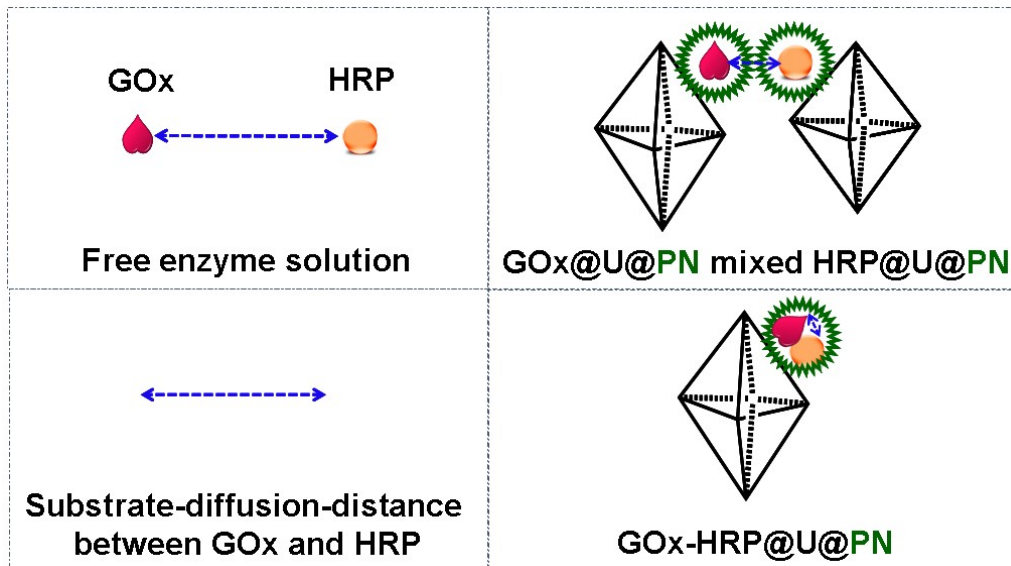


Fig. S11 Illustration of catalytic efficiency affected by the substrate-diffusion-distance between GOx and HRP in different catalytic systems.

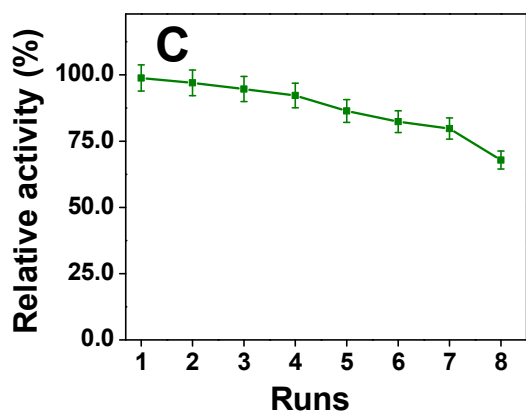
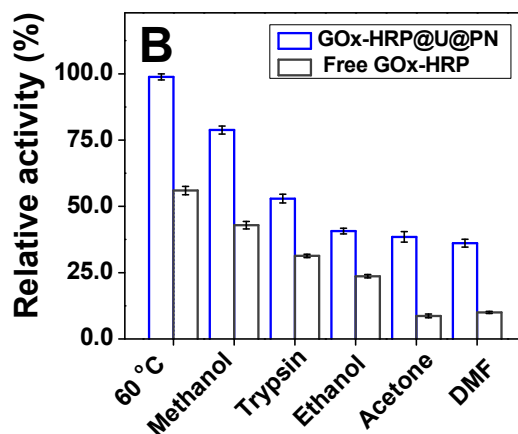
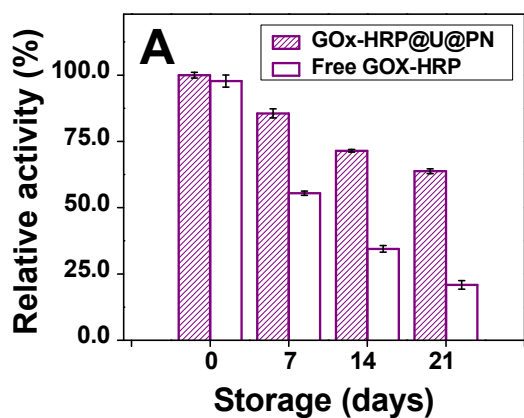


Fig. S12 Stabilities of GOx-HRP@U@PN and free GOx-HRP with storage (A) at 25 °C for three weeks; (B) heated up to 60 °C or incubated with trypsin or exposed to different organic solvents (20%) at 37 °C. (C) Reusability of the proposed GOx-HRP@U@PN.

Table S1 Recovery of the proposed method*

Rat serums	Found (mM)	Added (mM)	Detected (mM)	Recovery \pm RSD (%)
1	0.485	0.10	0.602	102.9 \pm 0.30
		0.50	0.965	97.9 \pm 0.10
		0.80	1.229	95.5 \pm 0.21
2	0.763	0.10	0.875	101.3 \pm 0.35
		0.50	1.199	94.9 \pm 0.32
		0.80	1.428	91.3 \pm 0.45
3	0.363	0.10	0.459	99.1 \pm 0.78
		0.50	0.878	101.7 \pm 0.40
		0.80	1.114	95.7 \pm 0.51

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

Table S2 Comparison with the reported enzymes@MOFs composites-TMB system for glucose sensing

Enzymes@MOFs composites	V_{\max} (10^{-8} Ms⁻¹)	Tunable catalytic performance	Refs.
GOx@MIL-53	4.48	No	W. Dong, <i>et.al.</i> , <i>RSC Adv.</i> , 2015, 5, 17451
GOx@MOF-808	1.39	No	W. Dong, <i>et. al.</i> Talanta 2017, 167, 359
GOx@Fe-MOF	5.60	No	W. Xu, <i>et.al.</i> <i>ACS Appl. Mater. Interfaces.</i> , 2019, 11, 22096
GOx@MIL-53	2.28	No	J. Wang, <i>et. al.</i> <i>Anal. Chim. Acta</i> 2020, 1098, 148
GOx-HRP@U@PN	8.62	Yes	This work