Design of hollow GOx@Cu^{2+}-PA integrated nanozyme for self-cascading colorimetric sensing of glucose

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1. Experimental

1.1. Reagents and materials

2-methylimidazole (2-MIM) was obtained from Hebei Bailingwei Super Fine Material Co., Ltd. Zinc nitrate hexahydrate ($Zn(NO_3)_2 \cdot 6H_2O$), phytic acid (PA, 70%), copper nitrate trihydrate ($Cu(NO_3)_2 \cdot 3H_2O$), acetic acid, sodium acetate, hydrogen peroxide (H_2O_2) and 3,3',5,5' - tetramethylbenzidine (TMB) were purchased from Macklin Co., Ltd. Polyvinylpyrrolidone (PVP) was obtained from Shanghai Sinopharm Chemical Reagent Co., Ltd. Tris-HCl was obtained from Shanghai Yuanye Biotechnology Co., Ltd. Glucose, Fructose and Sucrose were purchased from Beijing Hwrk Chemical Technology Co., Ltd. Glucose oxidase (GOx) was purchased from Aladdin Biochemical Technology Co., Ltd. The ultrapure water was sourced from the Millipore purification system (18.2 MΩ).

1.2. Apparatus

The X-ray diffraction (XRD) patterns were recorded using a D/max 2550 VB/PC diffractometer (Rigaku, Japan). Scanning electron microscopy (SEM) was carried out on FEI Quanta 400 FEG (American FEI). X-ray photoelectron spectroscopy (XPS) data were obtained by Thermo ESCALAB 250XI electron spectrometer (Thermo, America) under 150 W Al Kα radiation. Fourier Transform Infrared spectroscopy (FTIR) was recorded on the Nicolet iS50 FT-IR spectrophotometer. Ultraviolet-visible (UV-vis) absorption spectra were measured using a Cary 60 spectrophotometer (Agilent, USA). Electron spin resonance (ESR) was carried out on the A300-10/12 Bruker (Germany). Thermogravimetric analysis (TGA) was conducted using LABSYS evo TG-DSC/DTA (France) instruments.

1.3. Steady-state kinetics study

Under the optimal experimental conditions, the cascade enzymatic activity exhibited by GOx@Cu^{2+}-PA was investigated. Specifically, the apparent steady-state kinetic parameters were estimated while keeping the other concentrations of the substrates unchanged. The kinetic parameters were calculated based on the Michaelis-Menten equation ($1/\upsilon = K_m /(V_{max}\,[S]) + 1/V_{max}$). Here, [S] represents the substrate concentration (TMB, H_2O_2 or glucose), V_{max} is the maximum reaction rate, K_m is the

Michaelis constant, and υ is the initial reaction rate. The kinetic parameters V_{max} and K_m can be obtained through the Lineweaver-Burk plot.

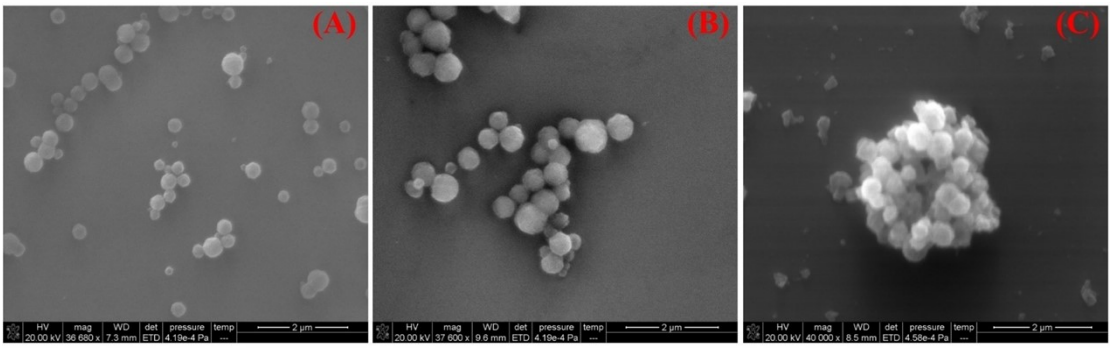

Fig. S1. SEM images of (A) ZIF-8, (B) GOx@ZIF-8 and (C) GOx@Cu^{2+}-PA.

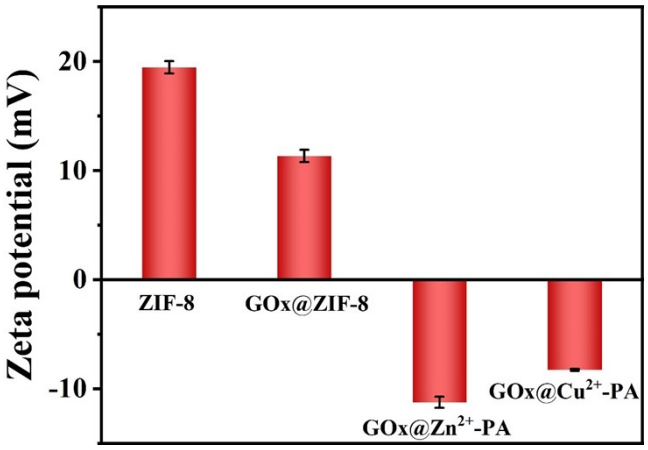

Fig. S2. Zeta potential diagrams of ZIF-8, GOx@ZIF-8, GOx@Zn^{2+}-PA and GOx@Cu^{2+}-PA.

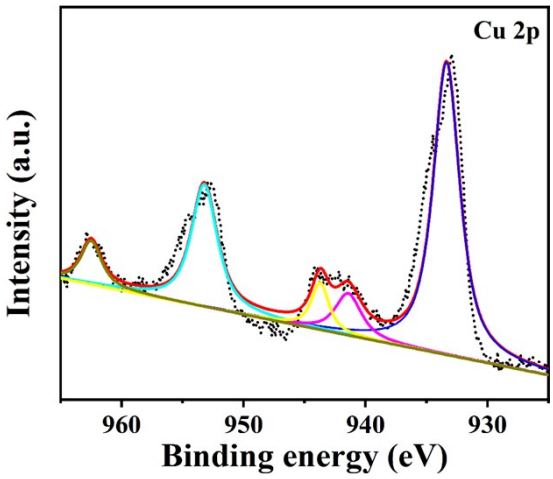

Fig. S3. Cu 2p high-resolution XPS spectra of GOx@Cu^{2+}-PA.

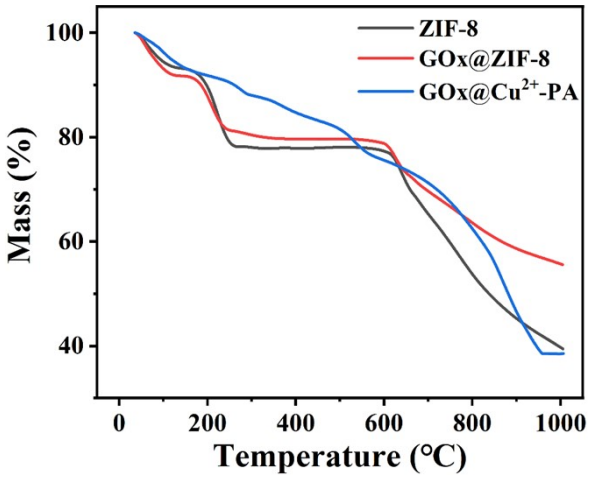

Fig. S4. TGA curves of ZIF-8, GOx@ZIF-8 and GOx@Cu^{2+}-PA in air.

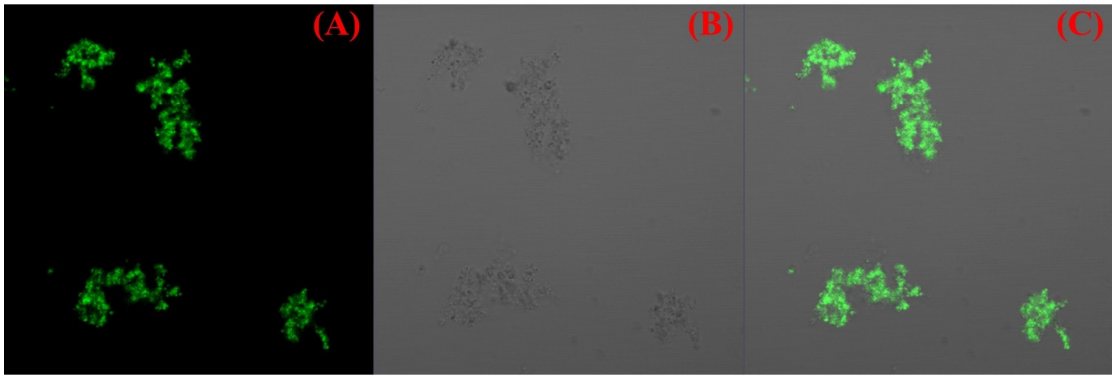

Fig. S5. (A) The CLSM image; (B) bright-field image; (C) along with their corresponding merged image of GOx@Cu^{2+}-PA marked with FTIC.

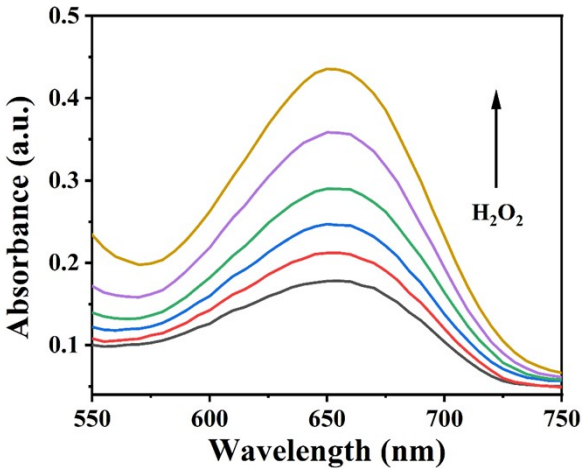

Fig. S6. The feasibility of using GOx@Cu^{2+}-PA to detect glucose.

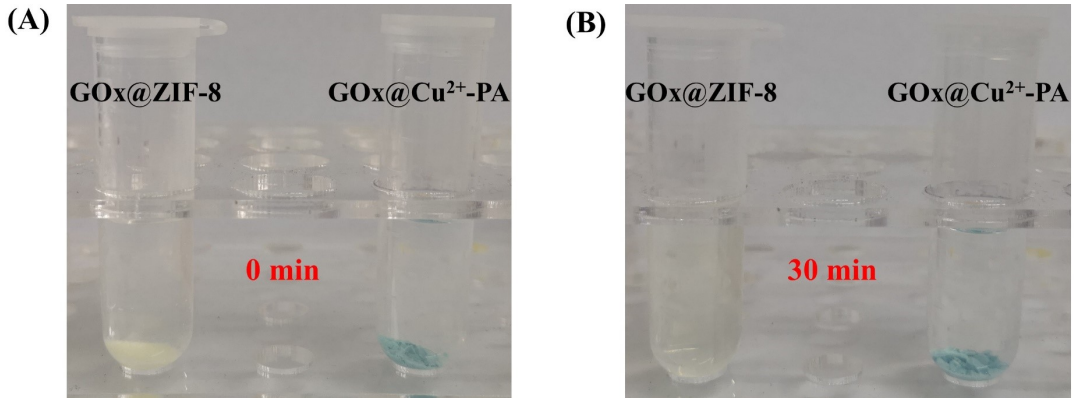

Fig. S7. Photographs of GOx@ZIF-8 and GOx@Cu^{2+}-PA (A) before and (B) after the incubation in a pH 3.5 acetic acid buffer solution for 30 min.

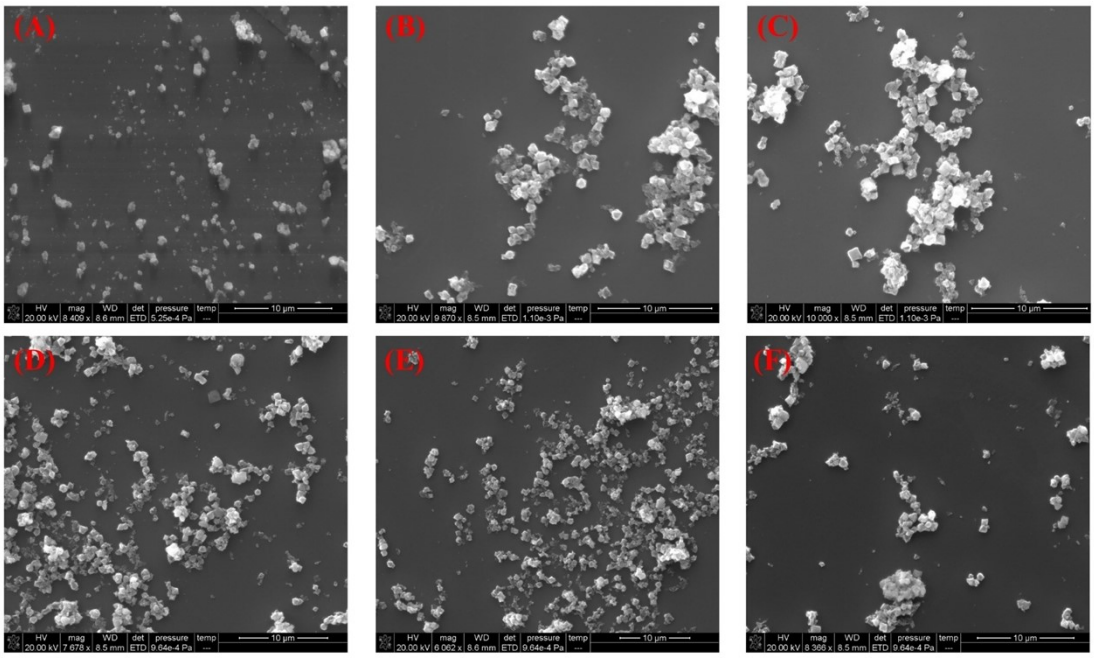

Fig. S8. SEM images of (A) untreated GOx@Cu^{2+}-PA; GOx@Cu^{2+}-PA immersed for 30 min in buffer solutions with pH values of (B) 3.5; (C) 4.5; (D) 5.5; (E) 7.0 and (F) 9.0 respectively.

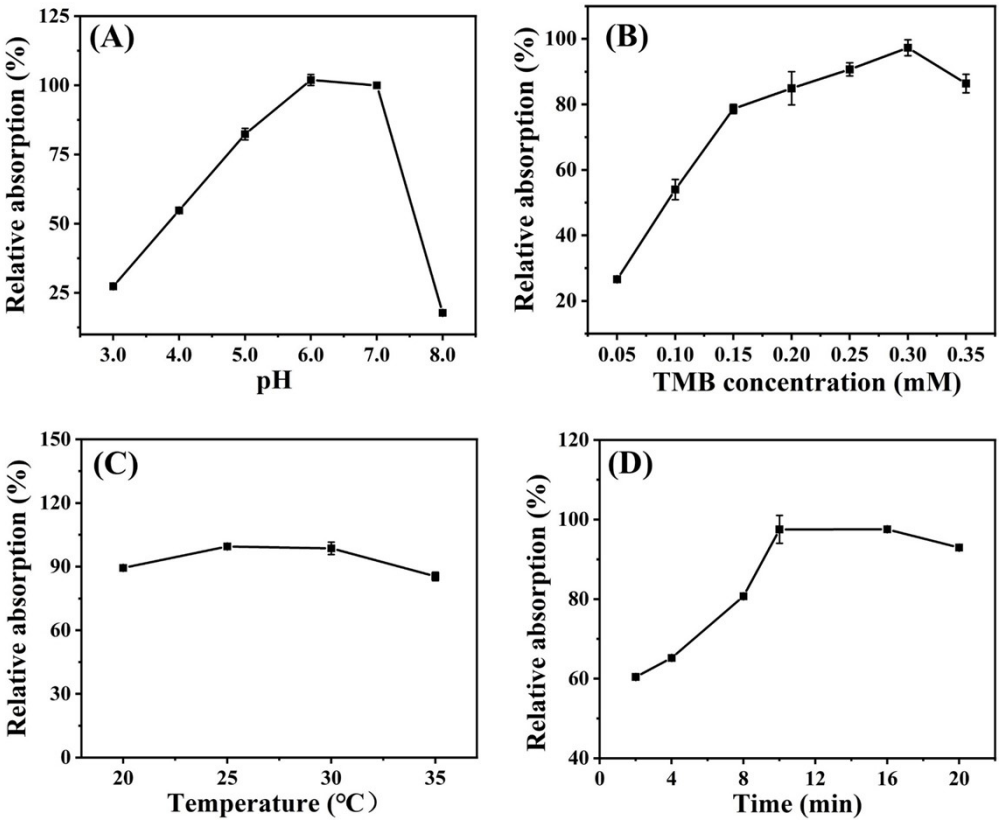

Fig. S9. The effects of (A) pH; (B) TMB concentration; (C) reaction temperature; (D) incubation time on the detection of glucose by GOx@Cu^{2+}-PA cascade nanozyme.

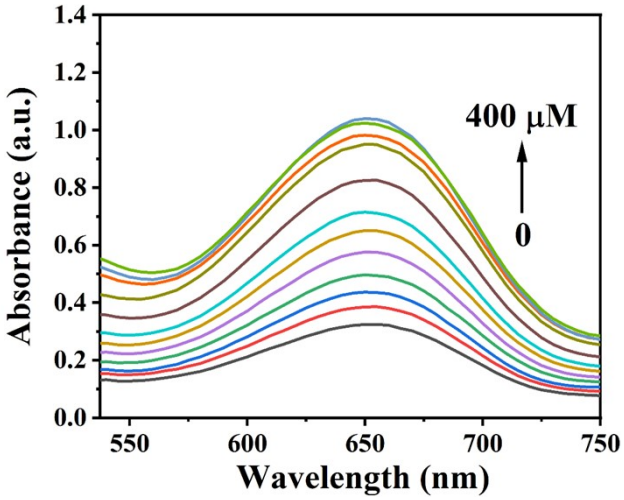

Fig. S10. The UV-vis absorption spectrum of the GOx@Cu^{2+}-PA-TMB system in the presence of different concentrations of glucose (0-400 μM).

Table. S1 Comparison of kinetic parameters of various nanozymes with peroxidase-mimicking properties.

Catalyst	Substance	K_m [mM]	V_{max} [10^{-8} M s^{-1}]	References
HRP	TMB	0.434	10.0	1
	H_2O_2	3.70	8.71	
	Glucose	0.37	20	
GOx@HP-MIL-88B-BA	TMB	0.22	6.8	2
	H_2O_2	0.38	7.2	
	Glucose	0.57	0.38	
GOx@MIL-100(Fe)-B	TMB	17.1	2.12	3
	H_2O_2	0.86	0.45	
	Glucose	3.3	2.02	
GOx@Fe-BTC	TMB	0.28	4.2	4
	H_2O_2	0.16	6.99	
GOx/Hemin@NC-ZIF	TMB	1.66	26.4	5
	H_2O_2	6.71	113.8	
	Glucose	0.05	5.8	
GOx@Cu^{2+} -PA	TMB	0.17	17.5	This work
	H_2O_2	2.2	16.8	

References

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