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Supplementary Information

Construction of peroxidase nanozyme based on Prussian Blue and its application in GSH colorimetric assay

Xiaocheng Lv, Ying Zhang, Yajin Zheng, Qianzi Tong, Yingying Zhang, Siyu Wang, Jing Chen, Xifu Liu*and Xueke Sun*

Ministry of Education Key Laboratory of Molecular and Cellular Biology, Hebei Anti-tumor Molecular Target Technology Innovation Center, College of Life Science, Hebei Normal University, Shijiazhuang, 050024, China.

***Corresponding author**.

E-mail Addresses： xfliu@hebtu.edu.cn (X.Liu), xuekesun@hebtu.edu.cn (X.Sun).

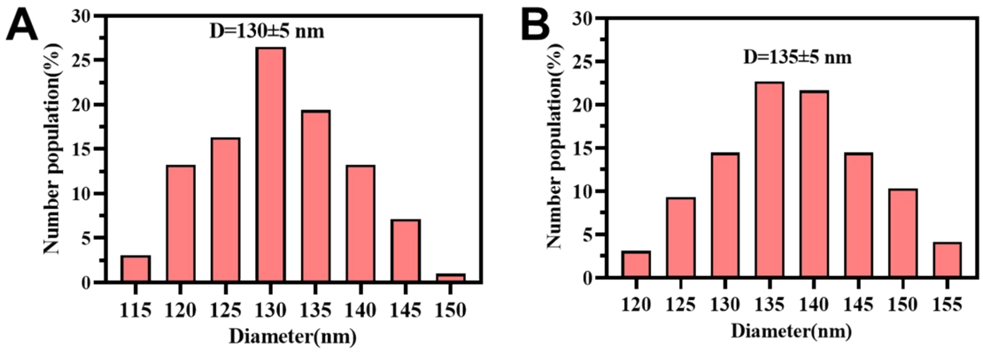

Figure S1 (A) Histogram of particle size distribution of (A) $mSiO_2$-APTES and (B) $mSiO_2$@PB.

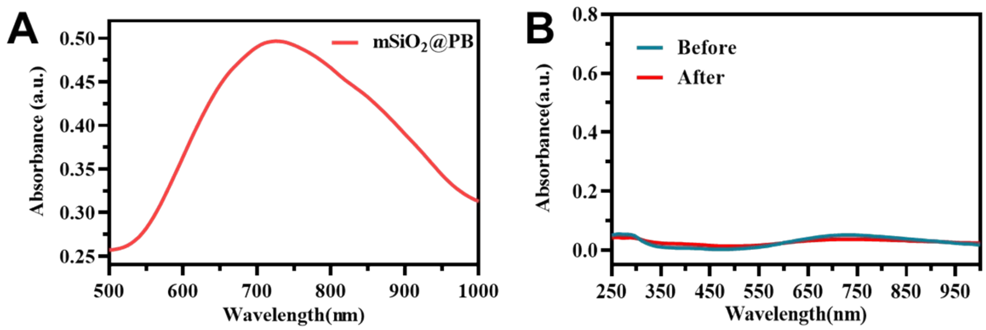

Figure S2 (A) UV-Vis absorption spectra of $mSiO_2$@PB in the NIR region. (B) Comparison of UV-vis absorption spectra of $mSiO_2$@PB aqueous solution (0.2 mg/mL) before and after 4 cycles of irradiation.

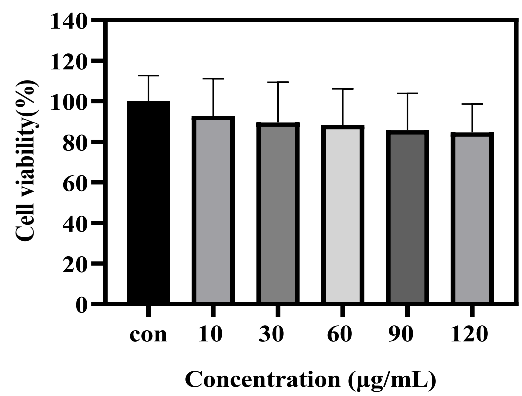

Figure S3 HT29 cell viability after $mSiO_2$@PB (0~120 μg/mL) treatment.

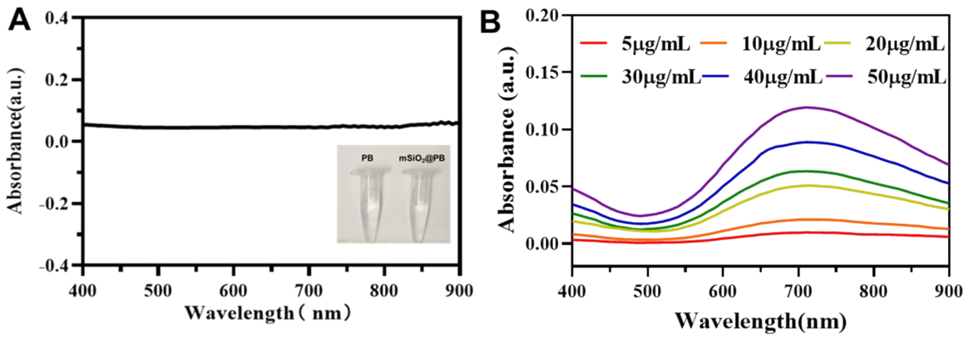

Figure S4 (A) UV-Vis absorption spectrum of $mSiO_2$@PB (5 μg/mL) (Inset: color comparison of beyond 5 μg/mL PB and 5 μg/mL $mSiO_2$@PB), (B) the UV-Vis absorption spectra of $mSiO_2$@PB at different concentrations (5-50μg/mL).

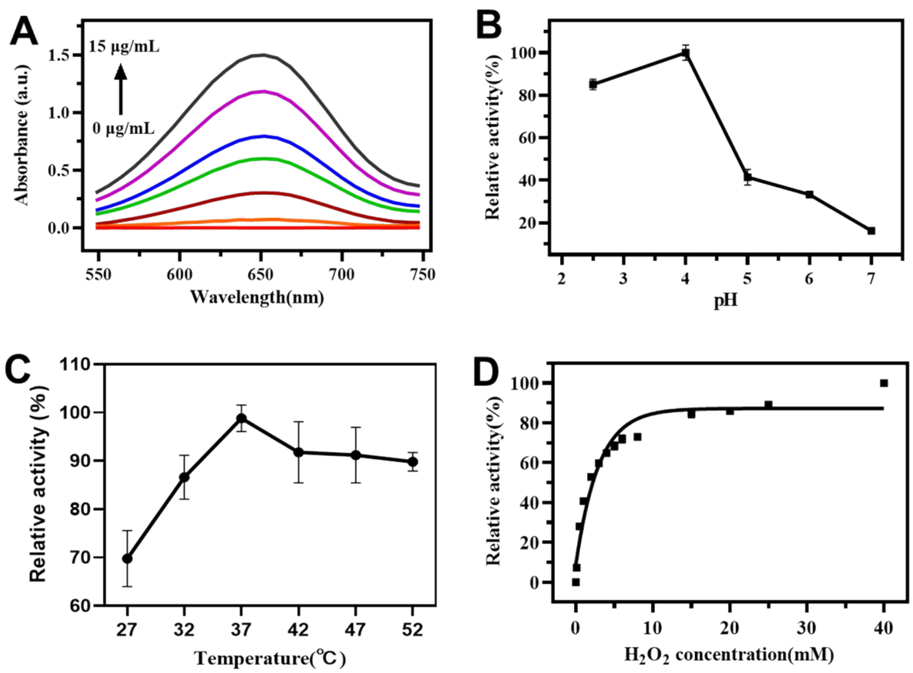

Figure S5 (A) UV-Vis absorption spectra of different concentrations of $mSiO_2$@PB (0-15 μg/mL) in the presence of H_2O_2, TMB, Effect of (B) pH (C) temperature (D) H_2O_2 concentration on the catalytic oxidation of TMB (the maximum point in each curve b, c, d is set to 100%).

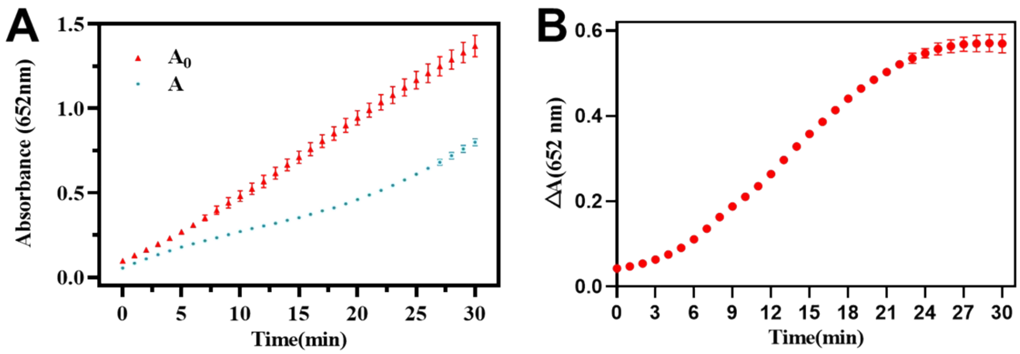

Figure S6 (A) Absorbance of $mSiO_2@PB$ at 652 nm in the presence of GSH (A, 40 μM) and in the absence of GSH (A_0) as a function of time, (B) $\Delta A=A_0-A$ versus time (t) in an "S"-shaped growth curve.

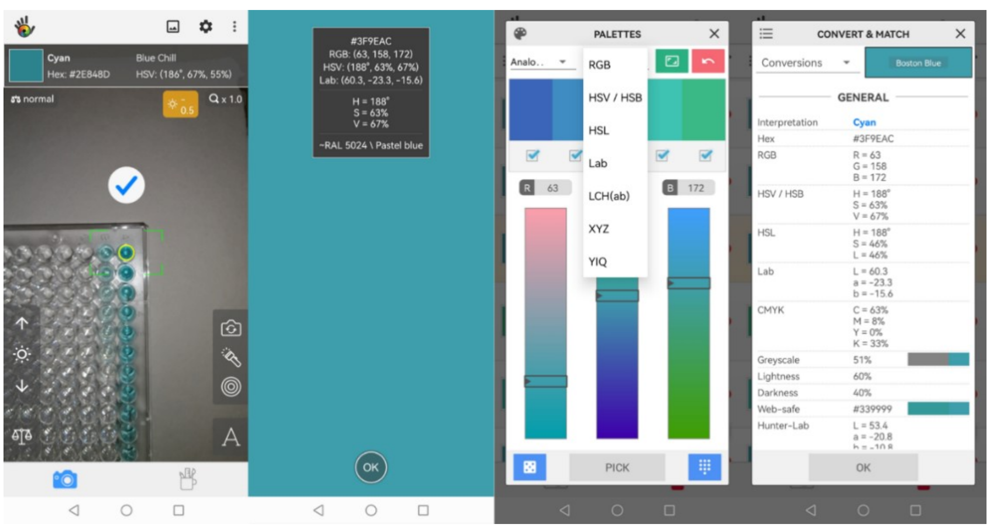

Figure S7 Screenshot of the operation interface of Color Grab application recording color parameter data processing.

Table S1 K_m values of different peroxide simulant

Enzymes	K_m（mM）		Ref.
	TMB	H_2O_2	
HRP	0.434	3.70	1,2
Cu NP/g-C_3N_4	0.389	9.27	3
$CdCo_2O_4$	0.317	0.325	4
D-ILE HNFs	2.41	1.02	5
CMNR	0.758	15.8	6
$mSiO_2$@PB	0.279	1.88	**This Work**

Table S2 Comparison of different methods for the detection of GSH

Materials	Detection method	Linear Range	LOD	Ref.
BODIPY-based probe	Fluorometric	0-200 μM	0.85 μM	7
conjugated polymer–Cu (II)	Fluorometric	0.1-15 μM	0.04 μM	8
CuPd@H-C_3N_4	Fluorometric	0.03-1.5 μM	0.0084 μM	9
PSMOF	Colorimetry	1-20 μM	0.68 μM	10
MnO_2 nanosheets	Colorimetry	1-25μM	0.3 μM	11
Co-Fe/MOF	Colorimetry	10-100 μM	5 μM	12
Gold nanoclusters	Colorimetry	2-25 μM	0.42 μM	13
UiO-66(NH_2)	Colorimetry	5-120 μM	0.31 μM	14
PtNPs/COF-300-AR	Colorimetry	0.4-4.0 μM	0.4 μM	15
$mSiO_2$@PB	Colorimetry	0.5-30 μM	0.15 μM	**This work**

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Abstract

Nanozymes demonstrate significant potential for various applications in multiple fields due to their mimetic activity, stability, ease of storage, and cost-effectiveness compared to natural enzymes. Prussian blue nanoparticles (PB NPs) exhibit exceptional catalytic activity and have a favorable preparation process, making them valuable substitutes for peroxide-mimetic enzymes. However, their utilization in colorimetric sensing is limited due to the inadequate stability of individual PB nanoparticles, tendency to aggregate in aqueous solutions, demanding purification conditions, and inherent color interference. In this study, we prepared and characterized a Prussian blue nanocomposite, $mSiO_2$@PB, which possesses a well-dispersed aqueous solution and eliminates the interference caused by its own color. Additionally, the $mSiO_2$@PB nanocomposite displayed excellent peroxidase-like activity and facilitated the color development reaction of 3,3',5,5'-tetramethylbenzidine (TMB) in the presence of H_2O_2. By leveraging the inhibitory effect of glutathione (GSH) on the oxidation of TMB, we constructed a sensitive biosensing platform for the colorimetric detection of GSH in physiological fluids. Furthermore, this study developed a portable analysis method for GSH detection using smartphones as detection terminals, capitalizing on their photo and colorimetric analysis capabilities. In conclusion, our work establishes an effective platform for the detection of GSH in physiological body fluids, thereby expanding its potential applications in biomedical analysis and monitoring.

Keywords: Prussian blue; Nanozymes; Peroxidase mimetic; Colorimetric sensing; Glutathione detection