Supporting Information

Sensitive Detection of Golgi Protein 73 by Magnetic Separation Combined with Dual Nanozyme MOF-818 and Magnetic Nucleus @ Bifunctional Shell (Fe_3O_4@PB-Au) Cascade Reaction Catalytic Amplification Strategy

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Experimental

Instruments and measurements.

UV-1750 ultraviolet-visible spectrophotometer (Shimadzu, Japan); F-4700 fluorescence spectrophotometer (Hitachi Scientific Instruments (Beijing) Co., Ltd.).

Determination of catechol oxidase activity of MOF-818 nanozyme

3,5-DTBC were selected to examine catechol oxidase activity by colorimetric assays, which were mixed with MOF-818 in 100uL of PBS (10 mM pH=8.0). The final concentrations of 3,5-DTBC and MOF-818 were 1 mM and 50 μg mL^{-1}, respectively. The absorption at λ_{max} = 415 nm (ε = 1900 $M^{-1}cm^{-1}$) characteristic of the formed o-quinone was measured over time.

Determination of peroxidase-like activity of Fe_3O_4@PB-Au nanozyme

The 0.5 mg mL^{-1} Fe_3O_4@PB-Au was added to a sodium acetate buffer solution (0.1 M pH=4.0) containing 2.5 mM TMB and 2.5 mM H_2O_2. The absorption at 652 nm was measured.

Kinetics of nanozyme composite nanozyme

The steady-state kinetic studies were accomplished by keeping the concentration of one substrate constant and increasing the concentration of only one other zymolyte. The kinetic studies of the oxidation of 3,5-DTBC were carried out by monitoring the concentration of 3,5-DTBQ at 415 nm by varying the concentration of 3,5-DTBC (range of 3-20 mM) while keeping the MOF-818 concentration constant. The reaction rate was found to follow Michaelis−Menten kinetics.

To study the effect of TMB (range of 0.5–15 mM) on the catalytic performance of using 50 μL of (2 mg/mL) Fe_3O_4@PB-Au and 50 μL of (5 mM) H_2O_2 in 2 mL of NaAc-HAc (0.1 M，pH=4.0) buffer solution. Measure the absorbance value of the system at 652 nm in the first 1 min after the start of the reaction is calculated. Similarly, to investigate the effect of H_2O_2 on the catalytic performance of Fe_3O_4@PB-Au, 50 μL of (2 mg/mL) Fe_3O_4@PB-Au and 50 μL of (5 mM) TMB in 2 mL of (0.1 M) NaAc-HAc (pH=4.0) buffer solution. Different concentrations of H_2O_2 (range of 2-20 mM) solution were added, and the catalytic performance was determined by measuring the change in absorbance at 652 nm for 1 min. According to the Lambert-Beer theorem, the Michaelis constant can be calculated from the Lineweaver–Burk double reciprocal curve, as outlined below:

$$\frac{1}{V_0} = \frac{K_m}{V_{max}} \times \left(\frac{1}{[S]} + \frac{1}{K_m}\right)$$

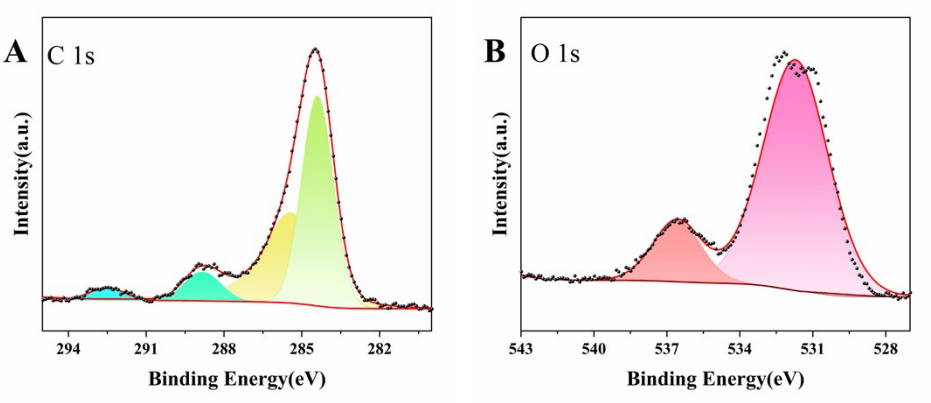

Fig. S1 High-solution of XPS spectra of (A) C 1 s in MOF-818. High-solution of XPS spectra of (B) O 1 s in Fe_3O_4@PB-Au.

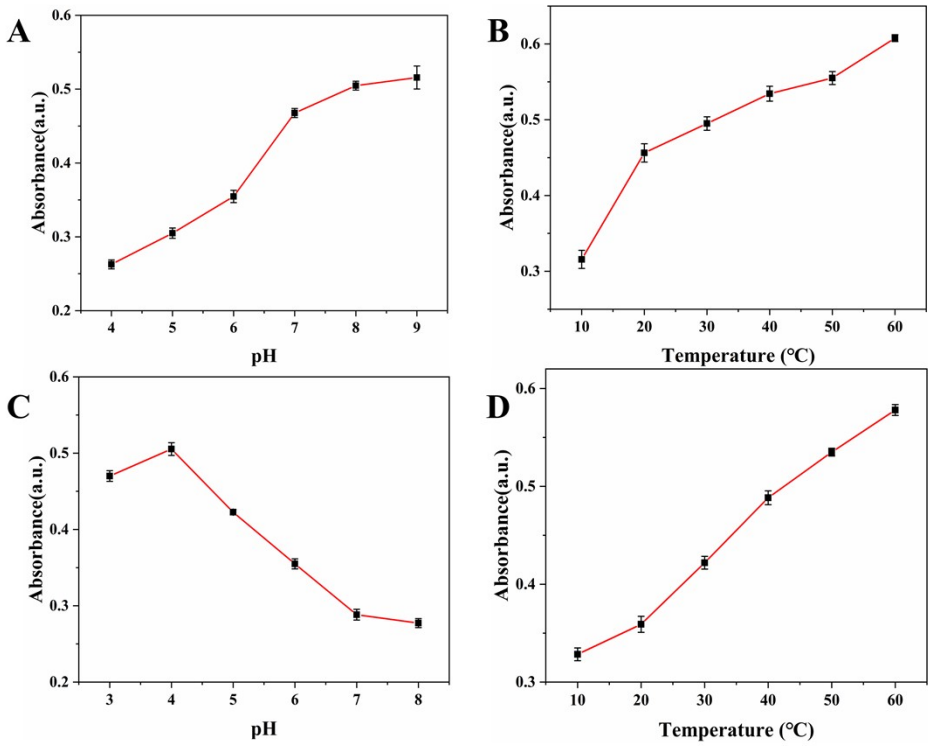

Fig. S2 Catechol oxidase-like activities of MOF-818 at different pH values (A) and temperatures (B). Peroxidase activity of Fe_3O_4@PB-Au at different pH values (C) and temperatures (D).

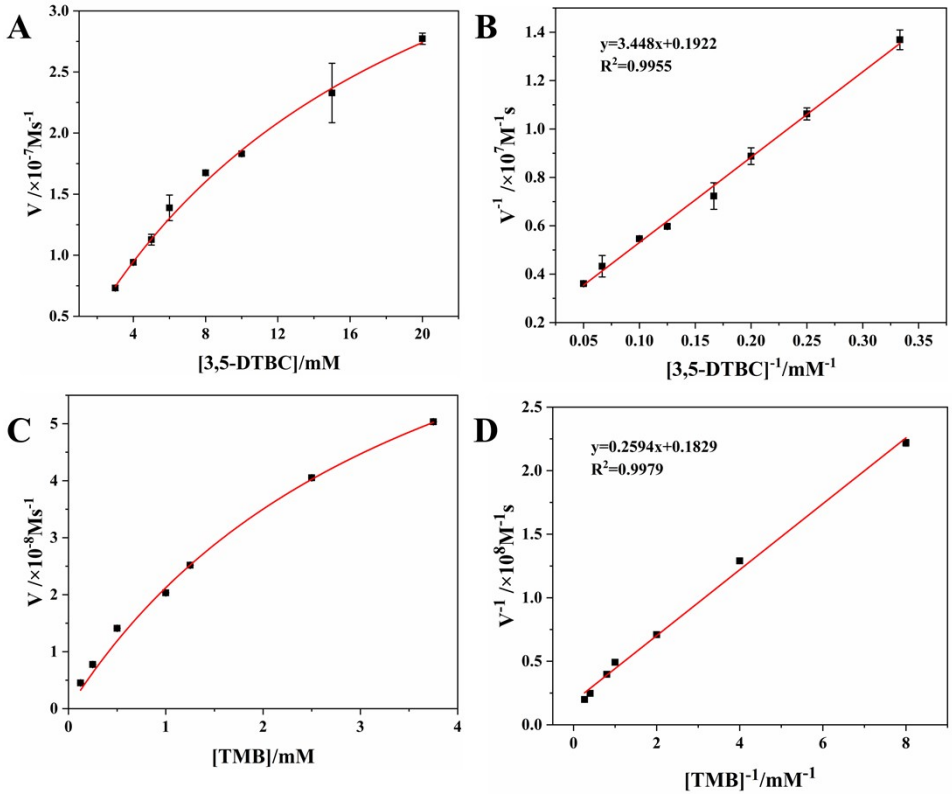

Fig. S3 (A) Michaelis−Menten curves for 3,5-DTBC. (B) Lineweaver−Burk plot for determination of kinetic constant of MOF-818 for 3,5-DTBC. (C) Michaelis−Menten curves for TMB. (D) Lineweaver−Burk plot for determination of kinetic constant of Fe_3O_4@PB-Au for TMB.

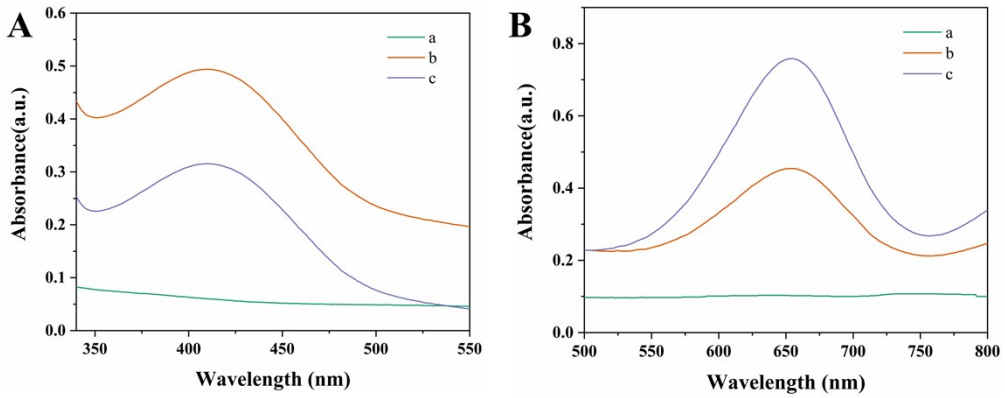

Fig. S4 (A) UV-Vis absorption spectra of MOF-818 bound to Apt1 (a: 3,5-DTBC, b: MOF-818, c: Apt1+MOF-818) (B) UV-Vis absorption spectra of Fe_3O_4@PB-Au bound to Apt2 (a: TMB, b: Fe_3O_4@PB-Au, c: Apt2+ Fe_3O_4@PB-Au)

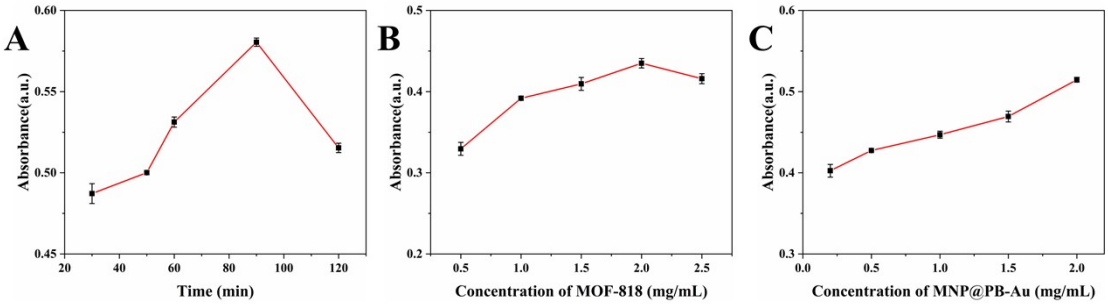

Fig. S5 Optimization of (A)Time (B)MOF-818, and (C) Fe_3O_4@PB-Au on the analytical performance of a dual nanozymatic colorimetric aptamer sensor

Table S1 Comparison of the peroxidase-like kinetic parameters of the Fe3O4@PB-Au nanozyme with other nanozymes

Catalyst	**K_m/mM**	**$V_{max}/10^{-8}$ Ms^{-1}**	**Ref.**
HRP	3.7	8.71	[1]
Fe_3O_4 NPs	154	7.98	[2]
Fe_3O_4@Cu	7.71	0.443	[3]
Fe_3O_4@SiO_2@Au	2.05	60.88	[4]
MoS_2/Fe_3O_4	2.50	3.30	[5]
MoS_2-CPBNPs	3.17	1.49	[6]
Fe_3O_4@PB-Au	1.06	2.57	This work

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