Dopamine–induced Au hydrogel nanozyme for enhanced biomimetic catalysis

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Experimental Section

Materials

$HAuCl_4$, $NaBH_4$ K_2HPO_4, KH_2PO_4, H_2O_2, HAc, NaAc, methyl red and NaCl were purchased from Sinopharm Chemical Reagent Co., Let (Shanghai, China). TMB, glucose, and HRP were obtained from Shanghai Aladdin Bio-Chem Technology Co., Ltd. Dopamine was purchased from Sigma-Aldrich.

Instruments

SEM image was obtained by a Quanta FEG250 field-emission environmental SEM (FEI, United States). TEM images were from Titan G260-300 (Thermo Fisher, United States). XPS measurements were performed by VG Multilab 2000 (Thermo Fisher, United States). XRD characterization was carried out by a D8 ADVANCE (Bruker, Germany). Fourier transform infrared spectroscopy was characterized by a TENSOR27 (Bruker, United States). All enzyme kinetics data and UV-vis spectra were performed by a multimode reader (Tecan Spark, Switzerland).

Preparation of Au hydrogel, Au-PDA, and Au hydrogel-HCl

Au hydrogel was prepared as follows: 400 μL of $HAuCl_4$ (100 mM) was dissolved in 30 mL ultrapure water containing 2.5 mg of dopamine and continuously stirred for 30 seconds after rapid injection of 2 mL of 50 mM $NaBH_4$ at 60 ℃. The obtained solution was changed from faint yellow to black. Finally, the black solution was kept motionless at 60 ℃ for 2 hours until the formation of the hydrogel. Au-PDA was prepared by the direct reduction of $HAuCl_4$ by dopamine. In detail, 400 μL of $HAuCl_4$ (100 mM) was dissolved in 30 mL of Tris-HCl buffer (pH=8.5). Next, 1 mL of dopamine (2.5 mg/mL) was rapidly added to the above solution and kept stirring for 1 hour. The obtained Au-PDA was washed/centrifuged at 13000 rpm for 10 minutes. Au hydrogel-HCl was prepared by using Au hydrogel as a precursor, following an HCl-treated procedure (1 M HCl, 48 hours) to remove the PDA on the surface of Au hydrogel.

Characterization of GOx-like activity

50 μL of Au hydrogel, Au-PDA, and Au hydrogel-HCl (2 mg/mL), 500 μL of glucose (10 mM) and 500 μL of PBS (0.01 M, pH 9.0) were mixed and incubated for 30 minutes at 37 ℃. Next, the mixture was centrifuged (13000 rpm, 3 minutes) to obtain the supernatant. The generated H_2O_2 in the supernatant (100 μL) was verified by the HRP (5 μg/mL, 10 μL)-TMB (1 mM, 100 μL) based chromogenic reaction in HAc-NaAc buffer (0.1 M, 100 μL, pH 4.0). The absorbance spectra were recorded by a multimode reader (Tecan Spark). Gluconic acid as another product was verified by methyl red induced colorimetric assay. In detail, methyl red solution (5 mM, 100 μL) was added into the supernatant for 10 minutes. The absorbance spectra of mixed solution were measured by a multimode reader (Tecan Spark).

Characterization of peroxidase-like activity

10 μL of Au hydrogel, Au-PDA, and Au hydrogel-HCl (2 mg/mL) were added into the mixture containing 100 μL of TMB (10 mM), 100 μL of H_2O_2 (1 M) and 100 μL of HAc-NaAc buffer (0.1 M, pH 4.0) and incubated for 5 minutes. The absorbance spectra were recorded by a multimode reader (Tecan Spark).

Kinetic analysis of GOx-like and peroxidase-like activity

Kinetic measurements of GOx-like property are confirmed by the color changes, which is indicated by the absorbance at 652 nm recorded by a multimode reader (Tecan Spark, Switzerland). In detail, 50 μL of Au hydrogel, Au-PDA, and Au hydrogel-HCl (2 mg/mL) were added into the mixture including 500 μL of different concentration of glucose and 500 μL of PBS (pH 9.0, 0.01 M) for 30 minutes. The supernatant (100 μL) obtained from above mixture were added into another solution containing 100 μL of HAc-NaAc (0.1 M, pH 4.0), 100 μL TMB (10 mM), and 10 μL of HRP (10 μg/mL). The final mixtures were detected rapidly time-scan mode at 652 nm. The kinetic data were calculated by a typical Michaelis–Menten curve as to $v= V_{max}[S]/(K_m+[S])$, where, v is the initial velocity, [S] is the

concentration of the substrate, K_m is the Michaelis−Menten constant, and V_{max} is the maximal reaction velocity.

Kinetic measurements of peroxidase-like property are confirmed by the color change, which is indicated by the absorbance at 652 nm recorded by a multimode reader (Tecan Spark, Switzerland). The kinetic data were detected by the changing concentration of TMB and H_2O_2. As for the kinetic data towards TMB, 10 μL of Au hydrogel, Au-PDA, and Au hydrogel-HCl (2 mg/mL) were added into the mixture including 100 μL of H_2O_2 (100 mM), 100 μL of HAc-NaAc (0.1 M, pH 4.0) and different concentration of TMB. Similarly, as for the kinetic data towards H_2O_2, 10 μL of Au hydrogel, Au-PDA, and Au hydrogel-HCl (2 mg/mL) were added into the mixture including 100 μL of TMB (1 mM), 100 μL of HAc-NaAc (0.1 M, pH 4.0) and different concentrations of H_2O_2. The kinetic data were calculated by a typical Michaelis–Menten curve as to $v= V_{max}[S]/(K_m+[S])$, where, v is the initial velocity, [S] is the concentration of the substrate, K_m is the Michaelis−Menten constant, and V_{max} is the maximal reaction velocity.

Characterization of biomimetic cascade catalysis

50 μL of Au hydrogel, Au-PDA, and Au hydrogel-HCl (2 mg/mL), 500 μL of glucose (100 mM) and 500 μL of PBS (0.01 M, pH 7.0) were incubated at 37 ℃ for 3 hours. After that, 100 μL of TMB (10 mM) was added to the above solution. Finally, the absorbance spectra were recorded by a multimode reader (Tecan Spark).

Colorimetric biosensing of glucose

50 μL of Au hydrogel (2 mg/mL) was mixed with various concentrations of glucose and then incubated for incubation 3 hours. Next, 100 μL of TMB (10 mM) was added to the above solution. Finally, the Au hydrogel was removed by centrifugation. The obtained supernatants were recorded by a multimode reader (Tecan Spark).

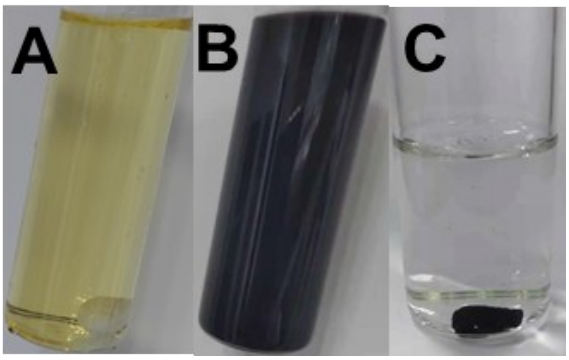

Figure S1. Digital pictures of Au hydrogel formation at different stages: (A) $HAuCl_4$ solution, (B) initial step after the addition of reducing agent, and (C) formation of Au hydrogel.

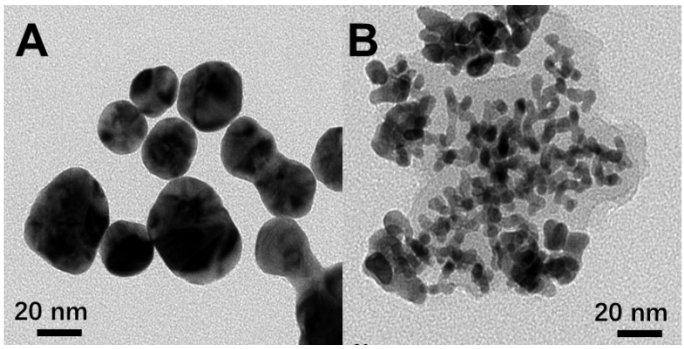

Figure S2. TEM images of (A) Au NPs and (B) Au-PDA.

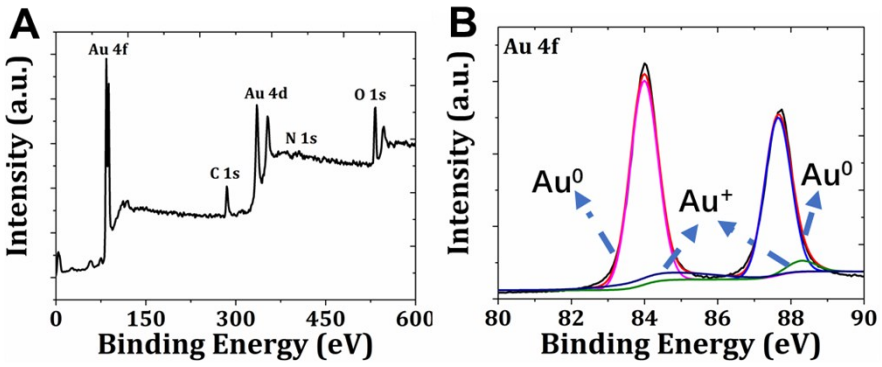

Figure S3. Full scan (A) and high resolution (B) XPS spectra of N 1s of Au hydrogel.

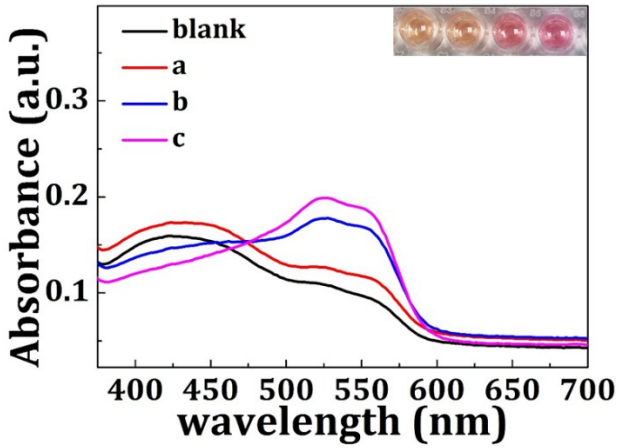

Figure S4. The UV-vis spectra of methyl red in Au nanozyme (a, Au-PDA; b, Au hydrogel-HCl; c, Au hydrogel)-glucose systems.

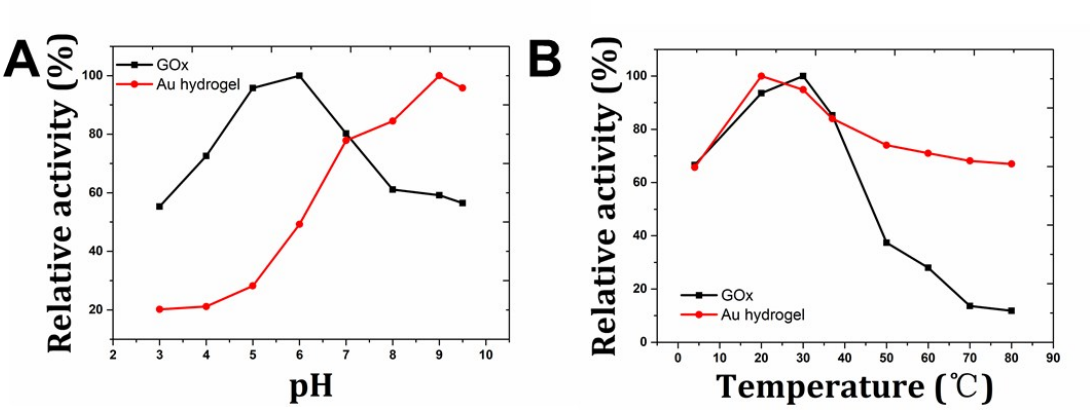

Figure S5. The effect of pH and incubation temperature on GOx-like activity of Au hydrogel and GOx.

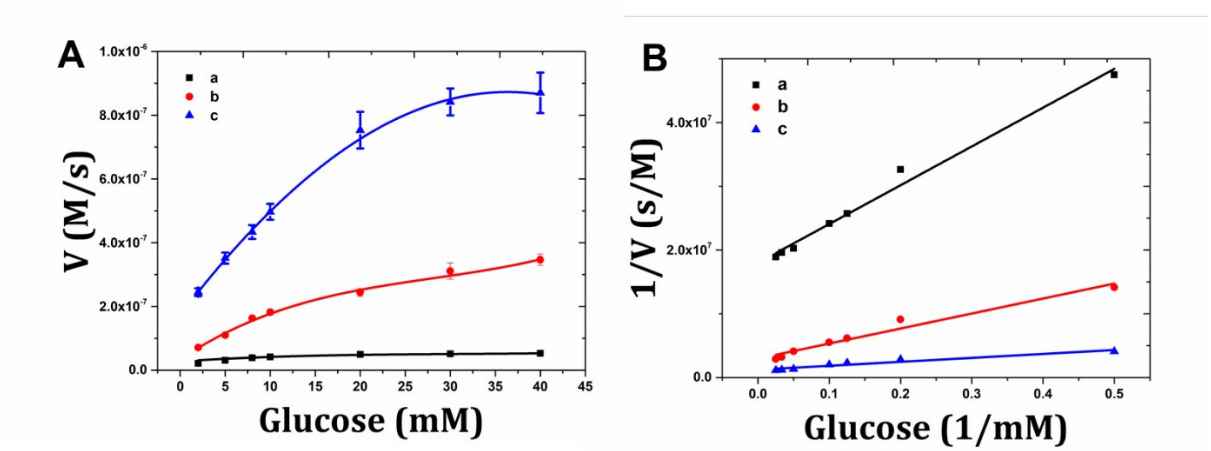

Figure S6. (A) GOx-like kinetic data of Au-PDA (a), Au hydrogel-HCl (b), and Au hydrogel (c). (B) The Lineweaver–Burk curves (1/V versus 1/[S]) of Au-PDA (a), Au hydrogel-HCl (b), and Au hydrogel (c).

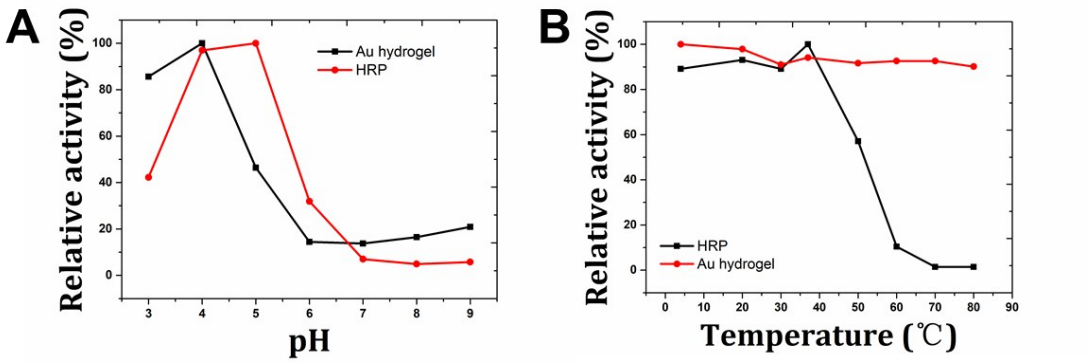

Figure S7. The effect of pH and incubation temperature on peroxidase-like activity of Au hydrogel.

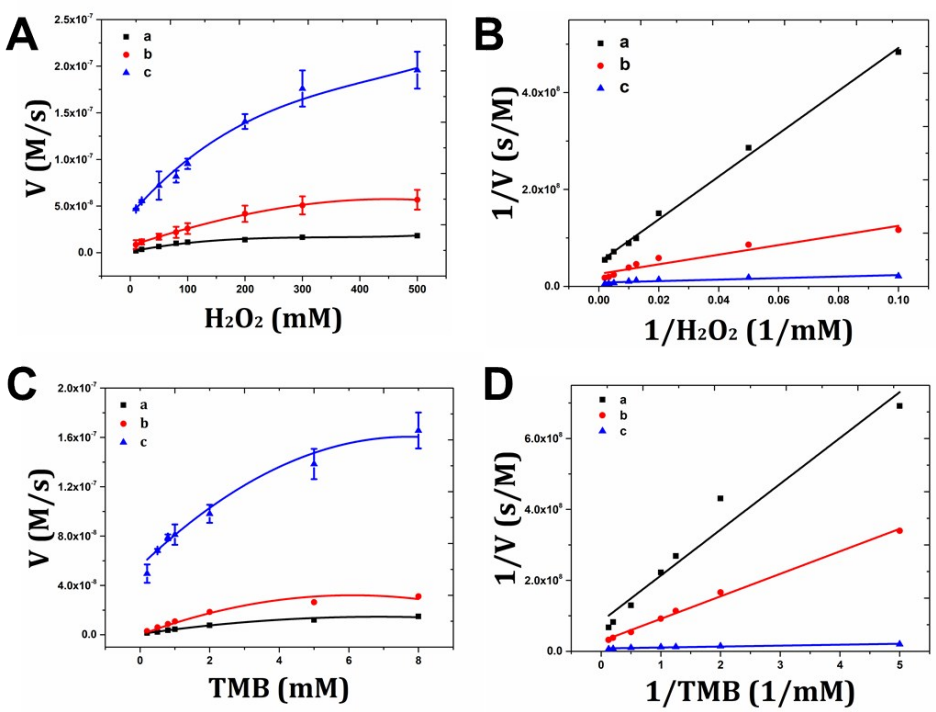

Figure S8. Peroxidase-like kinetics data of Au-PDA (a), Au hydrogel-HCl (b), and Au hydrogel (b) towards H_2O_2 (A) and TMB (C). (B) The Lineweaver–Burk curves (1/V versus 1/[S]) of Au-PDA (a), Au hydrogel-HCl(b), and Au hydrogel (c) towards H_2O_2 (B) and TMB (D).

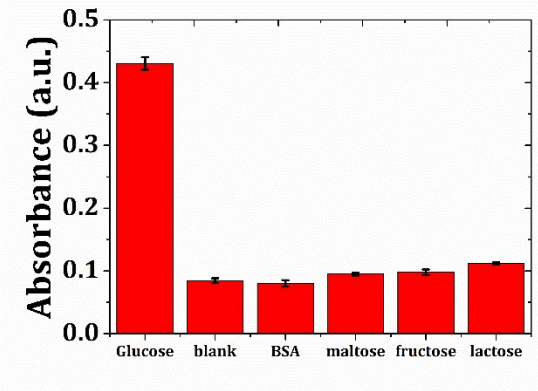

Figure S9. The selectivity of glucose biosensing by Au hydrogel-induced cascade reactions.

Table S1. The GOx-like kinetics data of Au-PDA, Au hydrogel-HCl and Au hydrogel.

Sample	K_m (mM)	V_{max} (10^{-8} M/s)
Au-PDA	3.39	5.56
Au hydrogel-HCl	7.91	33.6
Au hydrogel	4.98	82.1

Table S2. Peroxidase-like kinetics data of Au-PDA, Au hydrogel-HCl, Au hydrogel.

Sample	K_m H_2O_2 (mM)	V_{max} H_2O_2 (10^{-8} M/s)	K_m TMB (mM)	V_{max} TMB (10^{-8} M/s)
Au-PDA	89.52	2.01	1.53	1.18
Au hydrogel-HCl	38.67	3.98	2.25	3.56
Au hydrogel	19.92	12.8	0.32	12.30