Electronic Supplementary Information

In-site Encapsulating Gold "Nanowires" into Hemin-Coupled Protein Scaffolds through Biomimetic Assembly towards the Nanocomposites with Strong Catalysis, Electrocatalysis, and Fluorescence Properties

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Experimental Section

Materials and instruments

Hemin (Hem) with carboxyl groups (Hem-COOH, purity>90%),serum albumin (BSA), N-Hydroxysuccinimide (NHS), 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), phosphate buffer solution (PBS, pH 7.4), and chloroauricacid tetrahydrate ($HAuCl_4 \cdot 4H_2O$) were purchased from Aladdin Reagent Co., Ltd. (Shanghai, China). Hydrogen peroxide (H_2O_2), chitosan, dithiothreitol (DTT), and urea were obtained from Beijing Dingguo Biotechnology Co., Ltd (Beijing, China). Hem solution (i.e. 1.0 mg/mL) was prepared by dissolving the Hem powder in 0.10 M NaOH. All other reagents were of analytical grade. Ultra-purified water was used throughout the experiments.

Transmission electron microscopy (TEM, Tecnai G20, USA) imaging was operated at 100 kV to characterize the nanocomposites. UV-vis absorption spectra were recorded using UV-3600 spectrophotometer (Shimadzu, Japan) equipped with a thermostatted holder. Colorimetric measurements were performed using Infinite M200 PRO (TECAN, Switzerland). Fluorescence measurements were conducted using fluorescence spectrophotometer (F-7000, Hitachi, Japan) operated with both excitation and emission slit widths of 5.0 nm. Moreover, the electrochemical measurements were carried out using the electrochemical work station CHI830 (CH Instruments). Three-electrode system was applied consisting of glassy carbon (GC) working electrode, a Pt wire counter electrode, and an Ag/AgCl reference electrode.

Synthesis of dBSA-Hem scaffolds

The disassembled BSA (dBSA) was prepared according to a reported method with some modifications.[1]Typically, intact BSA solution (10 mL, 8.0 mg/mL) was introduced to the mixture containing 6.0 M urea and 2.0 mM EDTA to be stirred at room temperature for 20 min. Then, DTT solution (0.2 mL, 0.10 mM) was added, and the mixture was further stirred for additional 30 min under nitrogen atmosphere. Following that the as-prepared dBSA solution was purified by dialysis over night.

The catalytic Hem with carboxyl groups (Hem-COOH) was coupled onto the above dBSA with amine groups by the cross-linking chemistry. Typically, dBSA solution (10 mL, 4.0 mg/mL) was added into the Hem solution (1.0 mL, 2.0 mg/mL) containing 100 mM EDC, and 80 mM NHS to be vigorously stirred for 2 h at 37 °C. Furthermore, the resulting solution of dBSA-Hem scaffolds was purified by dialysis over night for cold standby.

Synthesis of dBSA-Hem-AuNCs

The nanocomposites of dBSA-Hem-AuNCs were prepared by following the procedure as schematically shown in **Scheme 1**. In a typical experiment, aqueous $HAuCl_4$ solution (1.0 mL, 10 mM) was added into the solution of dBSA-Hem scaffolds (10 mL, containing 2.0 mg/mL dBSA and 0.87 mg/mL Hem) to be stirred for 10 min. NaOH solution (0.50 mL, 1.0 M) was then introduced into the mixture to be incubated at 37 °C for 8 h. Subsequently,

the resulting dBSA-Hem-AuNCs were dialyzed overnight, and then re-dispersed in PBS to be stored at 0 °C. Accordingly, the synthesis of Hem loaded AuNCs (Hem-AuNCs) were performed by following the same procedure using 5.0 mg/mL Hem and 10 mM $HAuCl_4$ solution under the condition of alkaline at 37 °C for 8 h.

Colorimetric measurements

The chromogenic substrates consisting of TMB and H_2O_2 with different concentrations were used for the comparison of the peroxidase-like catalysis activities among dBSA-Hem-AuNCs, Hem, and Hem-AuNCs, each with Hem of 2.0μg/mL. All of the reaction products were monitored at 652 nm. The experimental conditions of TMB-H_2O_2 reactions catalyzed by dBSA-Hem-AuNCs were optimized under different pH values and temperatures. Moreover, steady state kinetic assays were comparably carried out for dBSA-Hem-AuNCs, Hem, and Hem-AuNCs (2.0 μg/mL Hem for each), where 4.5 mM H_2O_2 or 0.69 mM TMB was used alternatively at a fixed concentration of one substrate versus varying concentration of the second substrate. The Lineweaver–Burk plots by the double reciprocal of the Michaelis–Menten equation were thus performed to calculate the Michaelis constants.

Electrochemical measurements

An aliquot of dBSA-Hem-AuNCs was mixed with 2.0 mg/mL chitosan that was dissolved in 1.0% acetic acid. 5.0 μL of the above mixture was casted onto the surface of the pretreated GC electrodesand then dried at room temperature overnight to form the dBSA-Hem-AuNCs electrodes. Electrochemical measurements of cyclic voltammetry (CV) and / or linear sweep voltammetry (LSV) were used with potential range from – 0.7 V to 0.5 V at scanning rate of 0.05 V/s. A baseline correction of the resulting voltammograms was performed with the CHI software. The sensing stability of the electrodes under operative conditions was assessed by performing 100 consecutive CV cycles in PBS (pH 7.4) at a scan rate of 50 mV/s. Moreover, the electrocatalysis properties of the dBSA-Hem-AuNCs electrodes towards H_2O_2 were evaluated, with the reduction current responses recorded for different concentrations of H_2O_2 ranging from 2.0 μM to 2.2 mM,

in comparison to these electrodes modified separately with Hem and Hem-AuNCs under the same detection conditions.

Fluorimetric measurements

An aliquot of fluorimetric reagent of dBSA-Hem-AuNCs withthe certain amountswas separately mixed with 10 µL H_2O_2 samples (50 nM, 0.25 µM, 1.0 µM, 5.0 µM, 25 µM, and 100 µM H_2O_2) to be incubated for 10 min. Subsequently, the fluorescence spectra of the reaction mixtures were recorded depending on the quenching degrees of fluorescence induced by H_2O_2.

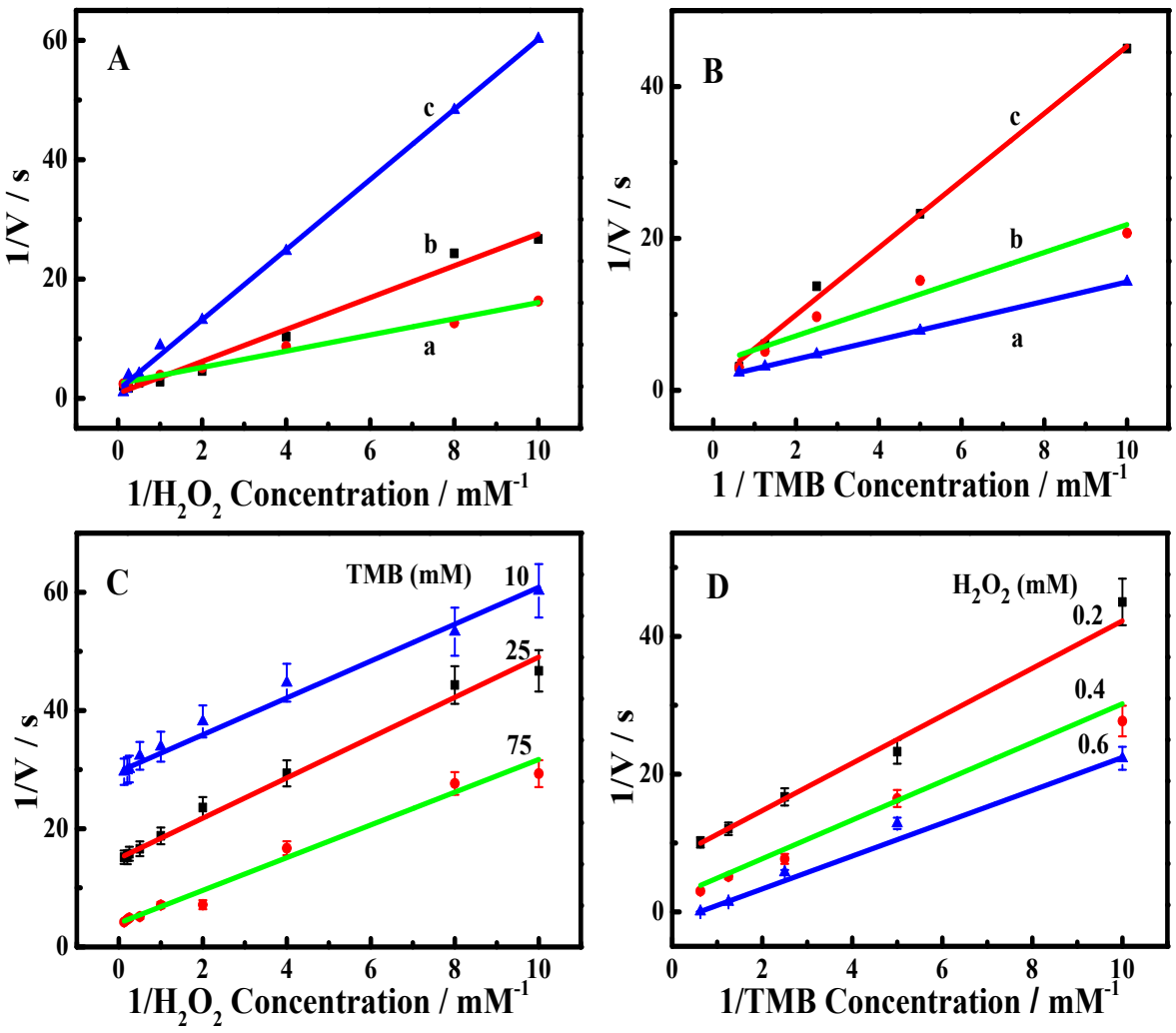

Fig. S1 Double-reciprocal plots of the comparison of catalysis activities among (a) dBSA-Hemin-AuNCs, (b) Hemin-AuNCs, and (c) Hem by using (A) various H_2O_2 concentrations at fixed 0.69 mM TMB, and (B) various TMB concentrations at fixed 4.5 mM H_2O_2; and double-reciprocal plots for kinetic catalysis of dBSA-Hemin-AuNCs using (C) various H_2O_2 concentrations at three fixed TMB concentrations, and (D) various TMB concentrations at three fixed H_2O_2 concentrations. The y-axis values are calculated from the observed colorimetric absorbance values.

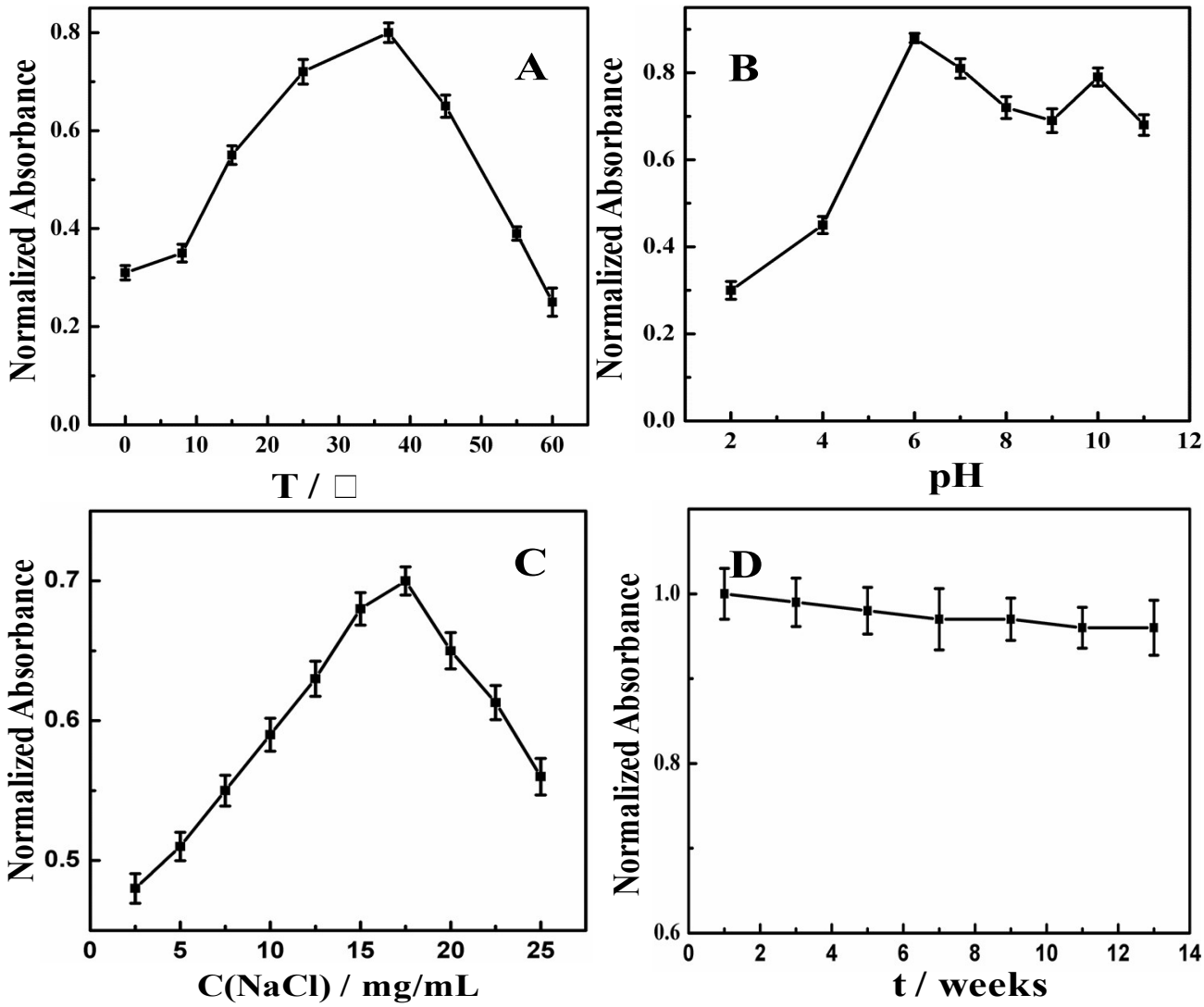

Fig. S2 The colorimetric catalysis performances of dBSA-Hemin-AuNCs nanocomposites depending on (A) different temperatures (2, 8, 15, 25, 37, and 50 °C), (B) different pH values (2, 4, 6, 8, 9, 10, and 11), (C) ion strengths in NaCl concentrations, and (D) the storage time, where the catalytic TMB-H_2O_2 reactions were performed.

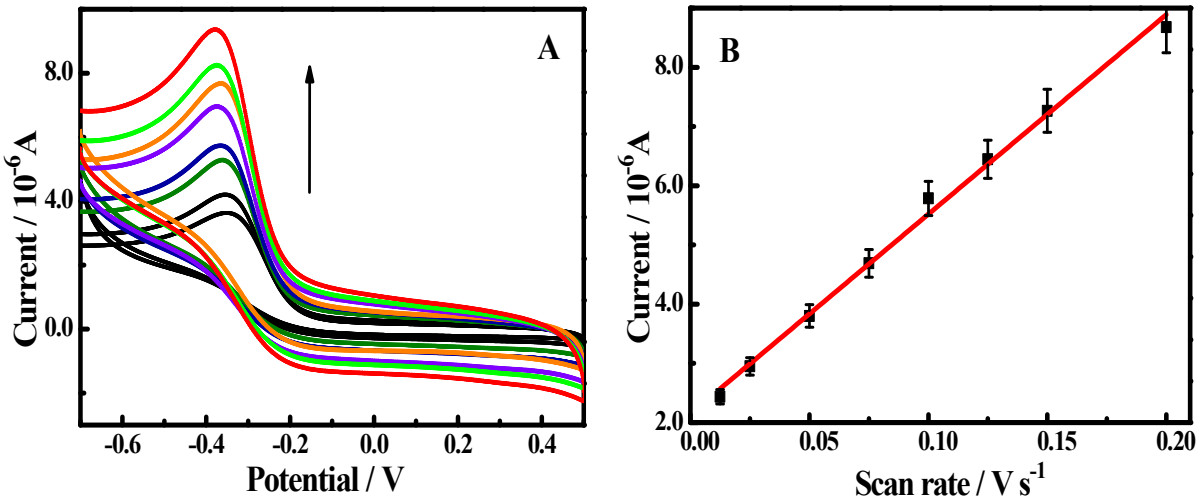

Fig. S3 Investigation of electrochemical kinetics for the electrodes modified with dBSA-Hemin-AuNCs by (A) cyclic voltammograms at different scanning speeds (12.5, 25, 50, 75, 100, 125, 150, and 200 mV/s) with the scanning range from - 0.70 V to 0.50 V, and (B) the relationship between the peak currents versus the scanning rates.

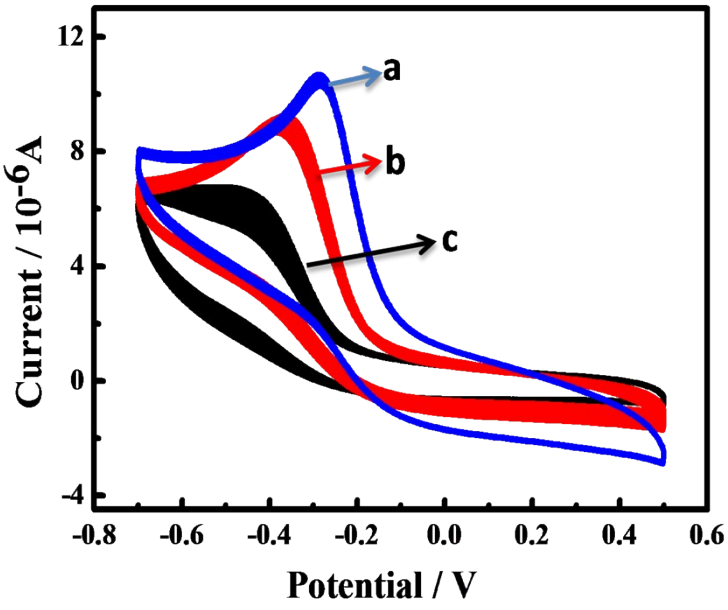

Fig. S4 Comparison of electrochemical sensing stabilities among the electrodes modified separately with (a) dBSA-Hem-AuNCs, (b) Hem-AuNCs, and (c) Hem (each containing 2.0 μg/mL Hem) with cyclic voltammograms of continuous scanning for 100 cycles in PBS.

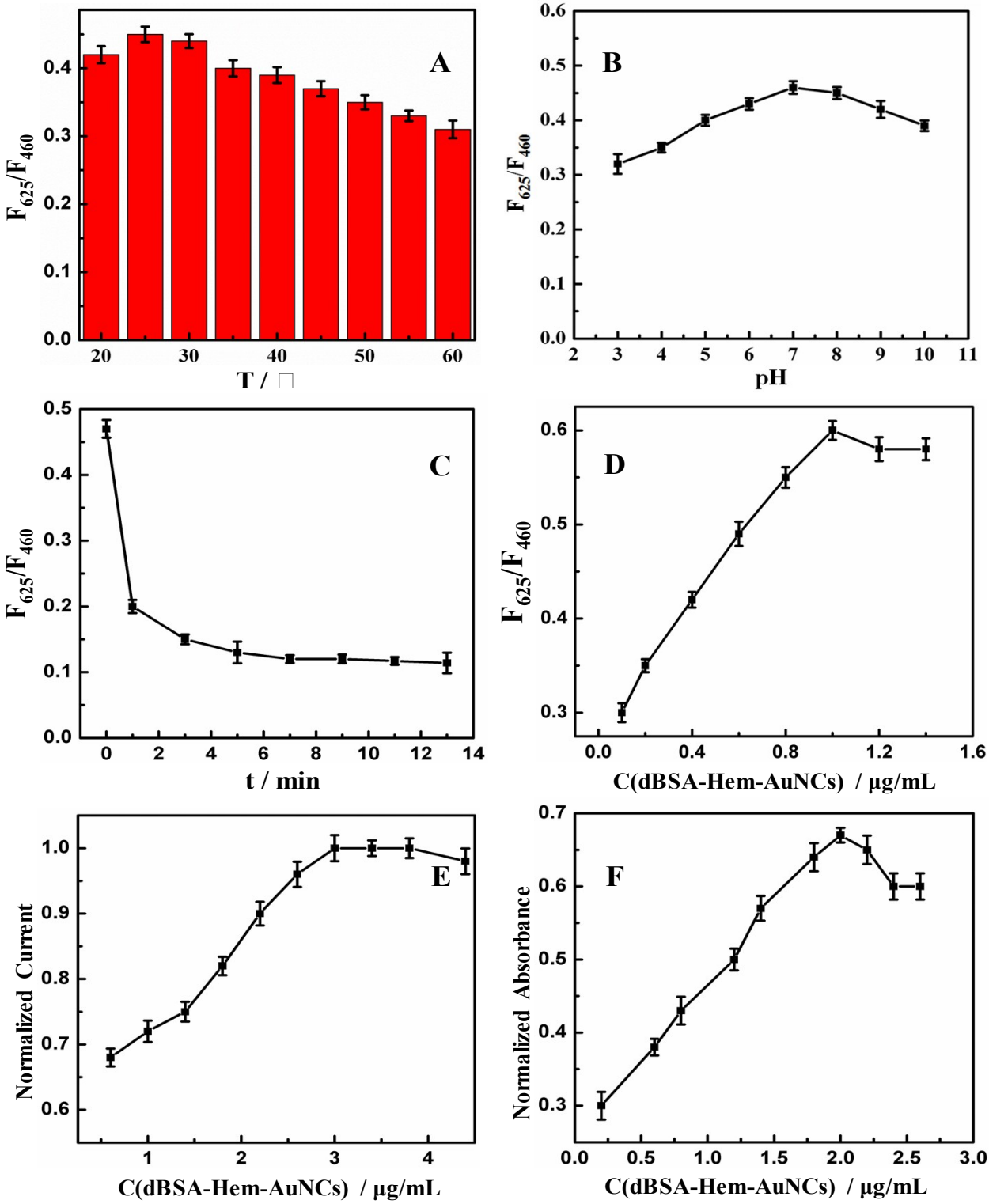
A
F_{625}/F_{460}
T / □
B
pH
C
t / min
D
C(dBSA-Hem-AuNCs) / μg/mL
E
Normalized Current
C(dBSA-Hem-AuNCs) / μg/mL
F
Normalized Absorbance
C(dBSA-Hem-AuNCs) / μg/mL

Fig. S5 The fluorescent responses of relative F_{625}/F_{460} ratios of dBSA-Hem-AuNCs to H_2O_2 (1.0 mM) depending on (A) reaction temperature (from 20 to 60 ^oC), (B) pH values (from 3 to 10), (C) the response time, and (D) dBSA-Hem-AuNCs concentrations. The dBSA-Hem-AuNCs–dependent H_2O_2 responses for (E) colorimetric and (F) electroanalytic measurements

Table S1 Comparison of dynamic catalysis parameters among dBSA-Hem-AuNCs, Hem-AuNCs, Hem, and documented HRP using TMB and H_2O_2 as the substrates.

Mimetic Enzyme	Substrate	K_m(mM)	$V_{max}\times10^{-8}$ ($M\cdot s^{-1}$)
Hem	TMB	5.055	0.917
Hem-AuNCs	TMB	4.800	0.634
dBSA-Hem-AuNCs	TMB	0.518	2.460
HRP[2]	TMB	0.434	10.00
Hem	H_2O_2	2.514	0.708
Hem-AuNCs	H_2O_2	2.445	0.954
dBSA-Hem-AuNCs	H_2O_2	0.842	1.402
HRP[2]	H_2O_2	4.700	8.710

1. Y. Wu, S. Chakrabortty, R. A. Gropeanu, J. Wilhelmi, Y. Xu, K. S. Er, S. L. Kuan, K. Koynov, Y. Chan and T. Weil, J. Am. Chem. Soc. 2010, 132, 5012-5014.
2. Gao, L.; Zhuang, J.; Nie, L.; Zhang, J.; Zhang, Y.; Gu, N.; Wang, T.; Feng, J.; Yang, D.; Perrett, S.; Yan, X. Nat. Nanotechnol. 2007, 2, 577-584.