Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Single Gold Nanoparticle-driven Heme Cofactor Nanozyme as Unprecedented Oxidase Mimetic

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Experimental section

Materials. Sodium borohydride ($NaBH_4$), NaOH, Hydrogen tetrachloroaurate tetrahydrate ($HAuCl_4$ $3H_2O$, 99%), sodium acetate, and acetic acid were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Hemin, 3,3′,5,5′-tetramethylbenzidine (TMB), 2,2′-azinobis (3ethylbenzthiazoline-6-sulfonic acid) diammonium salt (ABTS) were purchased from Sigma-Aldrich, Hydroethidine (DHE), FCLA Free Acid were from J&K Scientific. The ultrapure water was prepared with Milli-Q system (18.2 MΩ cm).

Instrumentation and Characterization. The transmission electron microscopy (TEM) characterizations were detected by Tecnai 12(Philips, Netherlands). Fourier transform infrared (FT-IR) spectra of samples were measured in the range 400-4000 cm^{-1} using Cary 610/670 FT-IR spectrophotometer (Variann, USA). X-ray photoelectron spectroscopy (XPS) analysis was performed by ESCALAB 250Xi spectrometer (Thermo Fisher, USA), UV-vis spectra were tested by Lambda 650 (PerkinElmer, USA). The fluorescence spectra were recorded with a F-7000 fluorescence spectrophotometer (Hitachi, Japan). Ultrapure water was obtained by a Milli-Q purification system (Millipore, MA).

Synthesis of Heme-AuNPs. 0.5 mM $HAuCl_4$ and 10 μL 1M NaOH were added in 5 mL water, followed by adding 1.2 mM $NaBH_4$ solution under vigorous stirring for 30 min. Then, AuNPs was stored at 4 ℃ in refrigerator. Hemin with different concentration was added the obtained Au colloid under magnetic stirring. 10 min later, the Heme-AuNP hybrid was obtained and stored at 4 ℃. Heme-AuNPs were purified by dialysis before use.

Oxidase-like Activity of Heme-AuNP. The oxidase-like activity of Heme-AuNPs was measured by a classical colorimetric assay with TMB as the substrate. In a typical experiment, 150 μL of Heme-AuNPs, 2754 μL of acetate buffer solution (0.2 M, pH 3.5), 96 μL of 25 mM TMB were added into a 5 mL tube. After incubation for 30 min at 35 ℃, the UV-vis spectrum of the system was monitored by Lambda 650 spectrophotometer.

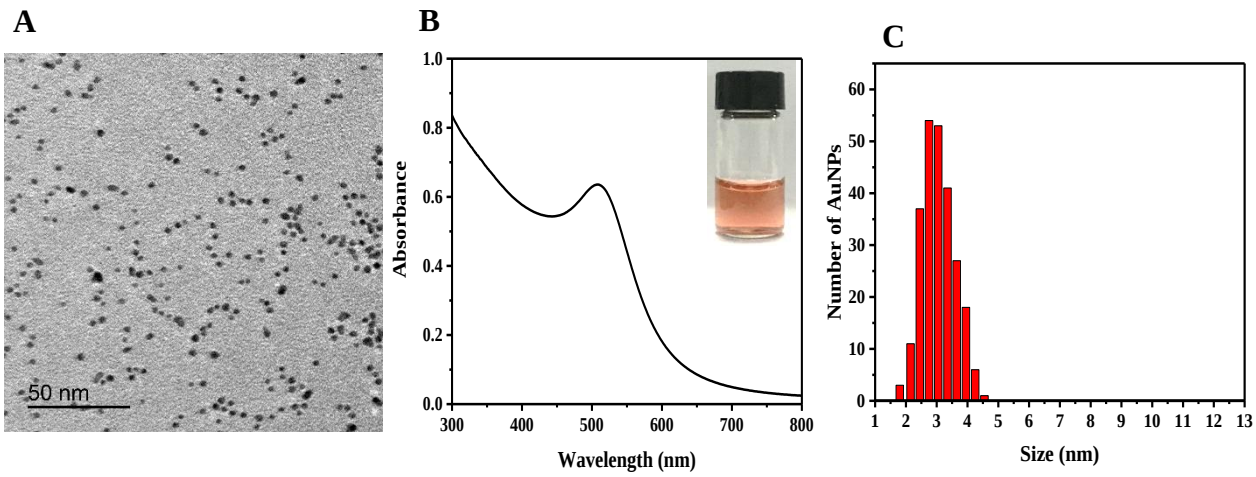

Fig. S1 TEM image (A) and UV-vis spectrum (B) of AuNPs. (C) The size distribution histogram for AuNP shown in A.

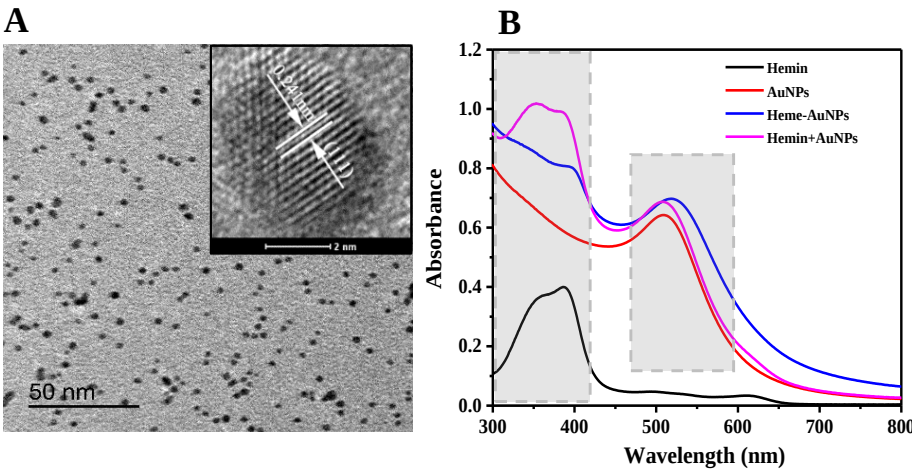

Fig. S2 TEM image (A) and the inset being the HRTEM image of AuNPs. (B) UV-vis spectra of AuNP, hemin, Heme-AuNP and hemin + AuNP.

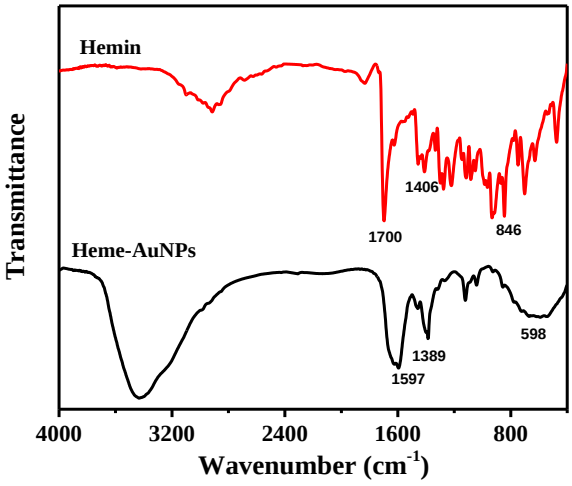

Fig. S3 FT-IR spectra of hemin and Heme-AuNP.

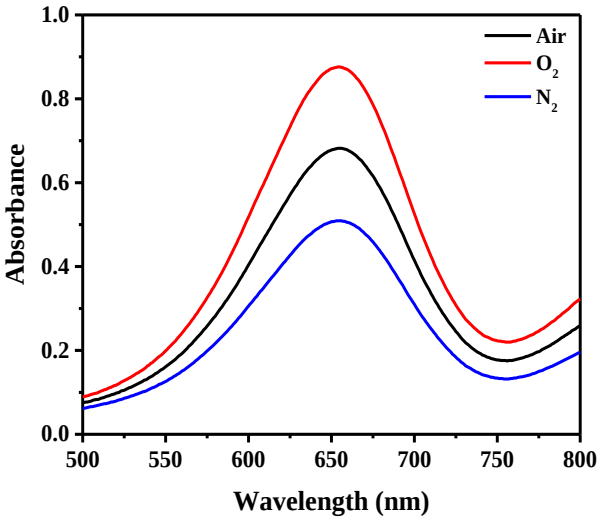

Fig. S4 UV-vis spectra of the oxidation of TMB catalyzed by Heme-AuNPs with testing solutions saturated by air, oxygen and nitrogen gases.

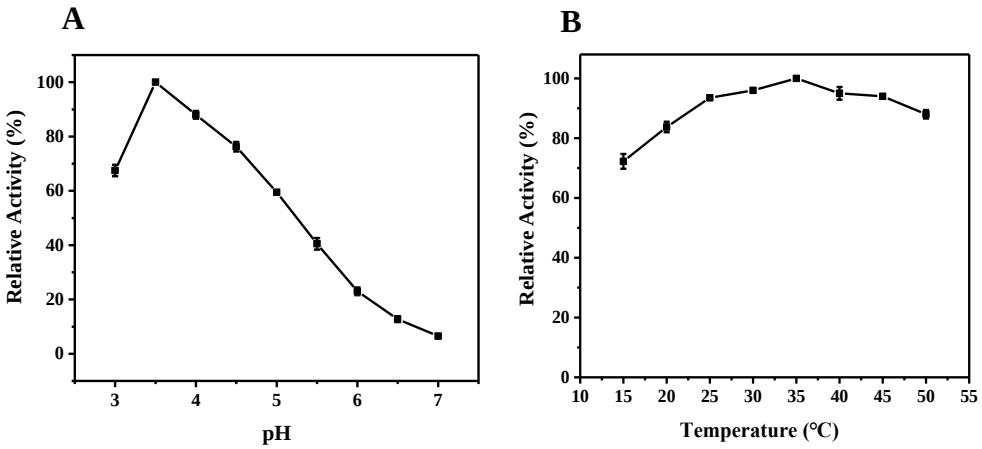

Fig. S5 Effect of pH (A) and temperature (B) on the oxidase-like activity of Heme-AuNPs.

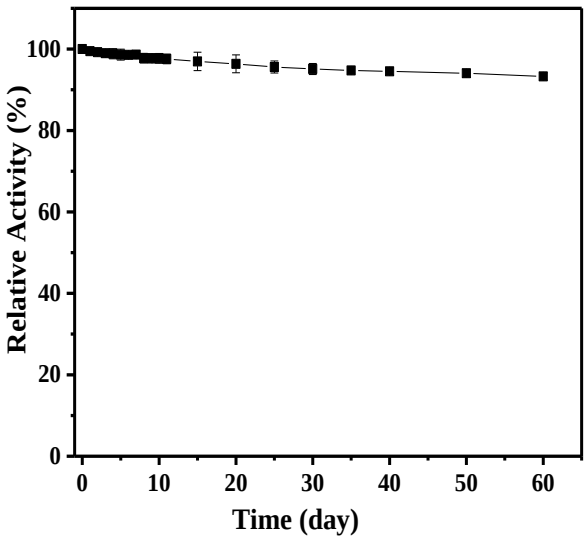

Fig. S6 The oxidase-like activity of Heme-AuNPs after stored for different time.

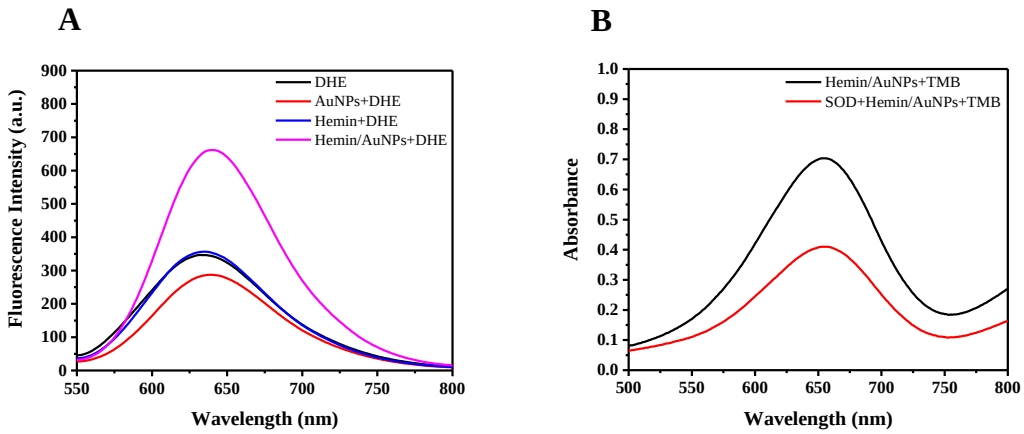

Fig. S7 (A) Fluorescent emission spectra of dihydroethidium to detect $O_2^{-\bullet}$ radicals. (B) Effect of SOD on the catalytic oxidation of TMB by Heme-AuNPs.

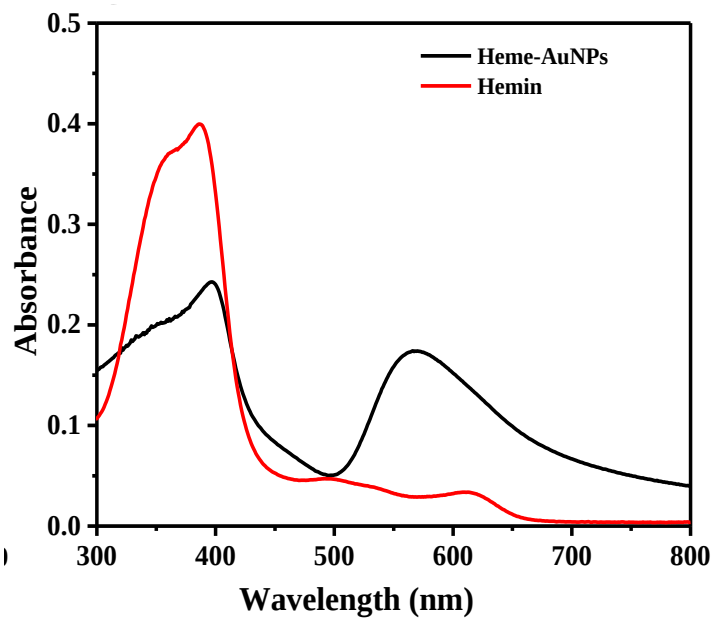

Fig. S8 The UV-vis spectra of heme without (red) and with AuNPs (black), [hemin] = 2.5 μM. The UV-vis spectrum of Heme-AuNPs was obtained with AuNP used as a reference.

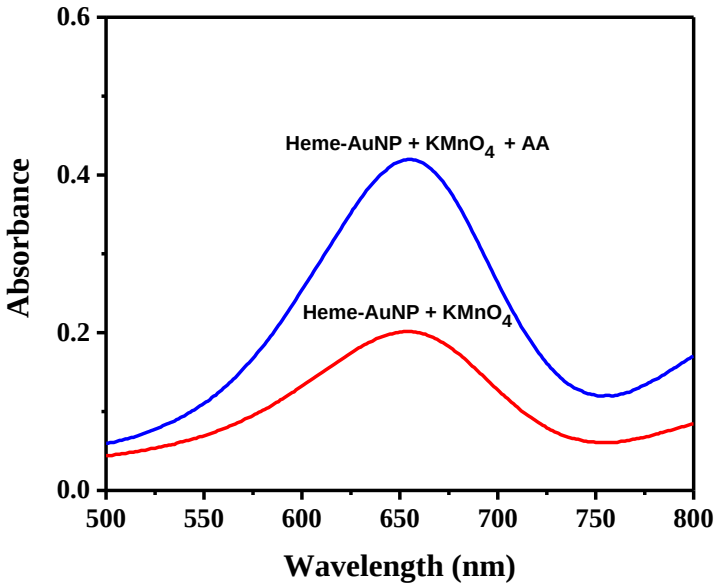

Fig. S9 Effect of AA on the oxidase-like activity of Heme-AuNPs treated with $KMnO_4$.

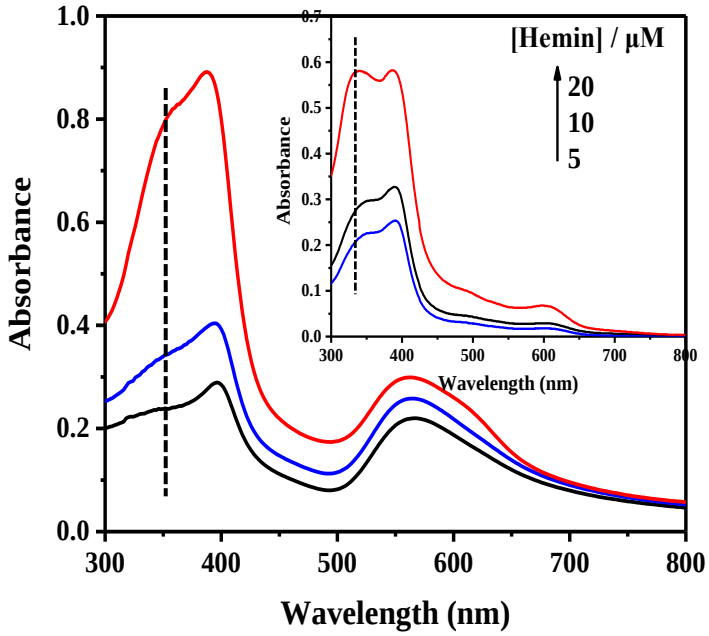

Fig. S10 UV-vis spectra profile of heme with different concentration with AuNPs, inset being the UV-vis spectra profile of hemin with different concentrations without AuNPs.

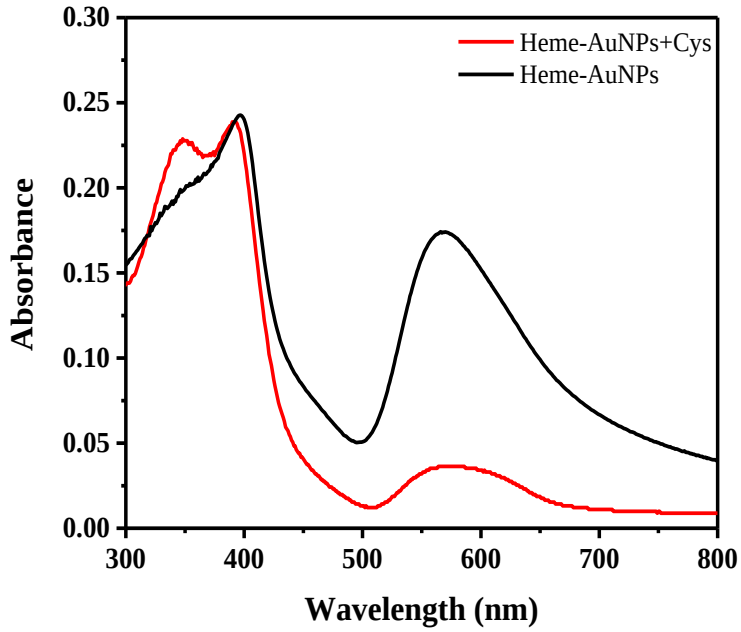

Fig. S11 The UV-vis spectra of heme in the presence of AuNPs (black) and of AuNP solution containing Cys (red).

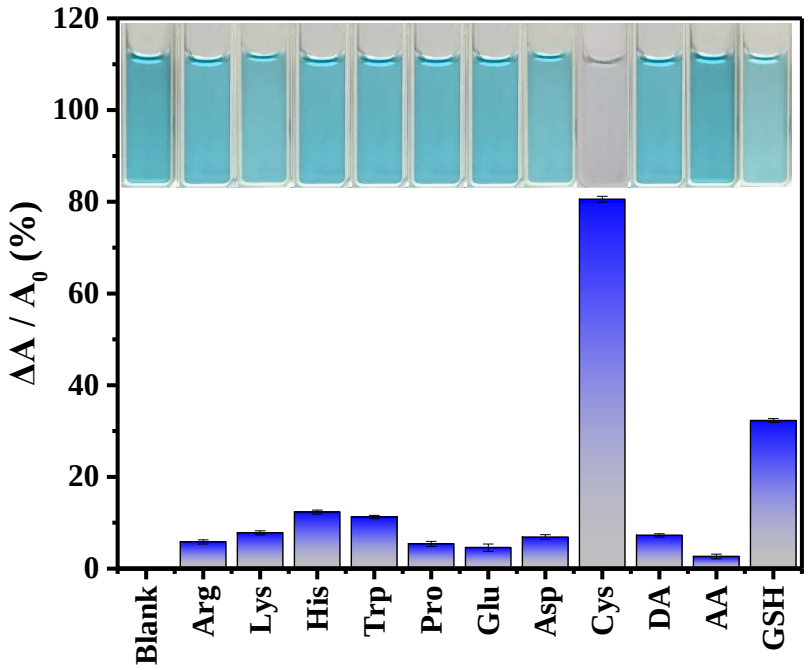

Fig. S12 The oxidase-like activity of Heme-AuNP in the presence of different 2.0 μM biomolecules.

Table S1. Comparison of the apparent Michaelis-Menton constant (K_m) and maximum reaction rate (V_{max}) of Heme-AuNP and other nanozymes.

Nanozyme	K_m (mM)	V_{max} (10^{-8} M s^{-1})	Reference
His@AuNCs	0.041	6.21	1
Nanoceria	0.42	10.04	2
Fe-N-C single-atom	1.81	0.0601	3
FeN_5 SA/CNF	0.148	75.8	4
PSMOF	0.165	13.9	5
Heme-AuNPs	0.168	1.68	This Work

Table S2. Comparison of different methods for cysteine detection based on nanozymes.

Nanozymes	Liner Range	LOD	Mimetic Enzyme	Reference
PtNPs/GO	25~5000 nM	1.2 nM	Peroxidase	6
$CuMnO_2$ nanoflake	25~300 μM	11.26 μM	Peroxidase	7
Pd NPs/CeO_2 nanotube	6~40 nM	2.9 nM	Peroxidase	8
Asp/Ce-nanotube	0.08~10 μM	33.2 nM	Peroxidase	9
FeCo-CNFs	1~20 μM	0.15 μM	Peroxidase	10
VS_4 submicrospheres	5~100 μM	2.5 μM	Peroxidase	11
POMOF/SWNT nanocomposites	1~80 μM	0.103 μM	Peroxidase	12
Mo-Co_3O_4 NTs	0.05~10 μM	24.2 nM	Peroxidase	13
MoS_2-QDs-AgNPs	1~100 μM	824 nM	Oxidase	14
Heme-AuNPs	15~750 nM	10 nM	Oxidase	This Work

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