Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

MOF-polymer composites with well-distributed gold nanoparticles for visual monitoring of homocysteine

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Experimental section

Materials and chemicals

Homocysteine (Hcy) and other amino acids were purchased from TCI Shanghai Co. Ltd. (Shanghai, China). UiO-66-NH_2 (U) was bought from Beijing Krre Technology Co., Ltd. (Beijing, China). Dimethylvinyloxazolinone (VD) was gotten from Beijing Institute of Coollight Fine Chemicals (Beijing, China). Tetrahydrofuran (THF) and *N,N*-dimethylformamide (DMF) were gotten from Concord Technology Co., Ltd. (Tianjin, China). $HAuCl_4$ was bought from Shenyang Jinke Reagent Factory (Shenyang, China). Trithiocarbonate (DDAT) was provided by Sigma-Aldrich (USA). Hydrogen peroxide (H_2O_2), 3,3′,5,5′-tetramethylbenzidine (TMB), *N*-2-hydroxypropyl methacrylamide) (H), 2,2-azobisisobutyronitrile (AIBN), 5.5'-dimethyl pyrroline *N*-oxide (DMPO), tetrahydrofuran (THF) and other chemicals were purchased from Beijing Innochem Technology Co. Ltd. (Beijing, China). Sodium acetate (NaAc) was obtained from Beijing Yili Fine Chemicals Co., Ltd. (Beijing, China). The aqueous solutions were prepared with Milli-Q water (Millipore, Bedford, MA, USA).

Instruments

The ultraviolet-visible (UV-*vis*) absorption spectra were recorded using a TU-1900 UV-*vis* double-beam spectrometer (Purkinje General, China). A 1.0 mL capacity cuvette with a 1.0 cm path length was used for measuring the UV-*vis* absorbance.

Fourier transform infrared (FT-IR) spectra were recorded by an FT-IR spectrophotometer (TENSOR-27, Germany).

The zeta potential measurements were carried out with a Zetasizer laser particle analyser (Zetasizer Nano ZS ZEN3600, British).

Transmission electron microscopy (TEM) and energy dispersive spectroscopy (EDS) were all implanted on a transmission electron microscope (JEM-2010, Japan electron optics laboratory, Japan) at a voltage of 200 kV.

Powder X-ray diffraction (PXRD) patterns were collected on a PANalytical Empyrean diffractometer (Empyrean, PANalytical B.V., Netherlands) at room temperature.

X-ray photoelectron spectroscopy (XPS) measurements were performed by an ESCALab220i-XL spectrometer (VG Scientific, U.K.) with XPS peak software 4.1.

Electron paramagnetic resonance (EPR) signals were measured by a Bruker ESP 300E spectrometer (Bruker, Rheinstetten, Germany) with a microwave bridge (receiver gain, 1×10^5; modulation amplitude, 2 Gauss; microwave power, 10 mW; modulation frequency, 100 kHz). A sample containing 0.5 M DMPO was transferred to a quartz capillary tube and placed in the EPR cavity. Under the UV-irradiation at 355 nm, EPR signals were detected

using DMPO as the spin trap.

Preparation of UVD

All of the glasswares were rinsed with aqua regia ($HCl:HNO_3$ = 3:1, v/v) and washed with ultrapure water. 50.0 mg of U was mixed with 1.4 mL VD in a glass-vial. After the mixture was stirred at room temperature for 12.0 h, the UVD was obtained for further preparation of UVD-PH.

Synthesis of PH

Poly(*N*-2-hydroxypropylmethacrylamide) (PH) was prepared *via* a reversible addition-fragmentation chain transfer polymerization (RAFT) method. Typically, 1.42 g H, 10.0 mg DDAT and 20.0 mg AIBN were added in a flask and mixed with 10.0 mL 1,4-dioxane. The flask was sealed under nitrogen after three freeze-evacuate-thaw cycles and put into an oil bath at 60 °C for 24.0 h. The precipitate was obtained by pouring the mixture into excess absolute ether while stirring and centrifuging at 10,000 rpm for 5.0 min, repeating the process of water dissolving, ether precipitating and centrifuging for three times. Finally, the resultant PH was dried at room temperature for 12.0 h and stored for further use.

Preparation of UVD-PH

Typically, 1.42 g H, 20.0 mg AIBN and 50.0 mg UVD were added into a flask with 20.0 mL 1,4-dioxane. The flask was sealed under nitrogen after three freeze-evacuate-thaw cycles and the mixture reacted under UV (365 nm) for 24.0 h. After the mixture was centrifuged at 10,000 rpm for 10.0 min, the precipitate was washed for three times with DMF, dried in an oven at 50 ^{o}C for 24.0 h, and the resultant UVD-PH was stored at room temperature for further use.

Synthesis of AuNPs@PH-on-U

Simply, 2.5 mL of $HAuCl_4$ (10.0 mM) and 2.5 mL PH (10.0 mg/mL) were mixed in a glass vial. Following the mixture was stirred at 25 °C for 2.0 min, 0.3 mL of NaOH (1.0 M) were added. Then the mixture reacted at 25 °C for 5.0 h to yield AuNPs@PH. 5.3 mL of the resultant AuNPs@PH and 20.0 mg U were added in a flask with 10.0 mL ethanol, the mixture was sonicated at 25 °C for 1.0 h. The precipitate AuNPs@PH-on-U was washed with ethanol for three times, centrifuged at 8,000 rpm for 5.0 min to remove any un-adsorbed AuNPs@PH.

Kinetics study of UVD-PH@AuNPs and PH@AuNPs-on-U

Steady-state enzyme kinetic parameters of UVD-PH@AuNPs-TMB-H_2O_2 system and PH@AuNPs-on-U-TMB-H_2O_2 system were calculated. The Michaelis-Menten curves were plotted and fitted to the double reciprocal Lineweaver-Burk equation (1):

$$1 / v = [(K_m / V_{max}) (1 / S) + (1 / V_{max})] \quad (1)$$

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.

Rat serum Hcy monitoring

The rat serums were obtained from Beijing Vital River Laboratory Animal Technology Co. Ltd. (Beijing, China). All experiments concerning with rat serums were performed in accordance with the institutional animal care and use guidelines of China (GB/T 27416-2014), and were complied with the guide for caring and using of laboratory animals from the Association for Assessment and Accreditation of Laboratory Animal Care.

After Hcy dissolved in physiological saline solution was injected into the abdominal cavity of rats (5.3 mg/kg), the controlled blank serum samples and five different serum samples (at 0 h, 0.5 h, 1.0 h, 2.0 h, 4.0 h, 6.5 h) were collected. The rat serum samples were pre-treated to eliminate the interferences-proteins. Simply, 0.1 mL of the rat serum samples was diluted by 0.1 mL of ethanol and mixed for 5.0 min. Consequently, the mixtures were centrifuged at 10,000 rpm for 10.0 min and the supernatant was collected and stored at 4°C for further analysis.

For detection of serum Hcy, the rat serum samples were 10-times diluted. 30.0 μL diluted rat serum samples was added into a mixture, which contained 2.72 mL HAc-NaAc buffer, 36.0 μL TMB, 90.0 μL H_2O_2 and 100.0 μL UVD-PH@AuNPs. Notably, for the serum samples after injection 6.5 h, 300.0 μL diluted rat serum samples was added into a mixture, which contained 2.42 mL HAc-NaAc buffer, 36.0 μL TMB, 90.0 μL H_2O_2 and 100.0 μL UVD-PH@AuNPs. After the mixture reacted at room temperature for 20.0 min, the UV-*vis* adsorption of oxTMB in the solution was measured at 650 nm.

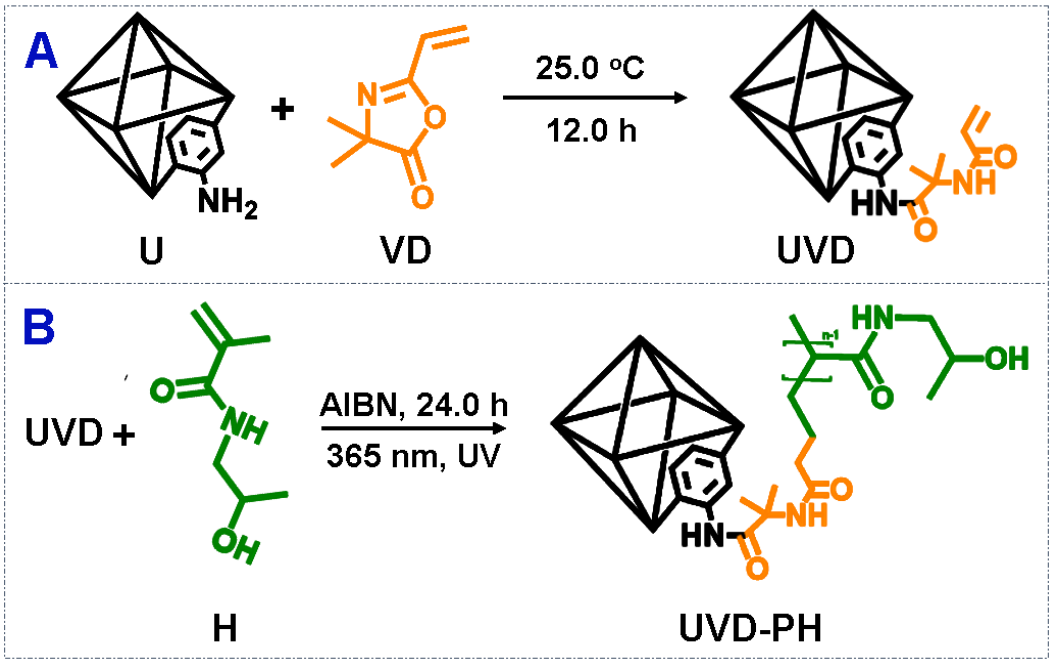

Fig. S1 Schematic diagram of synthesis of (A) UVD and (B) UVD-PH.

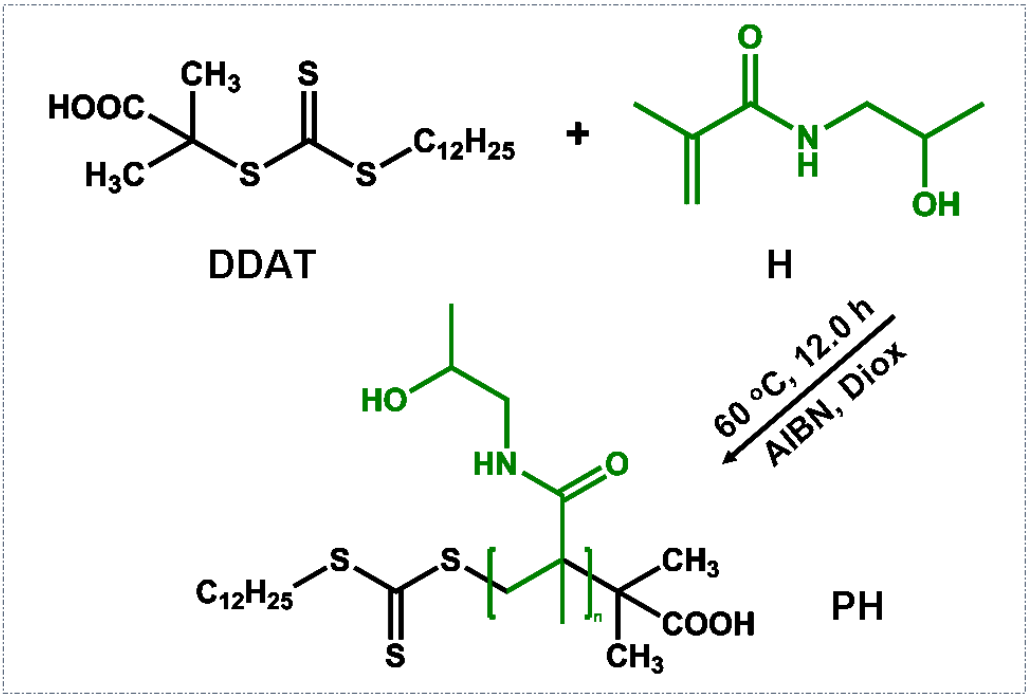

Fig. S2 Schematic diagram of synthesis of PH through RAFT polymerization protocol.

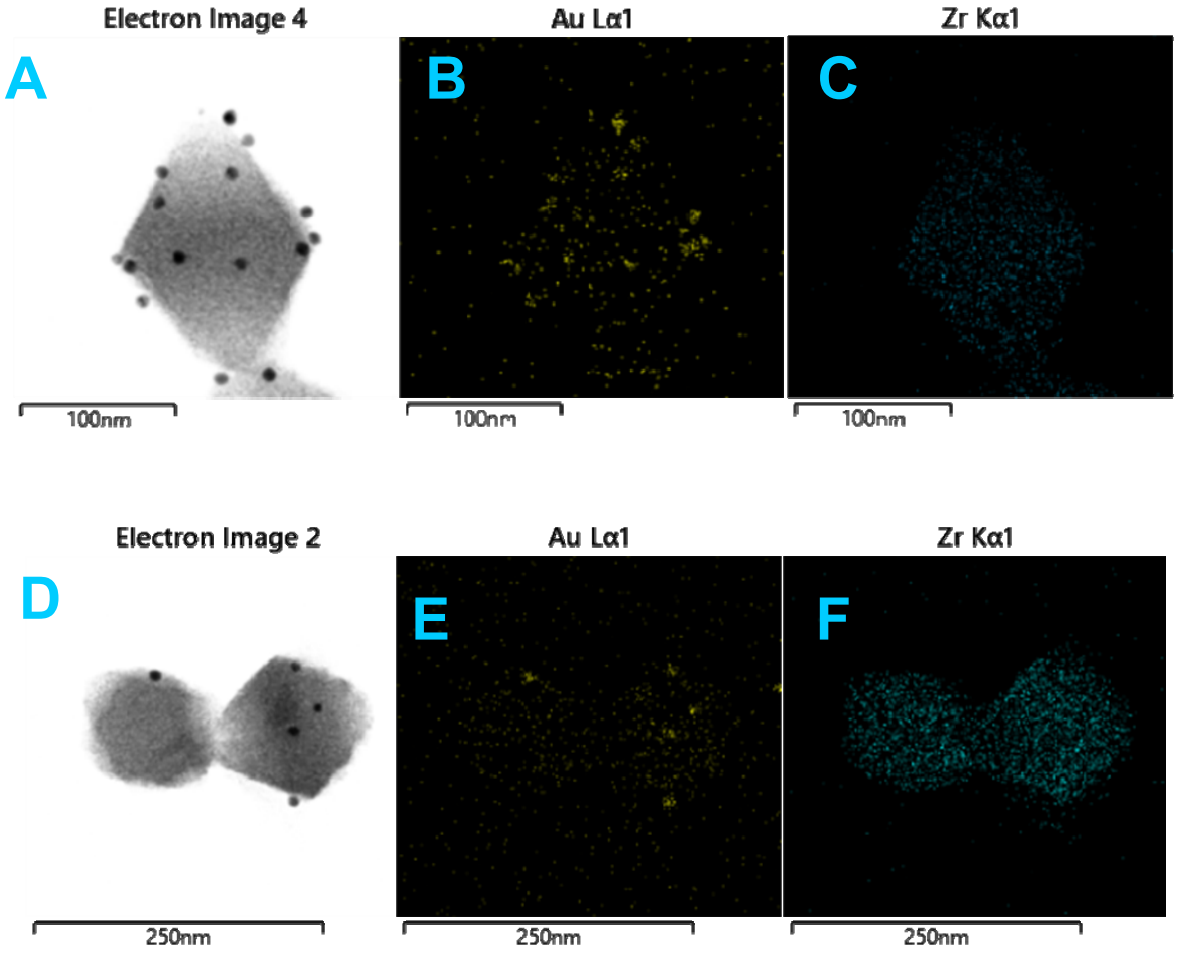

Fig. S3 EDS-mapping images of (A-C) UVD-PH@AuNPs and (D-F) PH@AuNPs-on-U.

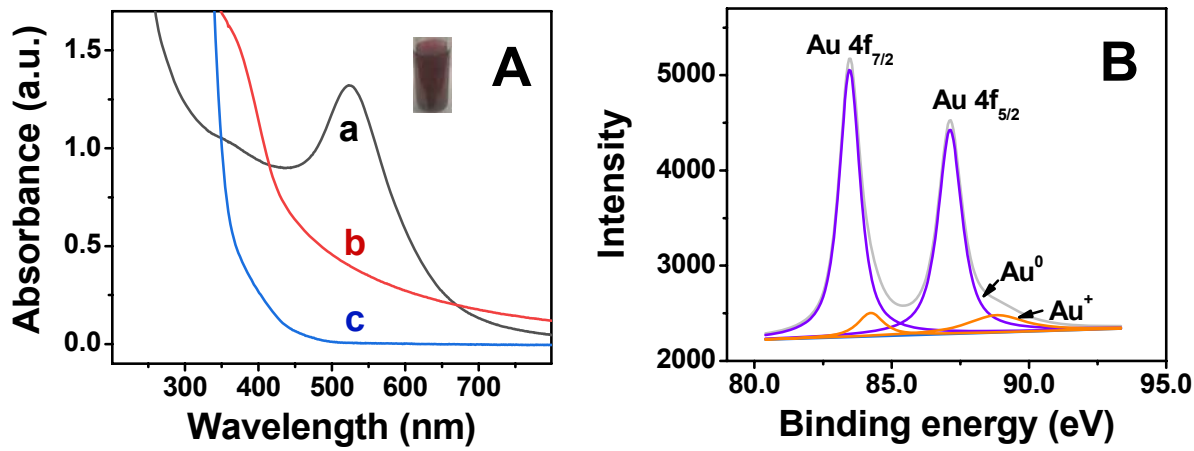

Fig. S4 (A) UV-*vis* of UVD-PH@AuNPs (a), UVD-PH (b) and $HAuCl_4$ (c); inset photo of UVD-PH@AuNPs taken under day light; (B) XPS spectra of Au 4f orbitals of UVD-PH@AuNPs.

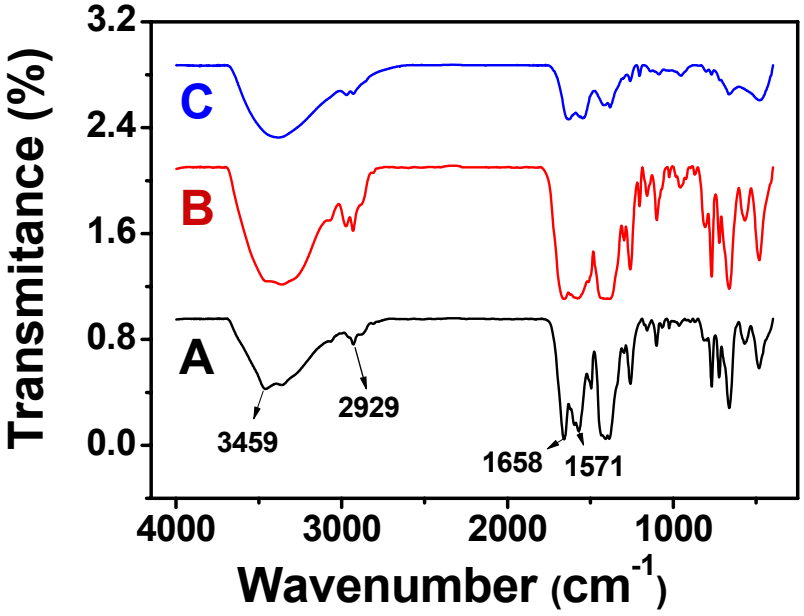

Fig. S5 FT-IR spectra of (A) U; (B) UVD-PH and (C) UVD-PH@AuNPs.

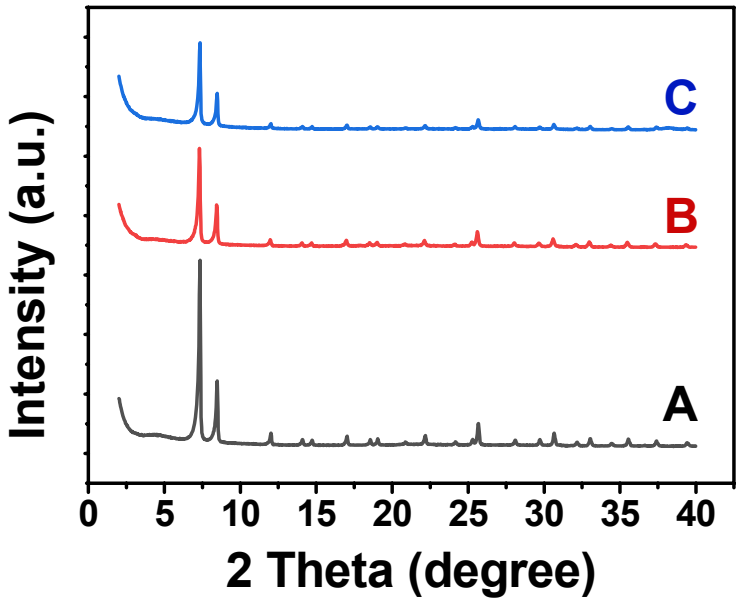

Fig. S6 PXRD patterns of (A) U; (B) UVD-PH and (C) UVD-PH@AuNPs.

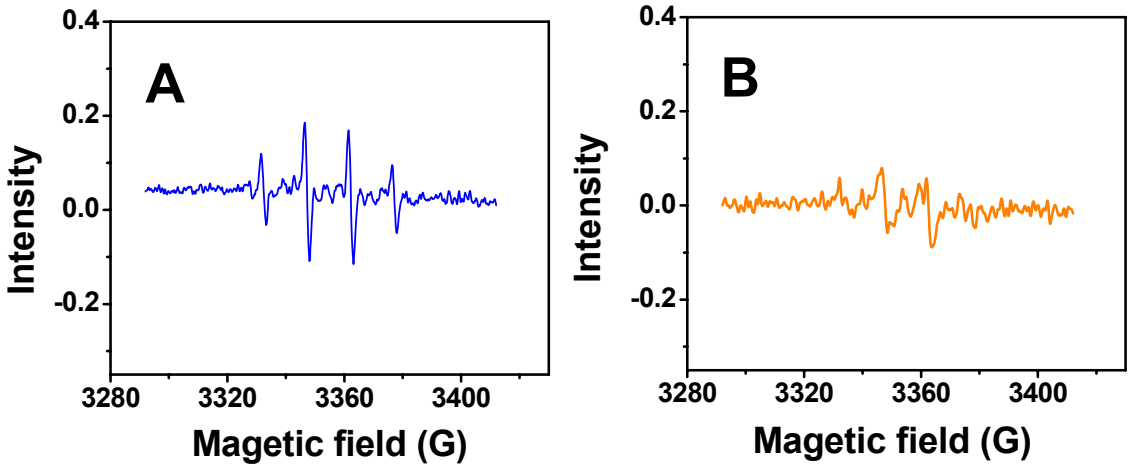

Fig. S7 EPR signals of (A) UVD-PH@AuNPs-DMPO-H_2O_2 and (B) PH@AuNPs-on-U-DMPO-H_2O_2. The concentrations of DMPO, UVD-PH@AuNPs, PH@AuNPs-on-U and H_2O_2 were 0.1 M, 0.1 mg/mL, 0.3 M and 0.25 μM, respectively.

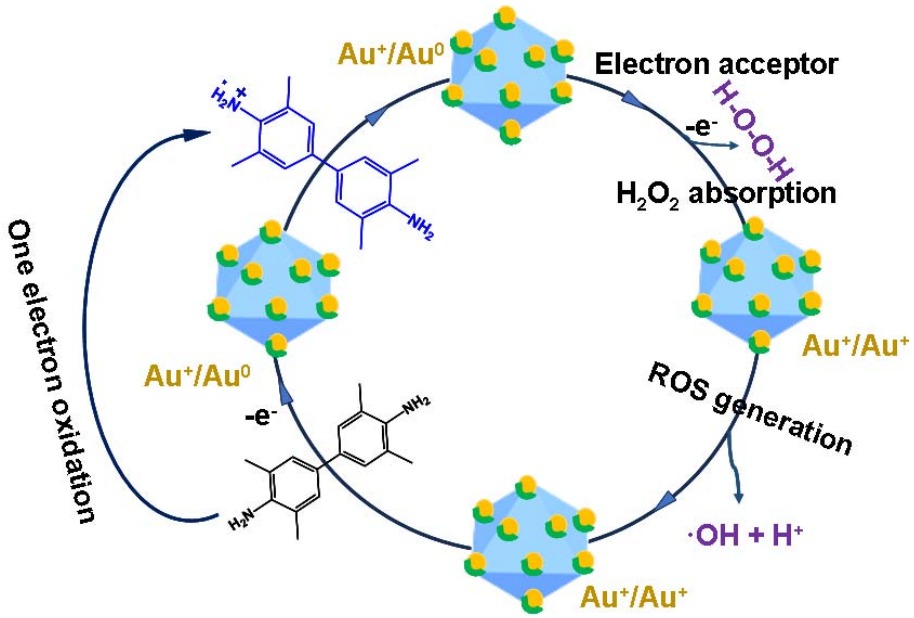

Fig. S8 Possible mechanism for the POD-like activity of UVD-PH@AuNPs.

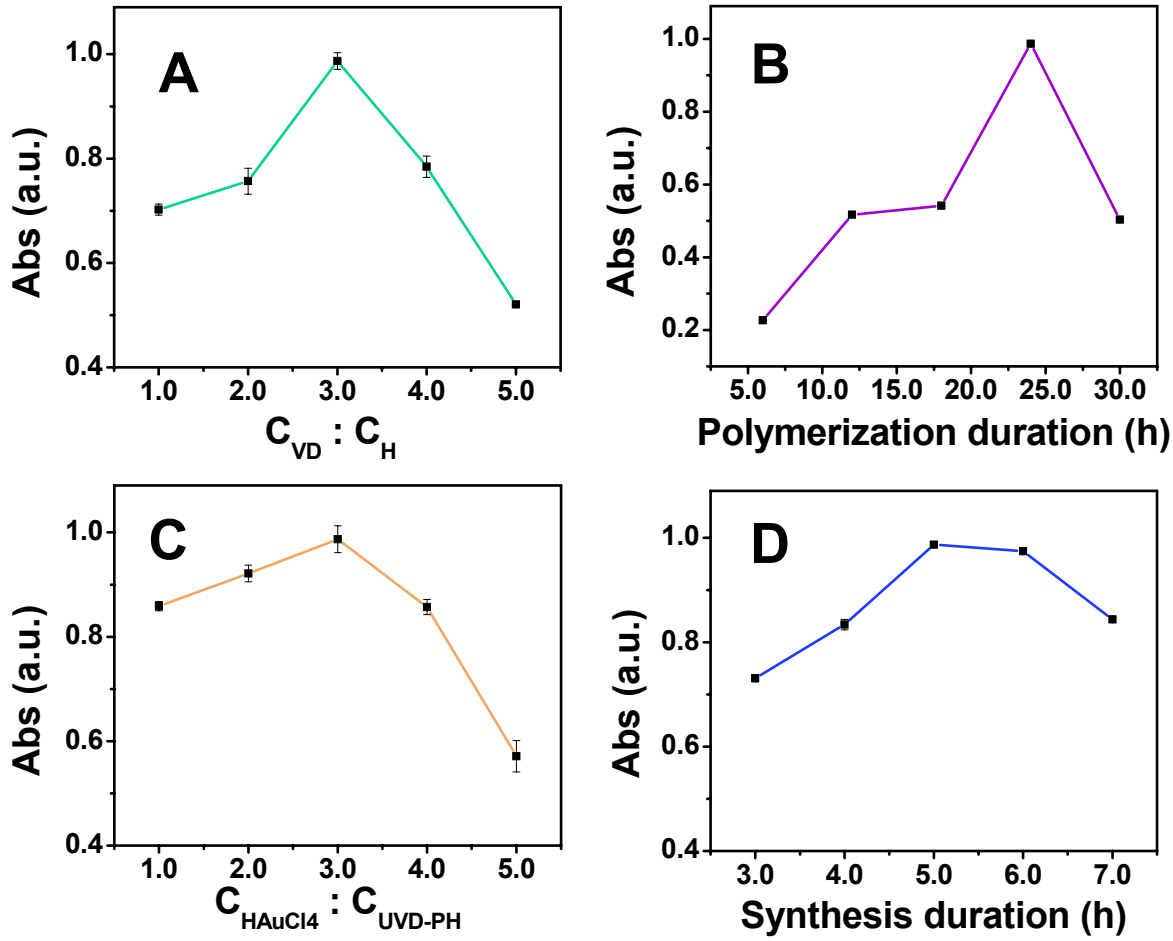

Fig. S9 Dependence of POD-like activity of UVD-PH@AuNPs on (A) concentration ratio of UVD-PH; (B) UVD-PH polymerization duration; (C) concentration ratio of UVD-PH to $HAuCl_4$ and PH-U (D) UVD-PH@AuNPs synthesis duration, (n=3). A_0 and A represented the UV-vis absorption of the UVD-PH@AuNPs-TMB-H_2O_2 system in the absence and presence of Hcy, respectively.

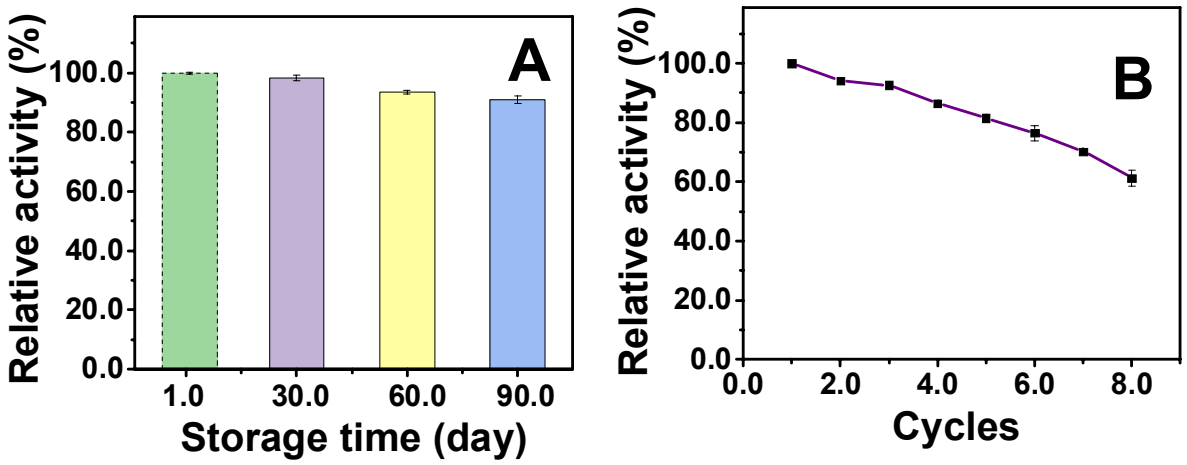

Fig. S10 (A) Change in POD-like catalytic activity of UVD-PH@AuNPs after storage for different days at room temperature. (B) Relative POD-like catalytic activity of UVD-PH@AuNPs in the TMB oxidation during the recycling processes.

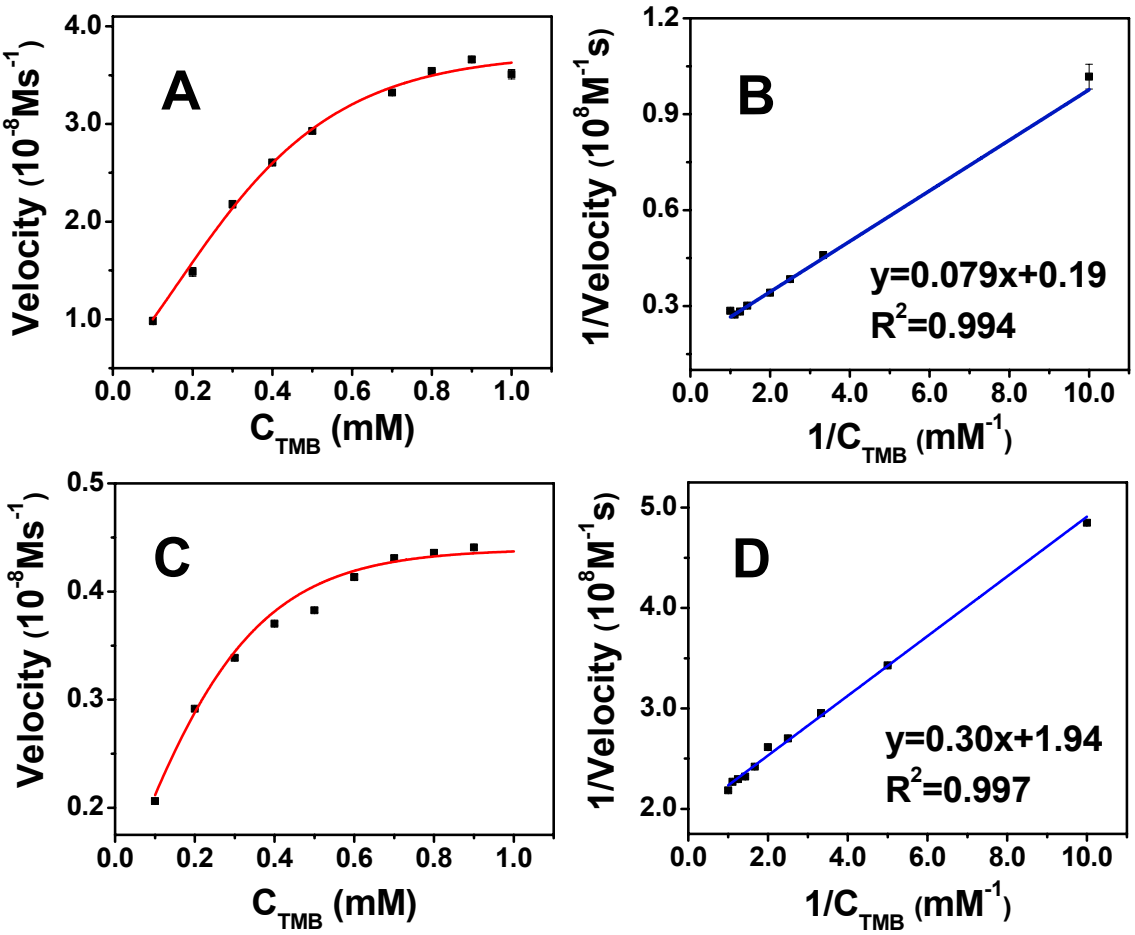

Fig. S11 Steady-state kinetics of UVD-PH@AuNPs (A and B) and PH@AuNPs-on-U (C and D) in the oxidation reaction of TMB in the presence of 0.30 M H_2O_2 (n=3).

Table S1 Comparison of kinetic parameters of the reported MOF-AuNPs based nanozymes

Nanozymes	K_m (mM)	V_{max} (10^{-8}Ms^{-1})	Ref.
Cu-MOF@AuNPs	0.78	1.06	X. Dang *et al.* Talanta 2020, 210, 120678
Cu-MOF@AuNPs	0.29	3.96	X. Liao *et al.* J. Phys. Chem. Lett. 2022, 13, 312
Cu-MOF@AuNPs	0.077	0.343	X. Hu *et al.* Food Chem. 2022, 376, 131906
UVD-PH@AuNPs	0.42	5.37	**This work**

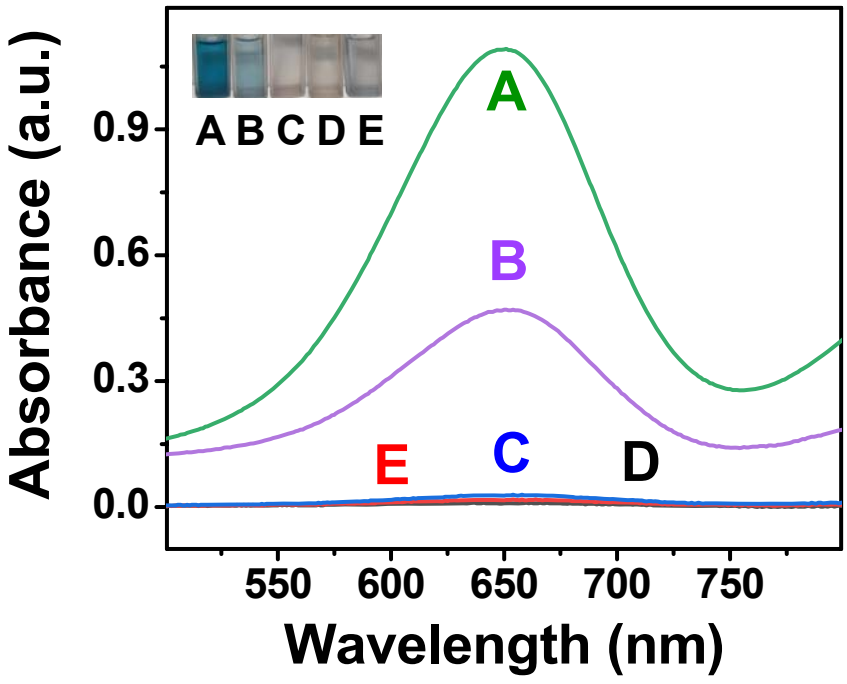

Fig. S12 UV-*vis* absorption spectra and inset photos taken under day light of (A) UVD-PH@AuNPs-TMB-H_2O_2; (B) UVD-PH@AuNPs-Hcy-TMB-H_2O_2; (C) Hcy-TMB-H_2O_2; (D) UVD-PH--TMB-H_2O_2 and (E) TMB-H_2O_2.

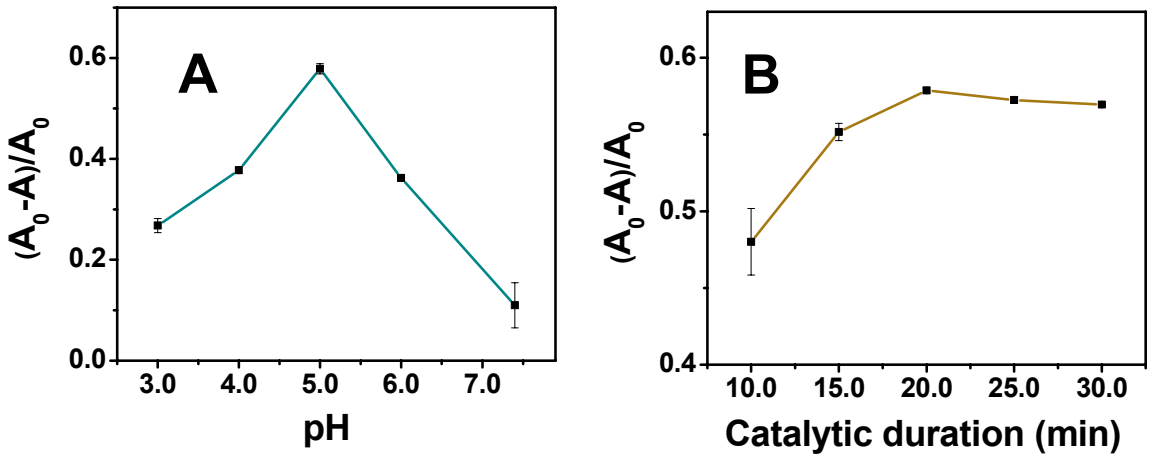

Fig. S13 Dependence of POD-like activity of PVD-PH@AuNPs on (A) buffer pH and (B) catalytic reaction duration (n=3). A and A_0 represented the UV-*vis* absorption of the UVD-PH@AuNPs-TMB-H_2O_2 system in the absence and presence of Hcy, respectively.

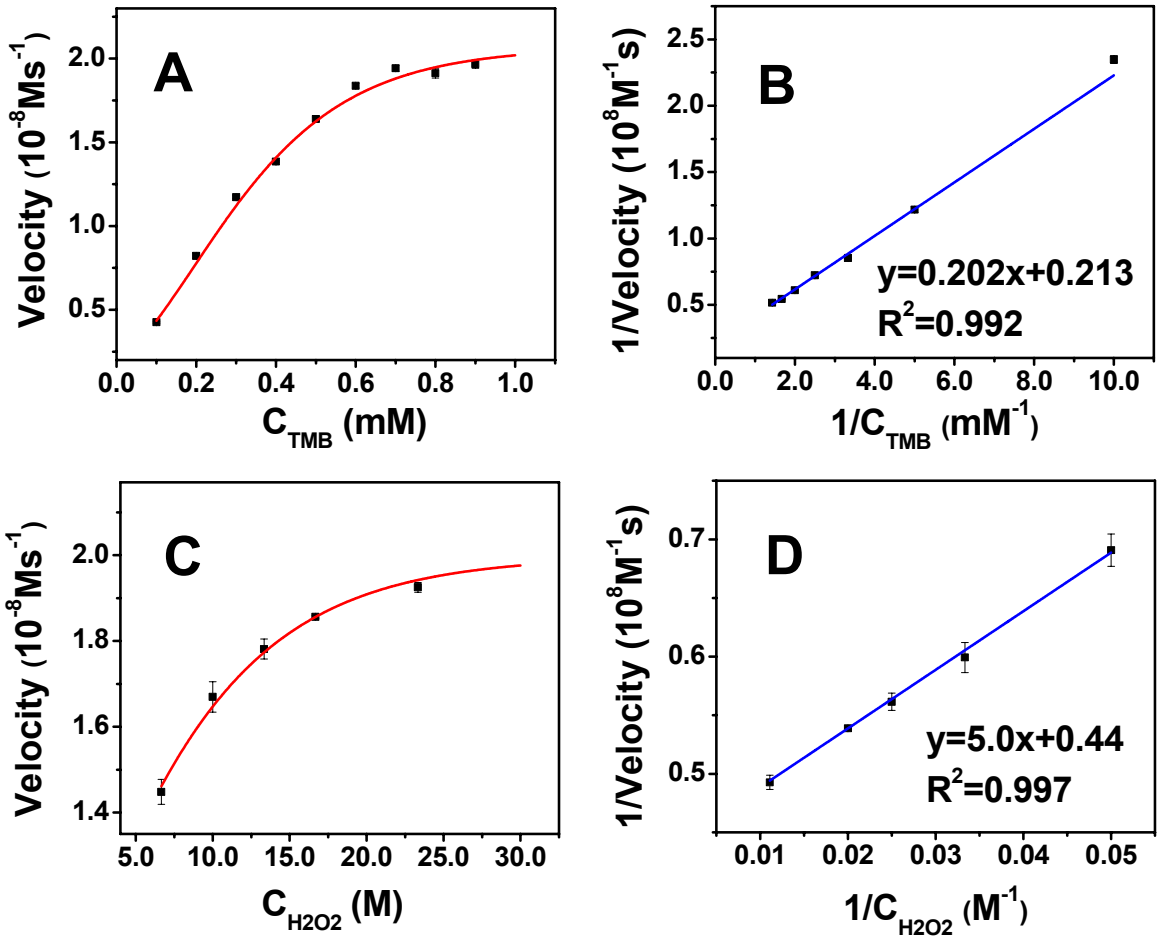

Fig. S14 The steady-state kinetics study of UVD-PH@AuNPs-Hcy with TMB (A and B) and H_2O_2 as the substrate (C and D), respectively.

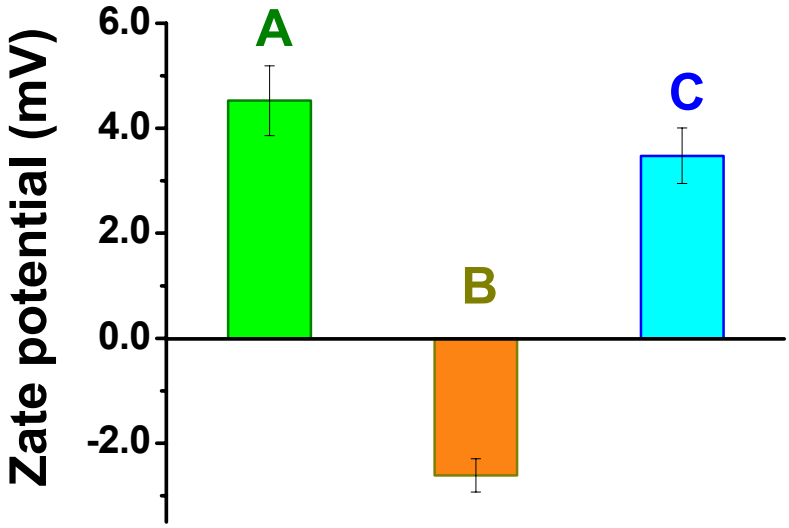

Fig. S15 The apparent zeta potentials of (A) UVD-PH@AuNPs; (B) Hcy and (c) UVD-PH@AuNPs-Hcy (n=3).

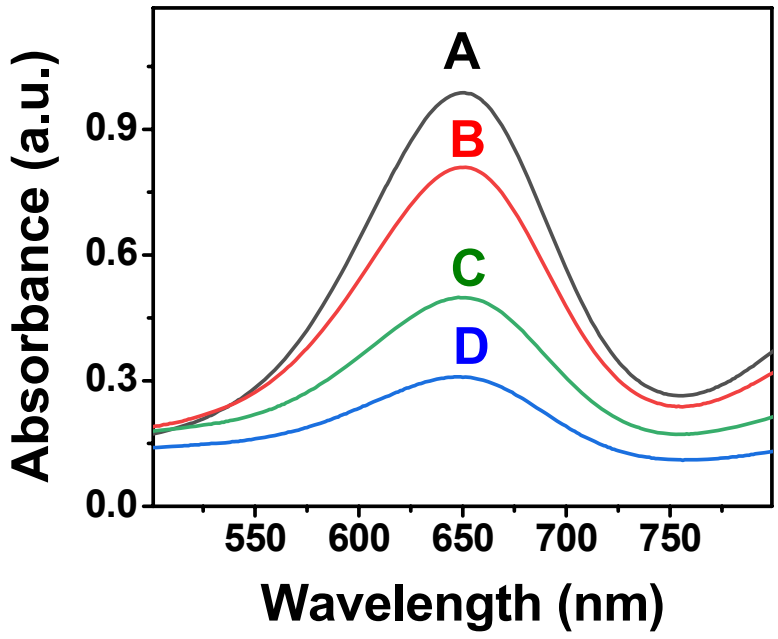

Fig. S16 Effect of ·OH inhibitor on the absorbance of UVD-PH@AuNPs-TMB-H_2O_2 and PH@AuNPs-on-U in the absence (A, C) and presence (B, D) of 2.0 mM t-tubyl alcohol.

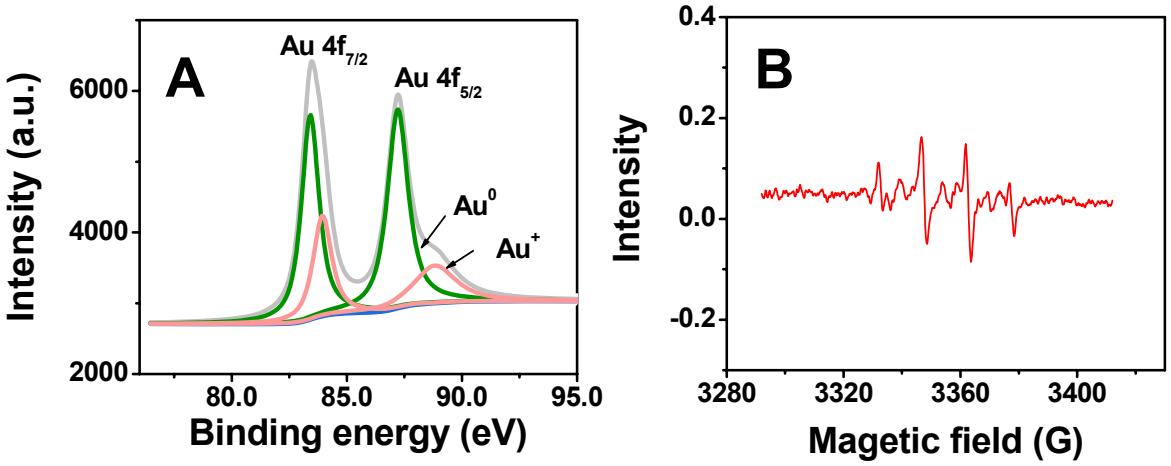

Fig. S17 XPS spectra of Au 4f orbitals of (A) UVD-PH@AuNPs-Hcy and (B) EPR signals of UVD-PH@AuNPs-Hcy-DMPO-H_2O_2. The concentrations of DMPO, UVD-PH@AuNPs, H_2O_2 and Hcy were 0.1 M, 0.3 mg/mL, 0.3 M and 10.0 μM, respectively.

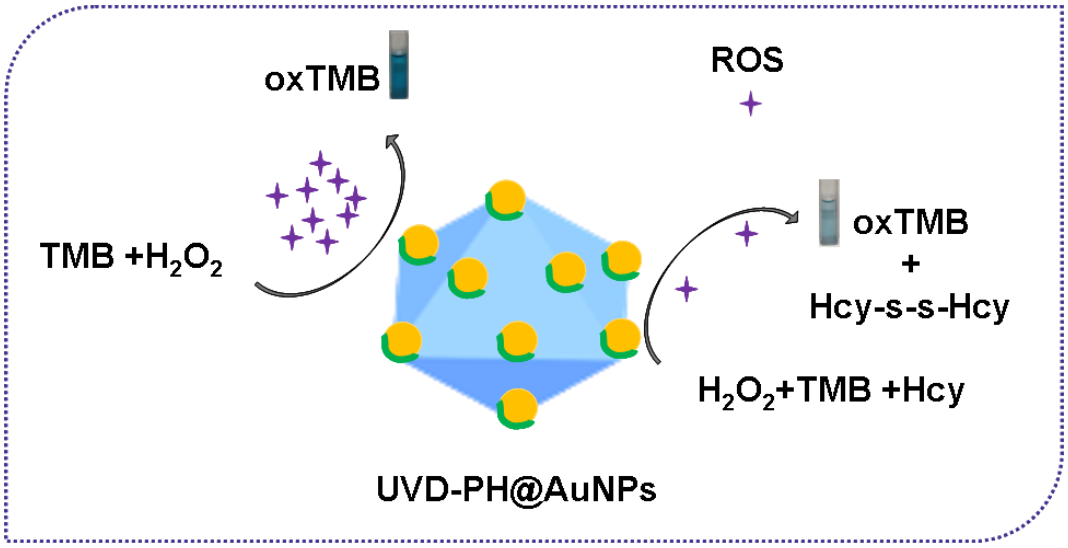

Fig. S18 Possible mechanism illustration of POD-like catalytic activity of UVD-PH@AuNPs inhibited by introducing of Hcy in the system.

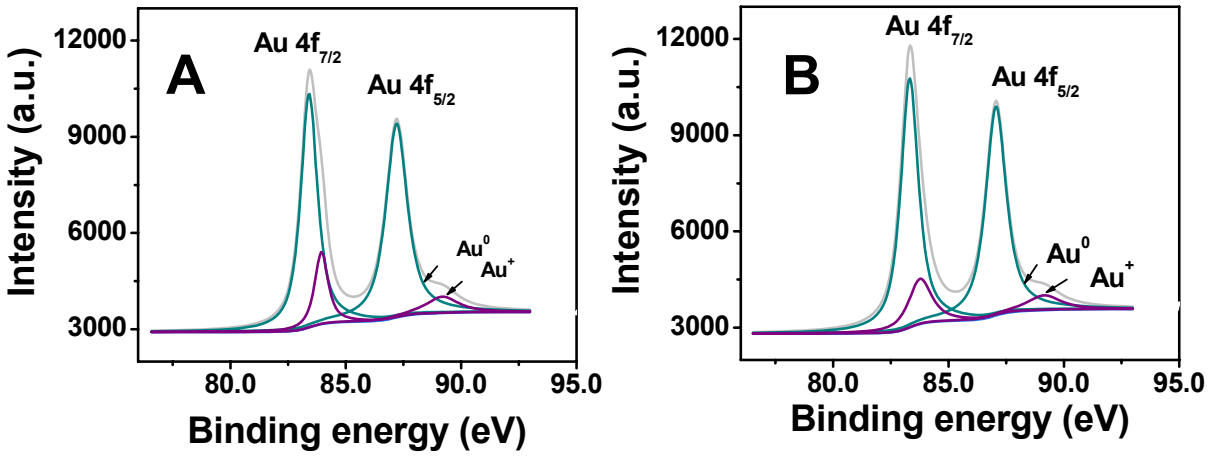

Fig. S19 XPS spectrums of (A) UVD-PH@AuNPs-Cys and (B) UVD-PH@AuNPs-GSH.

Table S2 Contents of Au^0 and Au^+ in different systems*

Nanozymes-test compounds	Content of Au^0 (%)	Content of Au^+ (%)
UVD-PH@AuNPs-Cys	84.4	15.6
UVD-PH@AuNPs-GSH	85.0	15.1
UVD-PH@AuNPs-Hcy	69.2	30.8
UVD-PH@AuNPs	88.6	11.4

* Content of Au^0 or Au^+ was obtained by calculation of the peak area (S) of $S_{Au0}/S_{(Au0 + Au+)}$ x100% or $S_{Au+}/S_{(Au0 + Au+)}$ x100%. The peak area of S_{Au0} or S_{Au+} or $S_{(Au0 + Au+)}$ in Fig. S19 were calculated using XPS peak software.

Table S3 Recovery of the proposed method*

Serums	Added (μm)	Found (μm)	Recovery (%)	RSD (%)
1	5.0	5.23	104.5	3.8
	10.0	9.73	97.3	0.8
	15.0	14.71	98.0	0.5
2	5.0	5.14	102.8	2.5
	10.0	0.94	93.8	2.9
	15.0	15.14	100.9	1.7
3	5.0	5.18	103.6	2.5
	10.0	9.99	99.9	1.3
	15.0	15.31	102.1	0.8

* Blank controlled rat serums were used for the recovery study (n=3).