Electronic Supplementary Material (ESI) for Analyst.


# Supporting Information

## Enhancing catalytic performance of MOFs-polymer@AuNPs based nanozymes for colorimetric detection of serum L-cysteine

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# Experimetal section

## Materials and chemicals

L-Cysteine (L-Cys) and other L-amino acids were purchased from TCI Shanghai Co. Ltd. (Shanghai, China). $HAuCl_4$ was bought from Shenyang Jinke Reagent Factory (Shenyang, China). UIO-66-$NH_2$ (U66) was bought from Beijing Krre Technology Co., Ltd. (Beijing, China). Dimethylvinyloxazolinone (VDMA) was gotten from Beijing Institute of Coollight Fine Chemicals (Beijing, China). Trithiocarbonate (DDAT) was provided by Sigma-Aldrich (USA). Acetonitrile (HCN), tetrahydrofuran (THF) and *N,N*-dimethylformamide (DMF) were gotten from Concord Technology Co., Ltd. (Tianjin, China). Hydrogen peroxide ($H_2O_2$), 3,3′,5,5′-tetramethylbenzidine (TMB), pepsin (Pep), 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). 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.).

Electron paramagnetic resonance (EPR) signals were measured by a Bruker ESP 300E spectrometer (Bruker, Rheinstetten, Germany) with a microwave bridge (receiver gain, $1\times10^5$; 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.

## Synthesis of PVDMA (PV)

All of the glasswares were rinsed with aqua regia (HCl : $HNO_3$ volume ratio = 3:1) and washed with ultrapure water. PV was synthesized by reversible addition-fragmentation chain transfer (RAFT) polymerization method. Under the protection of nitrogen, 10.0 mg DDAT, 1.2 mg AIBN 1.0 mL VDMA were added into a glass-vial with 3.0 mL of HCN. The mixture reacted at 70 $^{o}$C for 10.0 h, and then 30.0 mL of n-hexane was added. The resulted light yellow precipitates were washed for three times with pure water, dried in an oven for 24.0 h, and then were stored at room temperature for further analysis.

## Preparation of U66-PV

U66-PV was prepared through the amide reaction between lactone in PV and amino group residues in U66. 50.0 mg of PV and 50.0 mg of U66 were dissolved in a glass-vial with 3.0 mL of THF. The mixture was stirred at room temperature for 3.0 h, and then was centrifuged. The precipitates were washed for three times with pure water, dried in an oven for 24.0 h, and then were stored at room temperature for further analysis.

## Synthesis of U66-PV-Pep

The immobilization of Pep was achieved through the reaction between lactone in U66-PV and amino groups in Pep. 11.25 mg Pep and 20.0 mg U66-PV was added to 10.0 mL HAc-NaAc buffer (pH 3.0). After agitating for 2.0 h at 4 $^{o}$C, the product U66-PV-Pep was collected by centrifuging at 10000 rpm / min for 10.0 min and washed three times with HAc-NaAc buffer. Finally, 20.0 mg U66-PV-Pep was mixed with 6.0 mL HAc-NaAc buffer, the mixture was stored at 4 $^{o}$C for further analysis.

## Preparation of Pep@AuNPs

Pep@AuNPs was prepared with Pep as the reducing and capping agent. Simply, in a 10.0 mL-glass vial, 2.5 mL of $HAuCl_4$ (10.0 mM) and 2.5 mL of Pep (4.5mg / mL) aqueous solutions were mixed under gentle stirring at 25 $^{o}$C for 2.0 min. Then 0.25 mL of NaOH (1.0 M) was added, the mixture was stirred at 25 $^{o}$C for 3.0 h. After centrifuged at 10,000 rpm for 10.0 min, the Pep@AuNPs supernatant was collected and stored at 4 $^{o}$C for further use.

### Synthesis of Pep@AuNPs-on-U66

Freshly prepared Pep@AuNPs (5.25 mL) and 20.0 mg U66 dispersed in 10.0 mL ethanol were sonicated at 25 $^{o}$C for 1.0 h. The resulted Pep@AuNPs-on-U66 were washed with ethanol for three times, centrifuged at 8,000 rpm for 5.0 min to ensure complete removal of unimmobilized Pep@AuNPs.

### Measurement of L-Cys

Typically, the stock solution of L-Cys (30.0 μL) diluted at different concentrations was added to the reaction mixture of buffer (pH 3.0, 2.72 mL), TMB (36.0 μL), U66-PV-Pep@AuNPs (150.0 μL) and $H_2O_2$ (90.0 μL). The mixture was incubated at 25 °C for 20.0 min before

conducting the UV-vis absorption measurements.

**Metabolic assay of L-Cys in rat serum**

The proposed U66-PV-Pep@AuNPs-TMB-$H_2O_2$ system was applied to determination of L-Cys in the rat serum samples. 30.0 μL rat serums, U66-PV-Pep@AuNPs solution (150.0 μL), TMB (36.0 μL, 25.0 mM), $H_2O_2$ (90.0 μL, 10.0 M) and acetate buffer (2.72 mL, 12.0 mM, pH 3.0) were mixed. After the mixture was mixed and incubated at 25 $^oC$ for 20.0 min, the UV-vis absorption measurements were conducted.

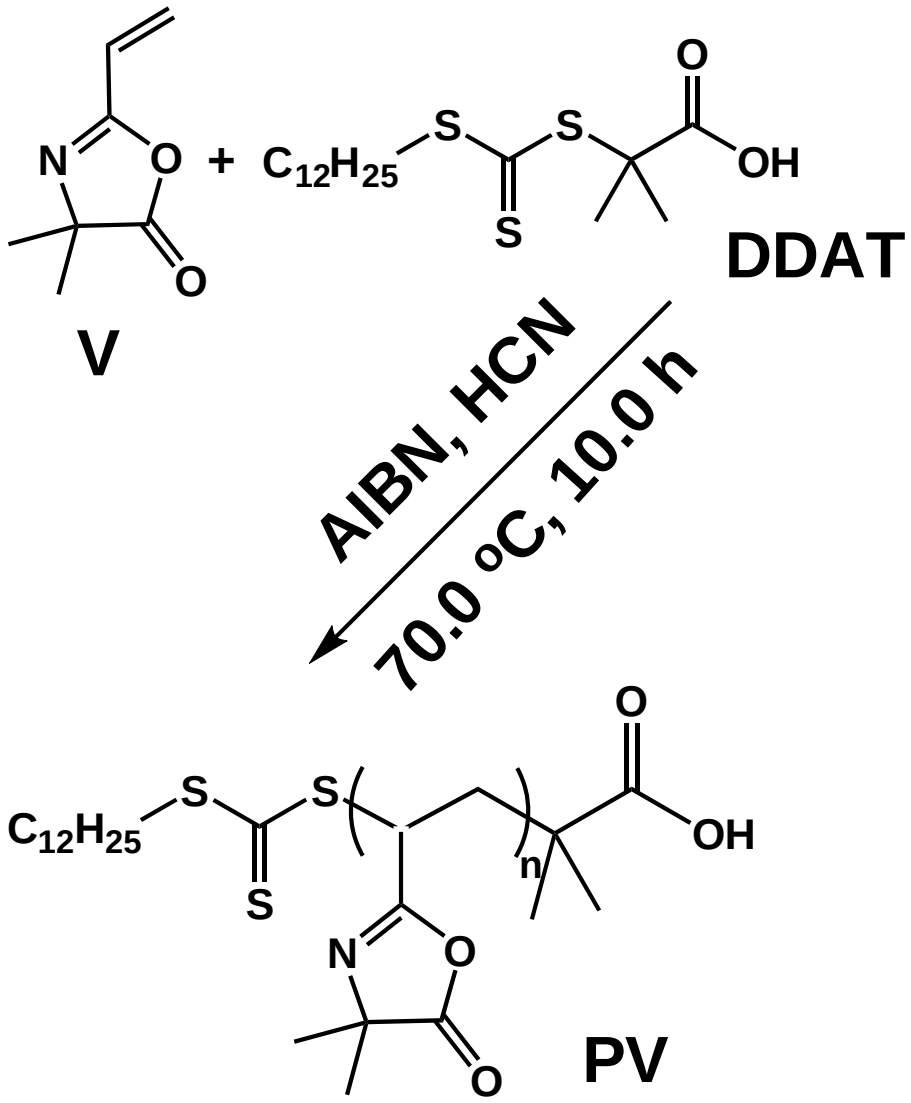


**Fig. S1.** Schematic diagram of the polymerization of PV.

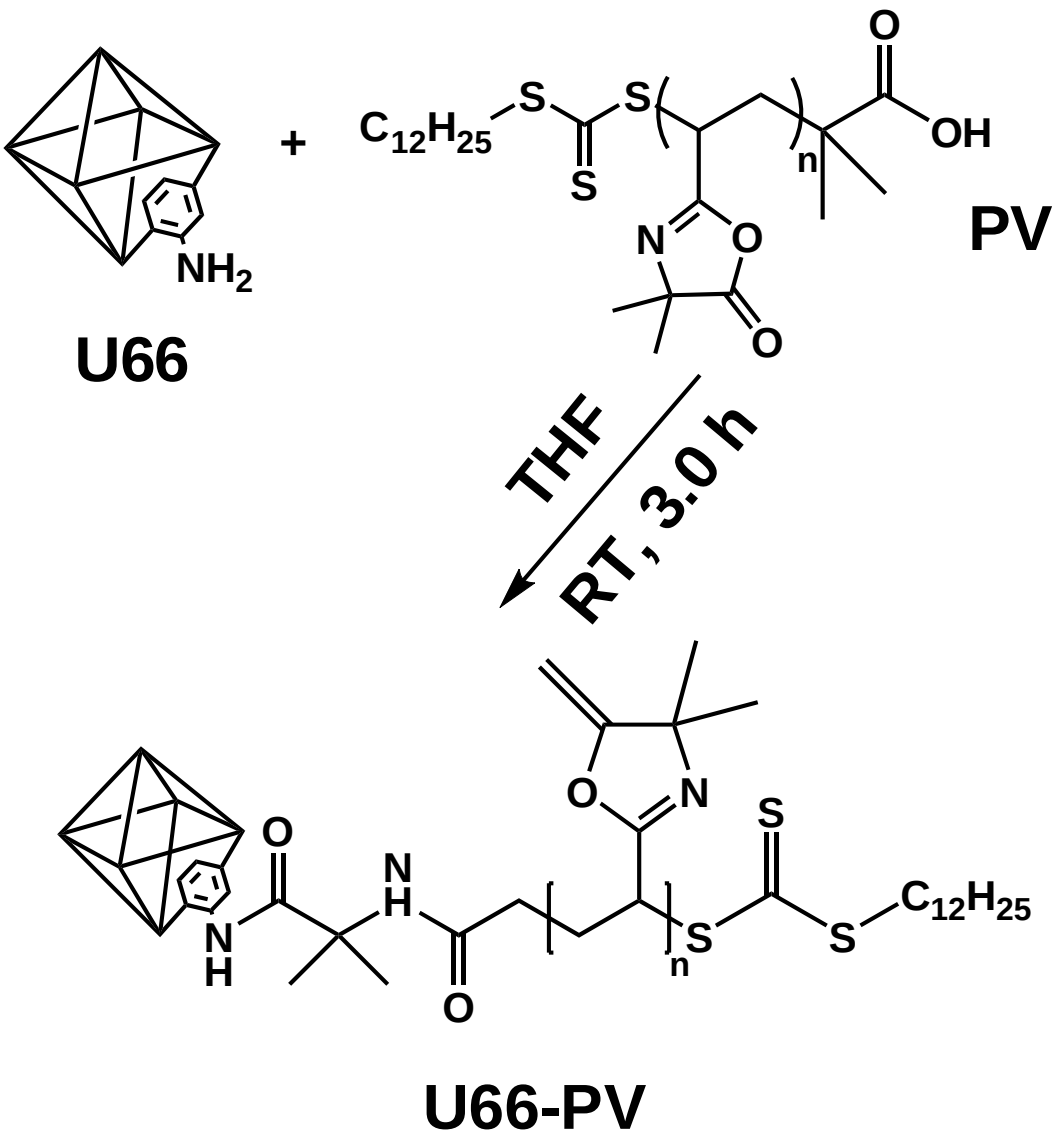


**Fig. S2.** Schematic diagram of the MOF-PV synthesis process.

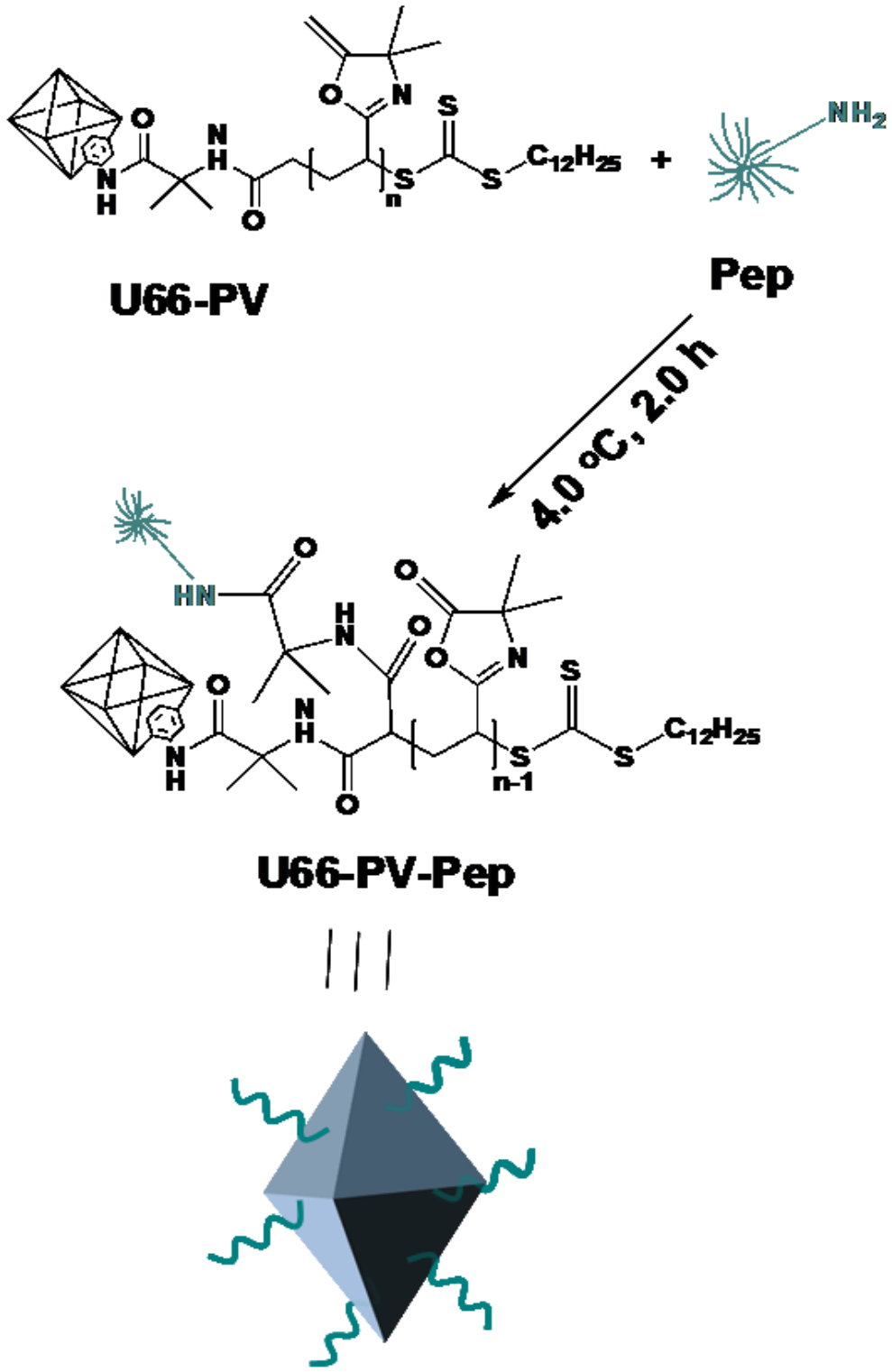


**Fig. S3.** The immobilization of pepsin with MOF-PV-P.

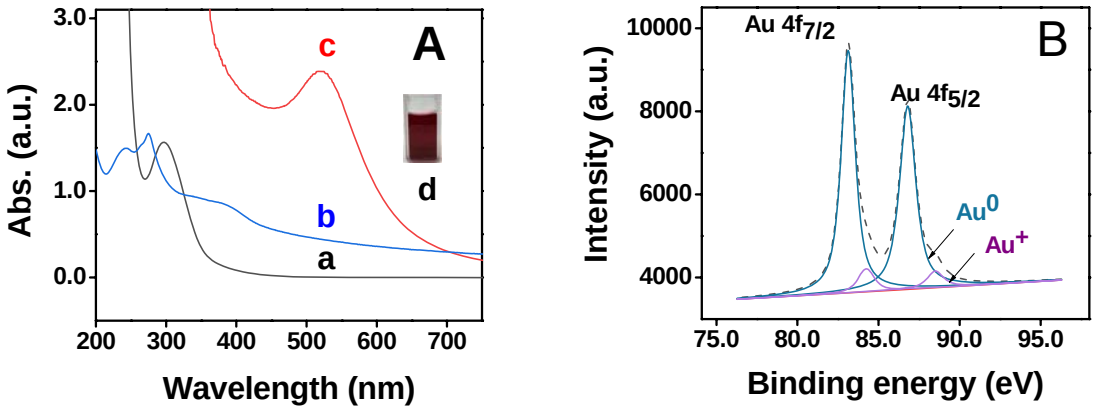


**Fig. S4.** (A) UV-vis of $HAuCl_4$ (a), U66-PV-Pep (b) and U66-PV-Pep@AuNPs (c); inset photo: U66-PV-Pep@AuNPs under daylight (d); (B) XPS spectra of Au 4f orbitals of U66-PV-Pep@AuNPs.

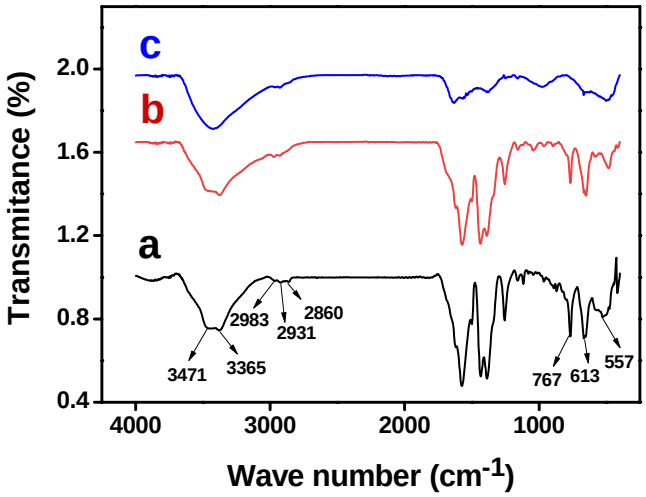


**Fig. S5.** FT-IR spectra of U66-PV (a); U66-PV-Pep (b) and U66-PV-Pep@AuNPs (c).

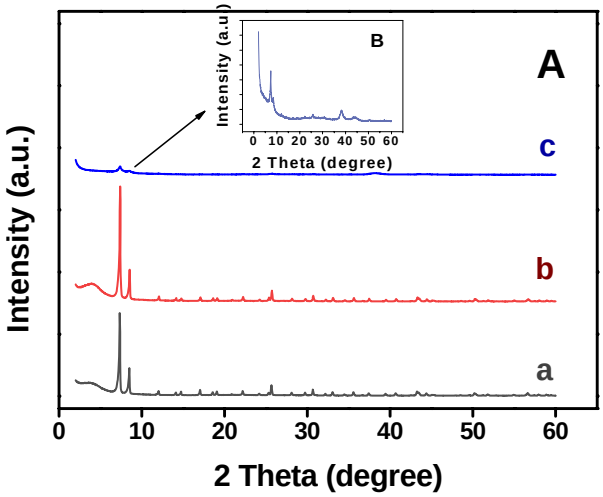


**Fig. S6.** PXRD patterns of (A) U66 (a), U66-PV (b) and U66-PV-Pep@AuNPs (c). The inset is the magnification of the corresponding to U66-PV-Pep@AuNPs (B).

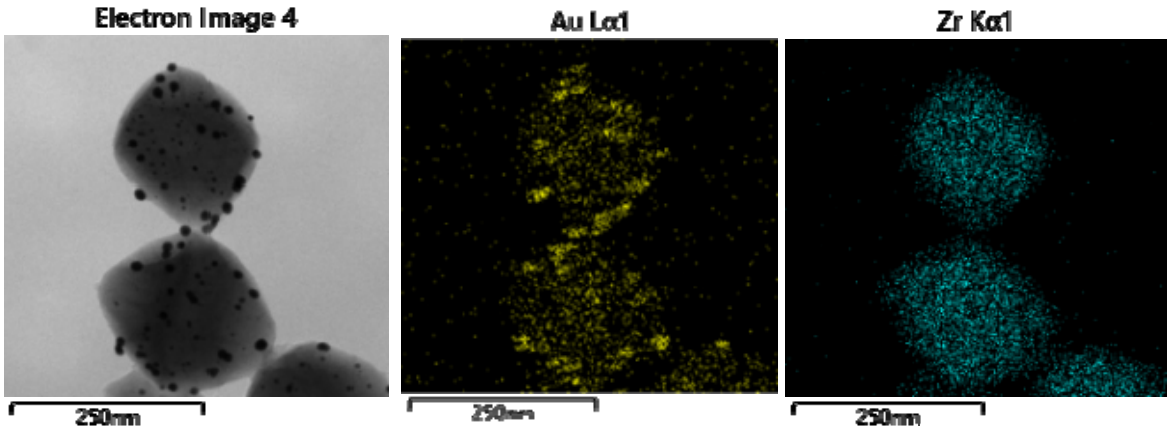


**Fig. S7.** EDS-mapping images of U66-PV-Pep@AuNPs.

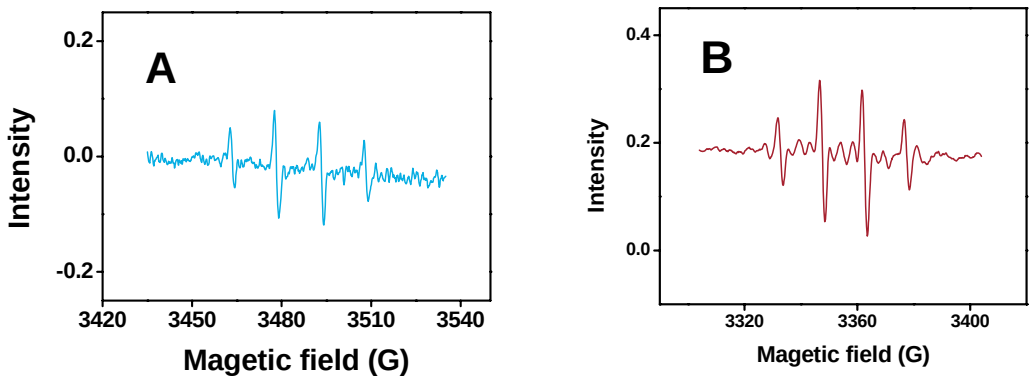


**Fig. S8.** EPR signals of (A) DMPO-$H_2O_2$ and (B) U66-PV-Pep@AuNPs-DMPO-$H_2O_2$. The concentrations of DMPO, U66-PV-Pep@AuNPs, $H_2O_2$ and L-Cys were 0.1 M, 0.1 mg/mL, 0.3 M and 0.25 μM, respectively.

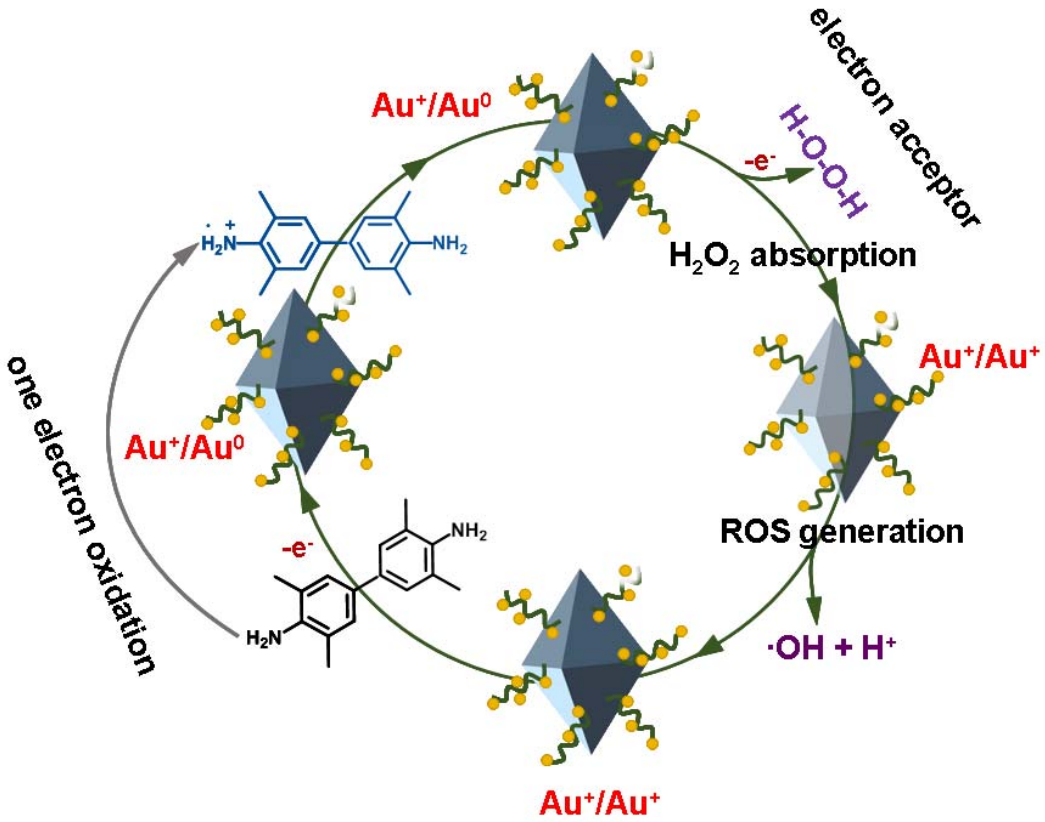


**Fig. S9.** Possible mechanism for the POD-mimetic activity of U66-PV-Pep@AuNPs.

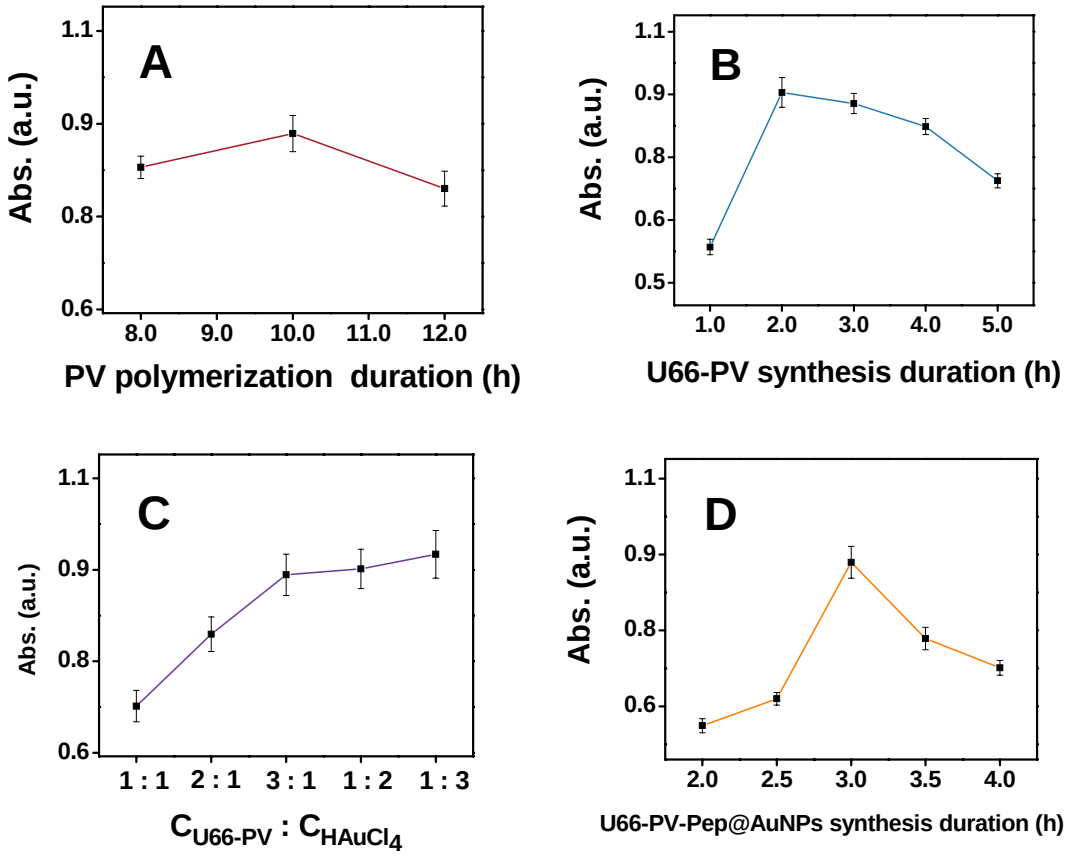


**Fig. S10.** Dependence of POD-mimetic activity of U66-PV-Pep@AuNPs on (A) PV polymerization duration; (B) U66-PV synthesis duration; (C) concentration ratio of U66-PV-Pep to $HAuCl_4$ and (D) U66-PV-Pep@AuNPs synthesis duration, (n=3).

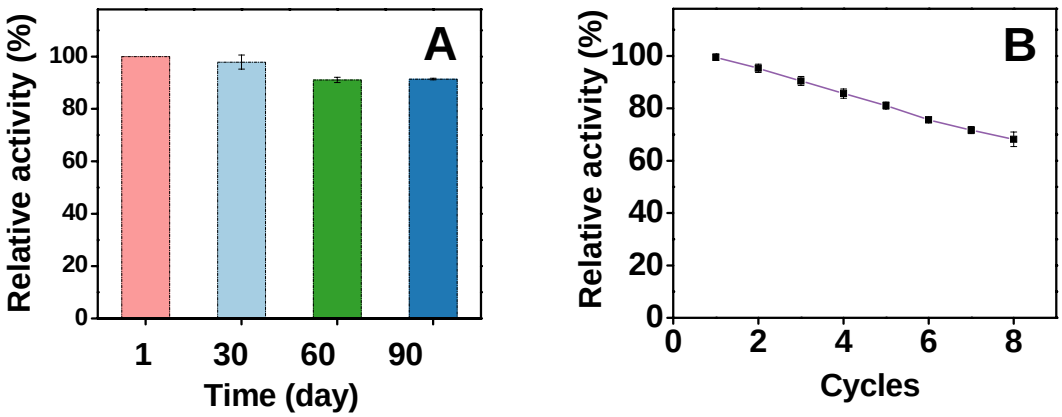


**Fig. S11.** Change in catalytic activity of U66-PV-Pep@AuNPs nanozymes after storage at room temperature (A). Relative catalytic activity of U66-PV-Pep@AuNPs in the TMB oxidation during the recycling processes (B).

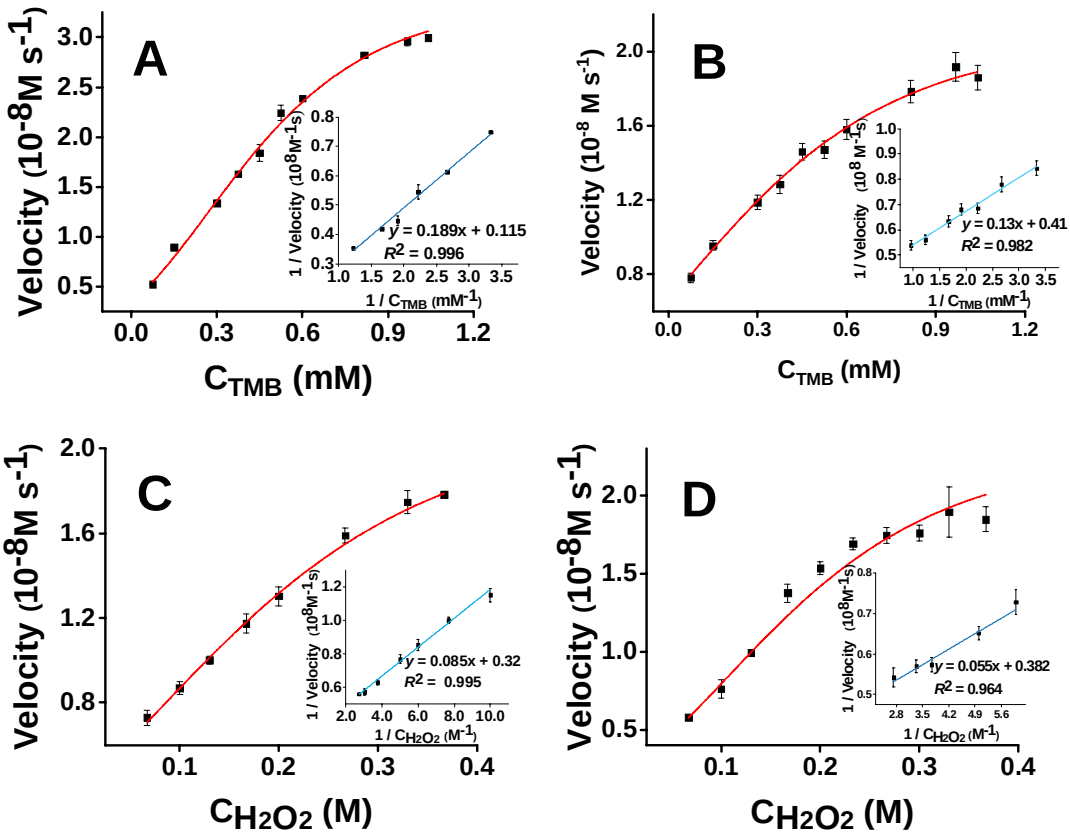


**Fig. S12.** The steady-state kinetics assays of the U66-PV-Pep@AuNPs (A, C) and Pep@AuNPs on U66 (B, D). Insets showed the Lineweaver-Burk plots of the Michaelis-Menten equations.

**Table S1** Comparison of kinetic parameters of the reported AuNPs based nanozymes

| Nanozymes | $K_m$ (mM) | $V_{max}$ ($10^{-8}$ M $s^{-1}$) | Ref. |
|---|---|---|---|
| **PDMAM-2@AuNPs** | 0.140 | 0.49 | Q. Ma, *et. al*. Anal. Bioanal. Chem., 2022, 414, 6047 |
| **CDs@AuNPs** | 0.058 | 1.86 | C. Zhang, *et. al*. RSC Adv., 2016, 6, 35280 |
| **PTA@AuNPs** | 0.954 | 1.15 | F. Shah, *et. al*. Colloids Surf., 2021, 198, 111478 |
| **G-$C_3N_4$@AuNPs** | 0.097 | 1.52 | N. Wu, *et. al*. Anal. Chim. Acta, 2019, 1091, 69 |
| **U66-PV-Pep@AuNPs** | 0.189 | 8.69 | **This work** |

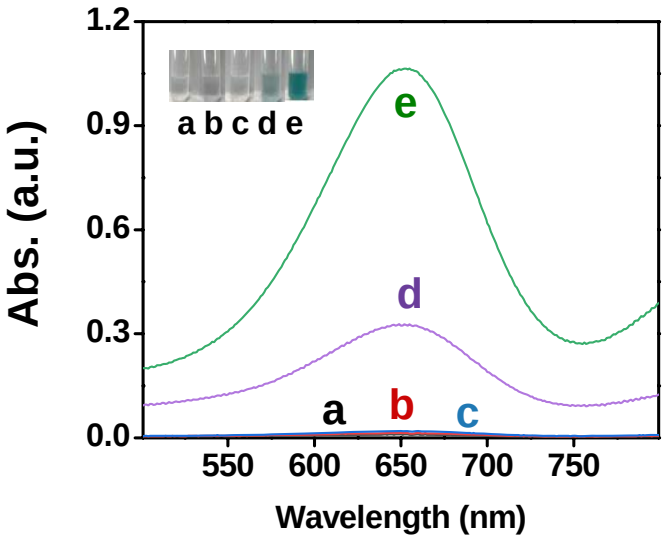


**Fig. S13.** The UV-*vis* absorption spectra and photos (insert) of different systems: (a) TMB-$H_2O_2$; (b) U66-PV-Pep-TMB-$H_2O_2$; (c) L-Cys-TMB-$H_2O_2$; (d) U66-PV-Pep@AuNPs-TMB-$H_2O_2$-L-Cys; (e) U66-PV-Pep@AuNPs-TMB-$H_2O_2$.

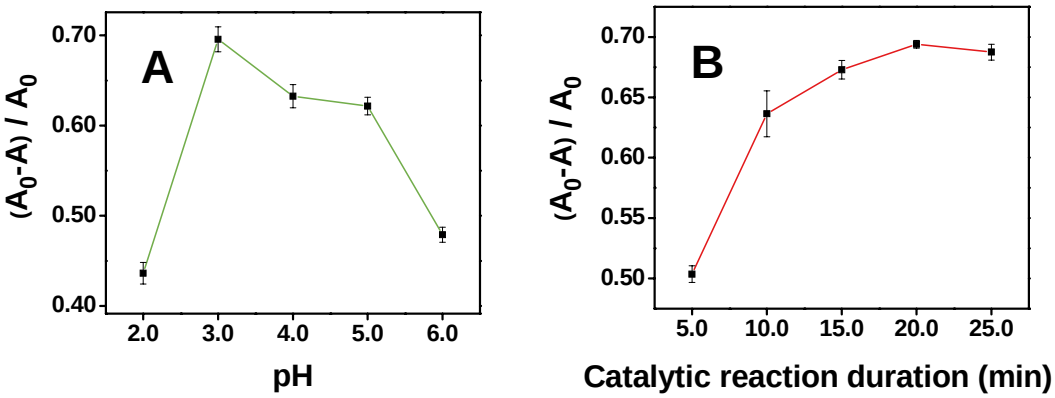


**Fig. S14.** Dependence of POD-mimetic activity of U66-PV-Pep@AuNPs on (A) buffer pH and (B) catalytic reaction duration (n=3).

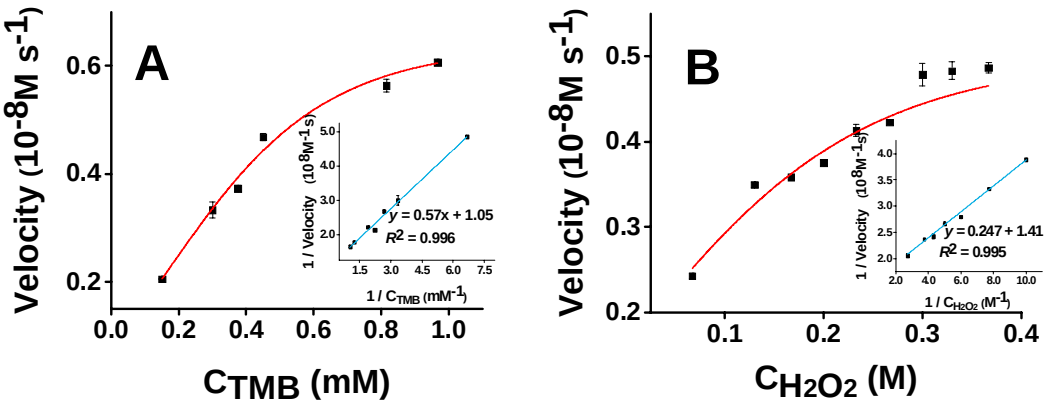


**Fig. S15.** The steady-state kinetics study of U66-PV-P@AuNPs-L-Lys with TMB (A) and $H_2O_2$ as the substrate (B). Insets showed the Lineweaver-Burk plots of the Michaelis-Menten equations.

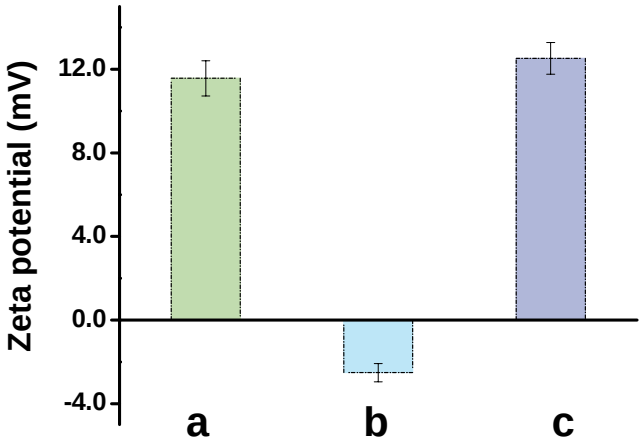


**Fig. S16.** The apparent zeta potentials of (a) U66-PV-Pep@AuNPs-L-Cys; (b) L-Cys and (c) U66-PV-Pep@AuNPs (n=3).

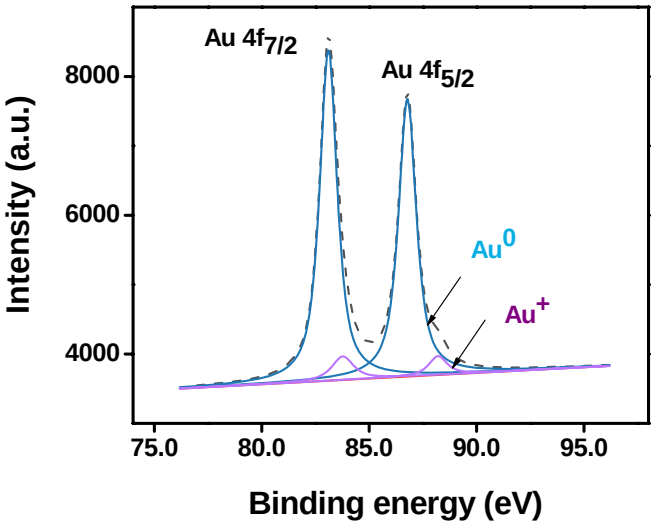


**Fig. S17.** XPS spectra of Au 4f orbitals of U66-PV-Pep@AuNPs-L-Cys.

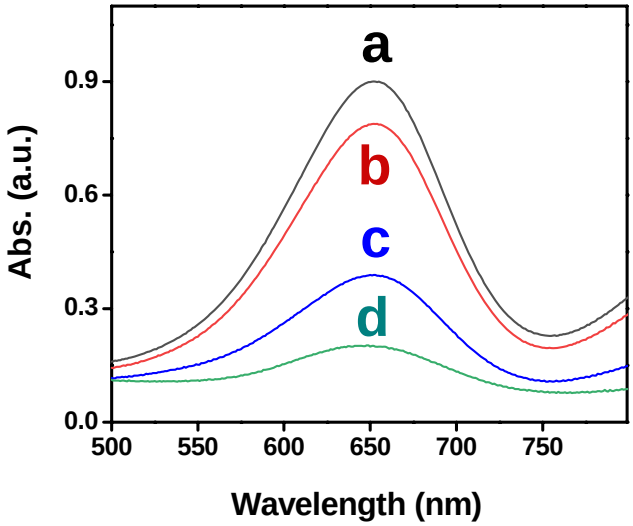


**Fig. S18.** Effect of ·OH inhibitors on the absorbance of U66-PV-Pep@AuNPs-TMB-$H_2O_2$ and Pep@AuNPs-on-U66 in the absence (a,c) and presence (b,d) of 2.0 mM t-tubyl alcohol.

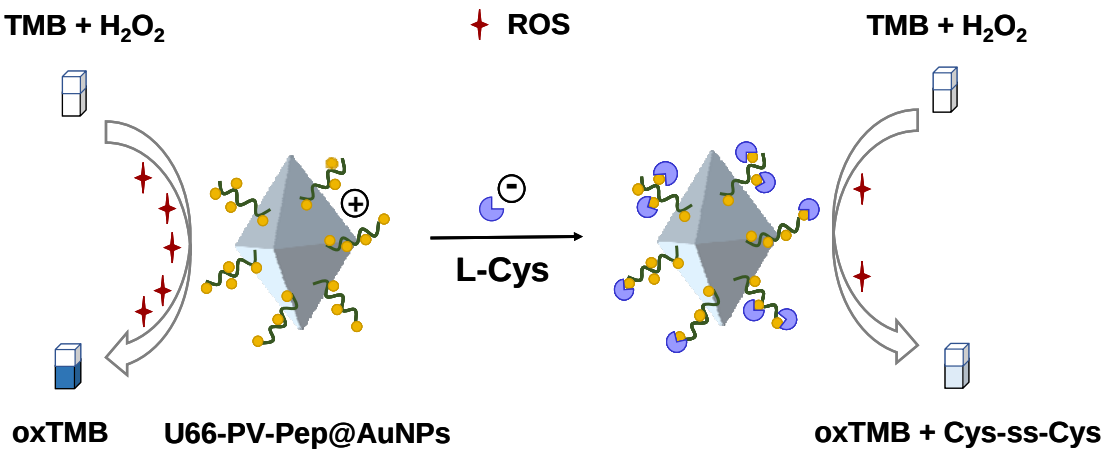


**Fig. S19.** Mechanism of L-Cys detection in U66-PV-Pep@AuNPs-TMB-$H_2O_2$ system.

**Table S2.** Recovery of the proposed assay*

| Serums | Added (μM) | Found (μM) | Recovery (%) | RSD (%) |
|---|---|---|---|---|
| 1 | 5.0 | 4.97 | 99.4 | 4.1 |
| | 10.0 | 9.61 | 96.1 | 3.1 |
| | 15.0 | 14.55 | 97.0 | 3.7 |
| 2 | 5.0 | 4.90 | 98.0 | 4.3 |
| | 10.0 | 8.83 | 104.3 | 6.2 |
| | 15.0 | 14.56 | 97.1 | 3.3 |
| 3 | 5.0 | 4.40 | 98.0 | 6.4 |
| | 10.0 | 9.02 | 90.2 | 2.7 |
| | 15.0 | 13.99 | 93.3 | 1.9 |

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