Electronic Supplementary Material (ESI) for Analyst.

Supporting information

Polymer capped gold nanoparticles as nanozymes with improved catalytic activity for monitoring of serum ciprofloxacin

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Experiments

Materials and chemicals

Ciprofloxacin (CIP) and 5,5'-dimethyl-1-pyrroline *N*-oxide (DMPO) was supplied ARK Pharm, Inc. (Shanghai, China). Acrylamide (AM) was gotten from Alfa Aesar Chemicals Co. Ltd. (Shanghai, China). Trithiocarbonate (DDAT) was obtained from Sigma-Aldrich Co. Ltd. (S. Lewis. MO, USA). $HAuCl_4$ was bought from Shenyang Jinke Reagent Factory (Shenyang, China). Sodium acetate (NaAc) was gotten from Beijing Yili Fine Chemicals Co., Ltd. (Beijing, China). Zinc sulfate ($ZnSO_4$), magnesium chloride ($MgCl_2$) and streptomycin (STR) were obtained from Aladdin Chemistry Company (Shanghai, China). Amino acids (Gly, L-Met, L-Pro, L-Arg, L-Ser, L-His, L-Val) were purchased from TCI Shanghai Co. Ltd. (Shanghai, China). Hydrogen peroxide (H_2O_2, 30.0%, w/w), 3,3',5,5'-tetramethylbenzidine (TMB), 2,2-azobisisobutyronitrile (AIBN), cetyltrimethylammonium chloride (CTAC), benzoquinone 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 potentials of PAM-4@AuNPs, CIP and PAM-4@AuNPs-CIP were carried out with a Zetasizer laser particle analyzer (Zetasizer Nano ZS ZEN3600, British).

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

Transmission electron microscopy (TEM) images were obtained using a transmission electron microscope (JEM-2010, Japan electron optics laboratory, Japan) at a voltage of 200 kV.

Electron paramagnetic resonance (EPR) signals were measured by a Bruker ESP 300E spectrometer (Bruker, Rheinstetten, Germany) with a microwave bridge (receiver gain, 1×105; 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 poly(acrylamide) (PAM)

All of the glasswares were washed with aqua regia (HCl : HNO_3 volume ratio = 3.0 : 1.0) and rinsed with ultrapure water. Typically, the PAM-1, PAM-2, PAM-3, PAM-4 and PAM-5 were prepared *via* reversible addition-fragmentation chain transfer polymerization (RAFT) method (Fig. S1A). Typically, 10.0 mM AM, 10.0 mg DDAT and 20.0 mg AIBN were added into 10.0 mL 1, 4-dioxanein a 20.0 mL-glass flask. Then the flask was sealed under nitrogen after three freeze-evacuate-thaw cycles, and then placed in an oil bath thermostatted at 60 ^{o}C for 4.0-12.0 h. The final polymers were obtained by pouring the reaction mixture into excess absolute ether while stirring, repeating the dissolving-precipitation three times, the polymer collected by filtration was dried in a vacuum oven at room temperature overnight, and stored at room temperature for further use.

Preparation of nanozymes

All of the glasswares were washed with aqua regia (HCl:HNO_3 volume ratio = 3:1) and rinsed with ultrapure water. The PAM-1@AuNPs, PAM-2@AuNPs, PAM-3@AuNPs, PAM-4@AuNPs and

PAM-5@AuNPs were prepared with PAM-1, PAM-2, PAM-3, PAM-4 and PAM-5 as the reducing and capping agent, respectively (Fig. S1B). Simply, in a 20.0 mL-glass flask, 2.5 mL of $HAuCl_4$ (10.0 mM), 0.25 mL of NaOH (1.0 M) and 2.5 mL of PAM-1 or PAM-2 or PAM-3 or PAM-4 or PAM-5 (2.0 mM) aqueous solutions were added and mixed under gentle stirring at 100 ^{o}C for 10 min. The PAM-1@AuNPs or PAM-2@AuNPs or PAM-3@AuNPs or PAM-4@AuNPs or PAM-5@AuNPs solution was centrifuged to remove the larger particles at 10,000 rpm for 10 min. Finally, the PAM-1@AuNPs or PAM-2@AuNPs or PAM-3@AuNPs or PAM-4@AuNPs or PAM-5@AuNPs supernatant was collected and stored at 4 ^{o}C for further use.

POD-like catalytic activity of PAM-4@AuNPs

The POD-like catalytic activity of PAM-4@AuNPs was surveyed through the oxidation with TMB (36.0 μL, 25.0 mM) as the chromogenic peroxidase substrate by the PAM-4@AuNPs catalyst (50.0 μL) in the presence of H_2O_2 (90.0 μL, 10.0 M) with an acetate buffer solution (2.80 mL, 12.0 mM, pH 4.0). The incubation time was 15.0 min at 25 °C for the reaction mixture, followed by ultraviolet-visible (UV-*vis*) absorbance measurements of the solution at 650 nm.

Effect of PAM chain length on the catalytic activity of the nanozymes

The effect of PAM chain length on the catalytic activity of PAM-1@AuNPs or PAM-2@AuNPs or PAM-3@AuNPs or PAM-4@AuNPs or PAM-5@AuNPs was obtained through the oxidation with TMB (36.0 μL, 25.0 mM) by the different PAM chain length modified AuNPs based catalysts (50.0 μL) in the presence of H_2O_2 (90.0 μL, 10.0 M) with an acetate buffer solution (2.80 mL, 12.0 mM, pH 4.0). Synthesis of PAM with different chain length (PAM-1 or PAM-2 or PAM-3 or PAM-4 or PAM-5) was controlled by different polymerization time (4.0 h, 6.0 h, 8.0 h, 10.0 h, 12.0 h) as shown in Table S1.

The molecular weight (M_W) of PAM and its polymerization of degree (DP) in different polymerization time was calculated by monomer conversion (Table S1) as described in Ref. [1]

Steady-state kinetic analysis of PAM-4@AuNPs

The Line weaver Burk plot drawn using the Michaelis-Menten equation was examined with respect to the change in the UV-*vis* absorbance at 650 nm. Enzymatic kinetics was tested for studying the relationship between the initial velocity of the PAM-4@AuNPs (catalyzed reaction) and the concentrations of TMB. The typical equation for Michaelis-Menten kinetics is: [2]

$$V_0 = V_{max}[S] / (K_m + [S]) \quad (1)$$

where V_0 is the initial velocity, V_{max} is the maximum velocity, K_m is the Michaelis-Menten constant and [*S*] is the concentration of the substrate.

CIP detection

CIP standard solutions (0.2-2.5 mM) were prepared. CIP solution (30.0 μL, 1.0 mM), PAM-4@AuNPs solution (50.0 μL), TMB (36.0 μL, 25.0 mM) and H_2O_2 (90.0 μL, 10.0 M) was mixed with sodium acetate buffer solution (2.80 mL, 12.0 mM, pH 4.0). The mixture was incubated at 25 ^{o}C for 15.0 min before conducting the UV-*vis* absorption measurements at 650 nm.

Metabolic assay of CIP in rat serum

Three male-Sprague-Dawley-rats (about 250 g) were gotten from Beijing Vital River Laboratory Animal Technology Co. Ltd. (Beijing, China). The controlled blank serum samples and five other serum samples (at 0 h, 0.5 h, 1.5 h, 3.5 h, 5.0 h, 7.0) were collected after 13.2 mg/kg CIP dissolved in physiological saline solution was injected into the abdominal cavity of rats. The rat serum samples were pre-treated to eliminate the interferences-proteins. Simply, 0.1 mL of the fresh rat serum samples was diluted by 0.1

mL of ethanol, which was incubated at 25 ^{o}C for 10.0 min. Consequently, the samples were centrifuged at 10,000 rpm for 10 min and the supernatant was collected and stored at 4 ^{o}C for further analysis.

The proposed colorimetric PAM-4@AuNPs-TMB-H_2O_2 system was applied to testing CIP in the rat serum samples. 30.0 μL rat serums, PAM-4@AuNPs solution (50.0 μL), TMB (36.0 μL, 25.0 mM), H_2O_2 (90.0 μL, 10.0 M) and acetate buffer (2.80 mL, 12.0 mM, pH 4.0) were mixed. After the mixture was mixed and incubated at 25 °C for 15.0 min, the UV-*vis* absorption measurements were conducted.

References

[1] R. Chang, X. Wang, X. Li, H. An and J. Qin, *ACS Appl. Mater. Interfaces,* 2016, **8**, 25544-25551.

[2] Y. Liu, D. Ding, Y. L. Zhen, and R. Guo, *Biosens. Bioelectron.* 2017, **92**, 140-146.

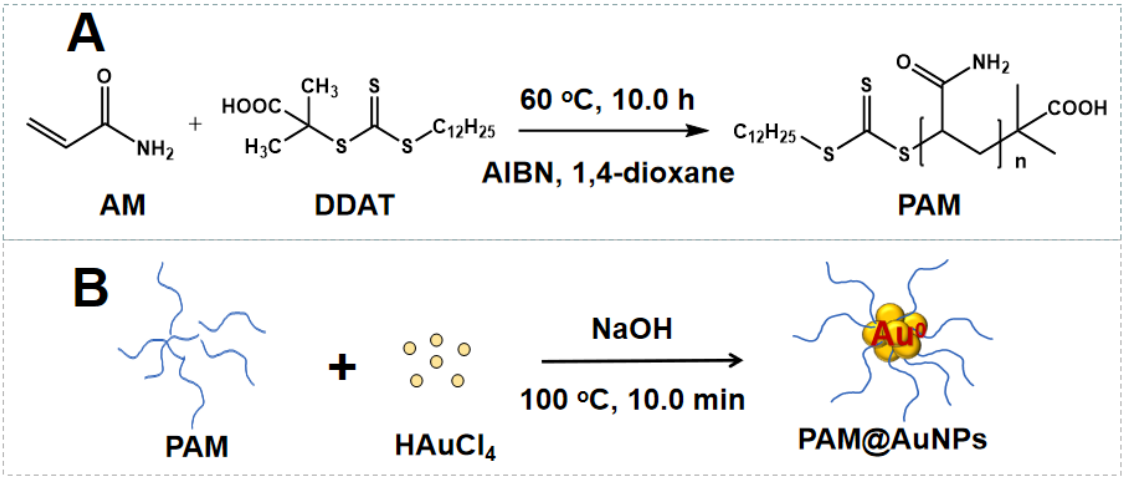

Fig. S1. Schematic diagram of the synthesis process of PAM (A) and PAM@AuNPs (B).

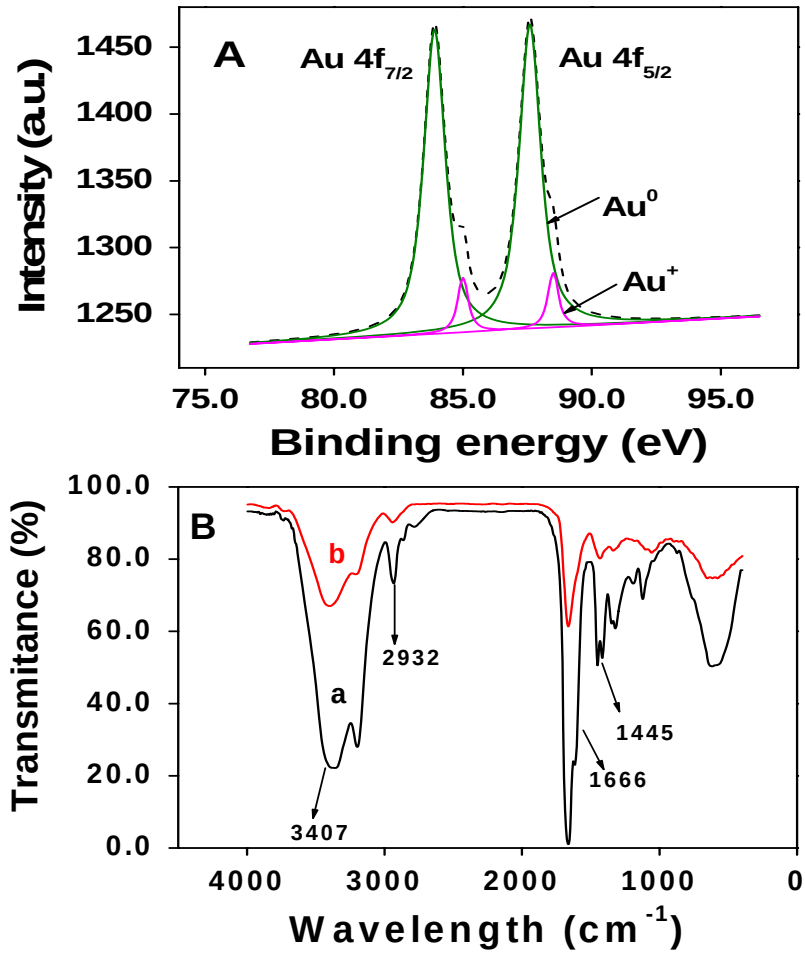

Fig. S2. (A) XPS spectra of Au 4f orbitals of PAM-4@AuNPs; (B) FT-IR spectra of PAM-4 (a) and PAM-4@AuNPs (b).

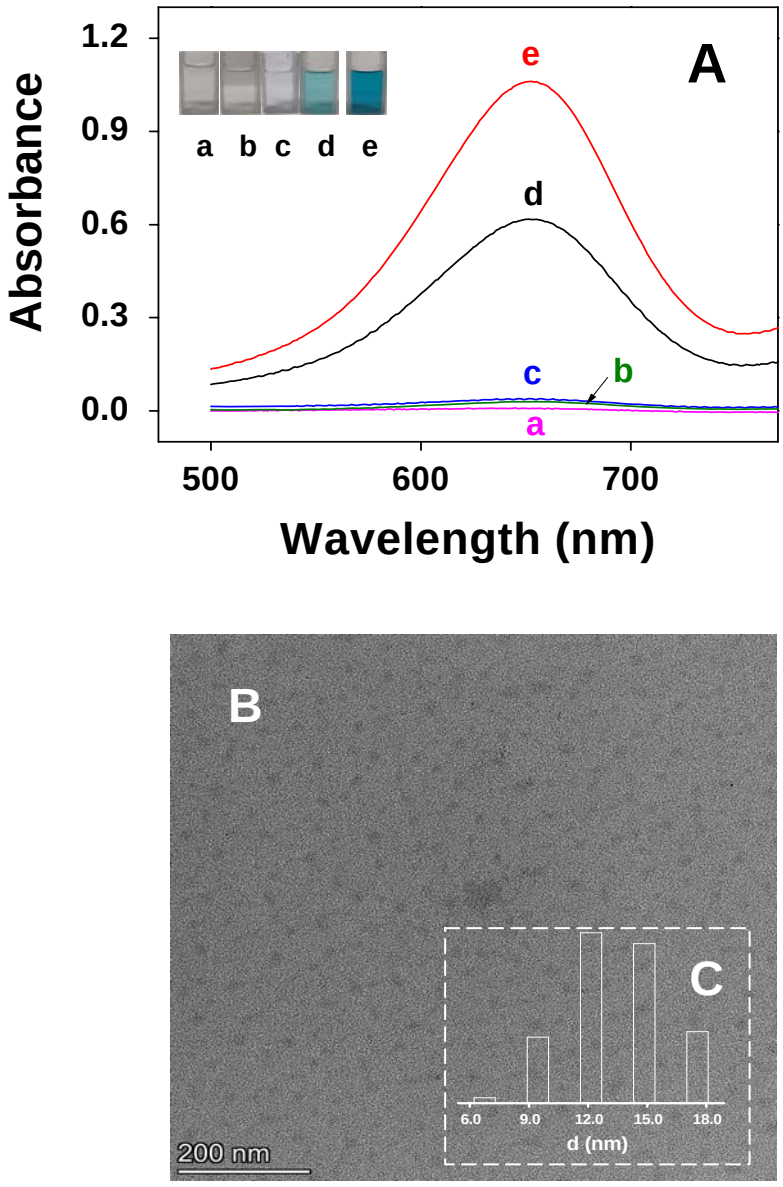

Fig. S3. (A) The UV-vis absorption spectra and photos of different systems: (a) TMB-H_2O_2; (b) TMB-H_2O_2-PAM; (c) TMB-H_2O_2-CIP; (d) PAM-4@AuNPs-TMB-H_2O_2; (e) PAM-4@AuNP-TMB-H_2O_2-CIP. (B) TEM image and (C) size distribution of PAM-4@AuNPs-CIP.

Table S1. The M_W and DP of PAMs prepared in different polymerization time

PAMs	Polymerization time (h)	Yield (g)	M_W (10^4 g mol^{-1})	DP
PAM-1	4.0	0.558	1.99	280
PAM-2	6.0	0.602	2.15	303
PAM-3	8.0	0.639	2.29	322
PAM-4	10.0	0.655	2.35	330
PAM-5	12.0	0.693	2.49	350

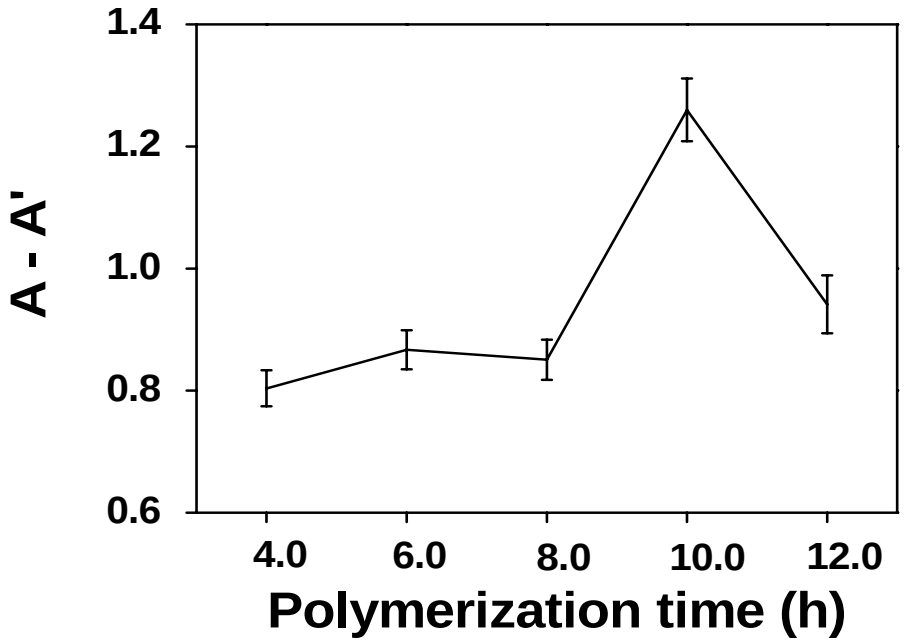

Fig. S4. Dependence of the POD-like activity of PAM-1@AuNPs, PAM-2@AuNPs, PAM-3@AuNPs，PAM-4@AuNPs or PAM-5@AuNPs on polymerization time of PAMs. A' and A represent the UV-*vis* absorption of the TMB-H_2O_2 system in the absence and presence of the nanozymes, respectively.

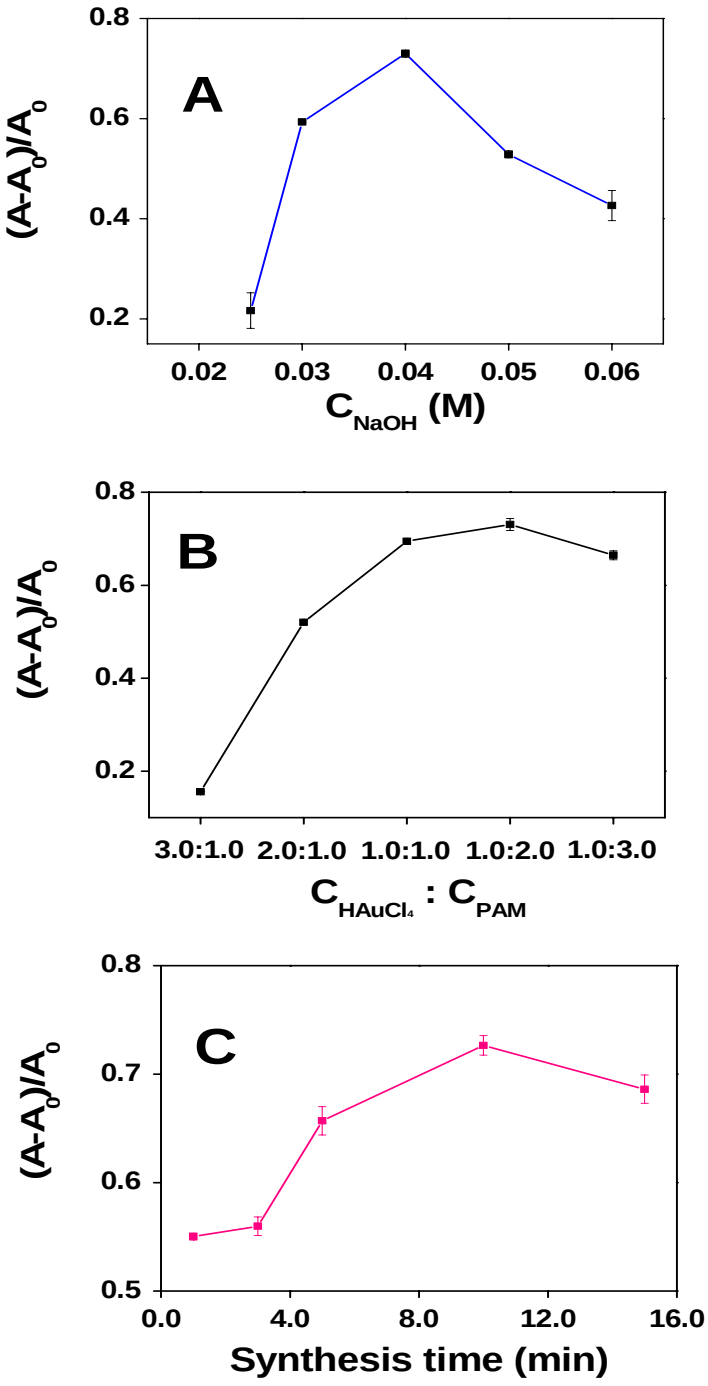

Fig. S5. Dependence of the POD-like activity of PAM-4@AuNPs on (A) concentration of NaOH; (B) concentration ratio of $HAuCl_4$ to PAM-4 and (C) synthesis time of PAM-4@AuNPs. A and A_0 represent the UV-*vis* absorption of the PAM-4@AuNPs-TMB-H_2O_2 system in the presence and absence of CIP, respectively.

Table S3. Comparison of the polymer@AuNPs based nanocatalysts synthesis conditions

Nanocatalysts	Synthesis temperature (^{o}C)	Synthesis time (h)	Reductants /stablizers	References
PANI-PSS@AuNPs	25	24.0	PANI-PSS/PANI-PSS	X. Liu, et al. *Nanoscale* 2014, **6**, 5223.
MMT-PANI@AuNPs	25	24.0	Citrate/MMT-PANI	Y. Xia, et al. *RSC Adv.* 2014, **4**, 20516.
PNIPAM@Au-AgNPs	25	0.55	Ascorbic acid /PNIPAM	D. Li, et al. *J. Nanopart. Res.* 2017, **19**, 377.
PVP@AuNPs	0	0.5	Sodium borohydride /PVP	B. Agrawal, et al. *J. Nanopart. Res.* 2021, **23**, 67.
PAM-4@AuNPs	100	0.17	PAM/PAM	**This work**

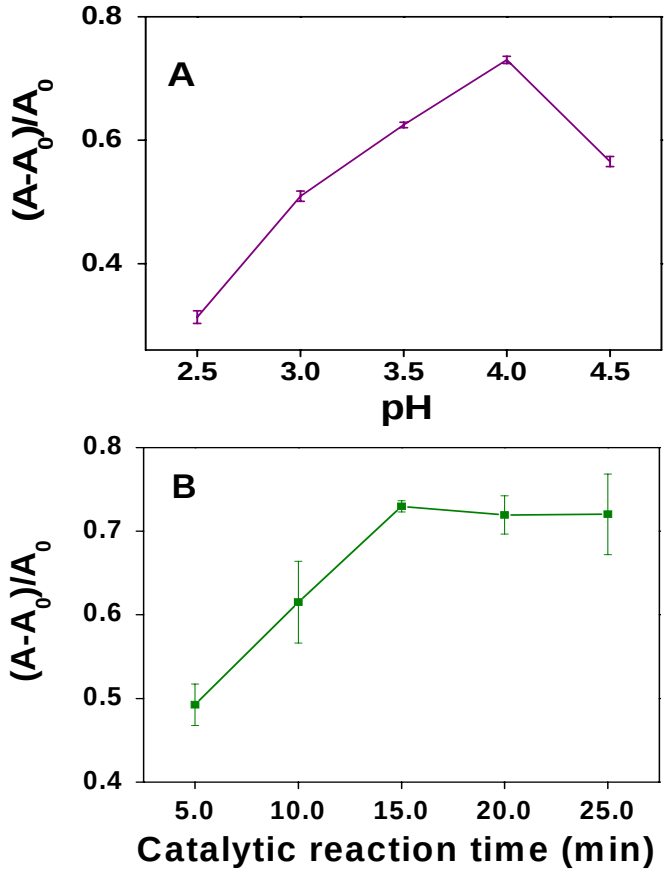

Fig. S6. Effect of (A) buffer pH and (B) incubation time on the POD-like activity of PAM-4@AuNPs. A and A_0 represent the UV-*vis* absorption of the PAM-4@AuNPs-TMB-H_2O_2 system in the presence and absence of CIP, respectively.

Table S3. Kinetics of the nanozymes with TMB as the substrate

Catalysts	K_m (mM)	V_{max} (10^{-8} M·s^{-1})
PAM-4@AuNPs	0.10	2.69
PAM-4@AuNPs-CIP	1.95	5.00

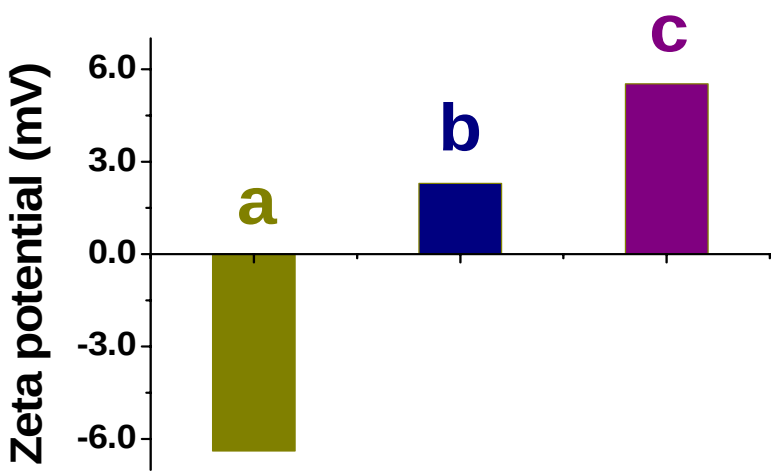

Fig. S7. The apparent zeta potentials of (a) PAM-4@AuNPs; (b) CIP and (c) PAM-4@AuNPs-CIP, respectively.

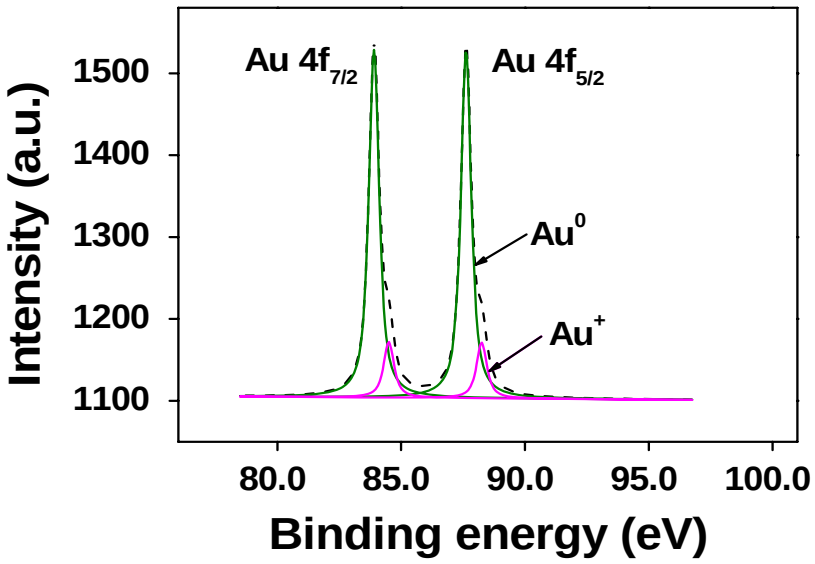

Fig. S8. XPS spectra of Au 4f orbitals of PAM-4@AuNPs-CIP.

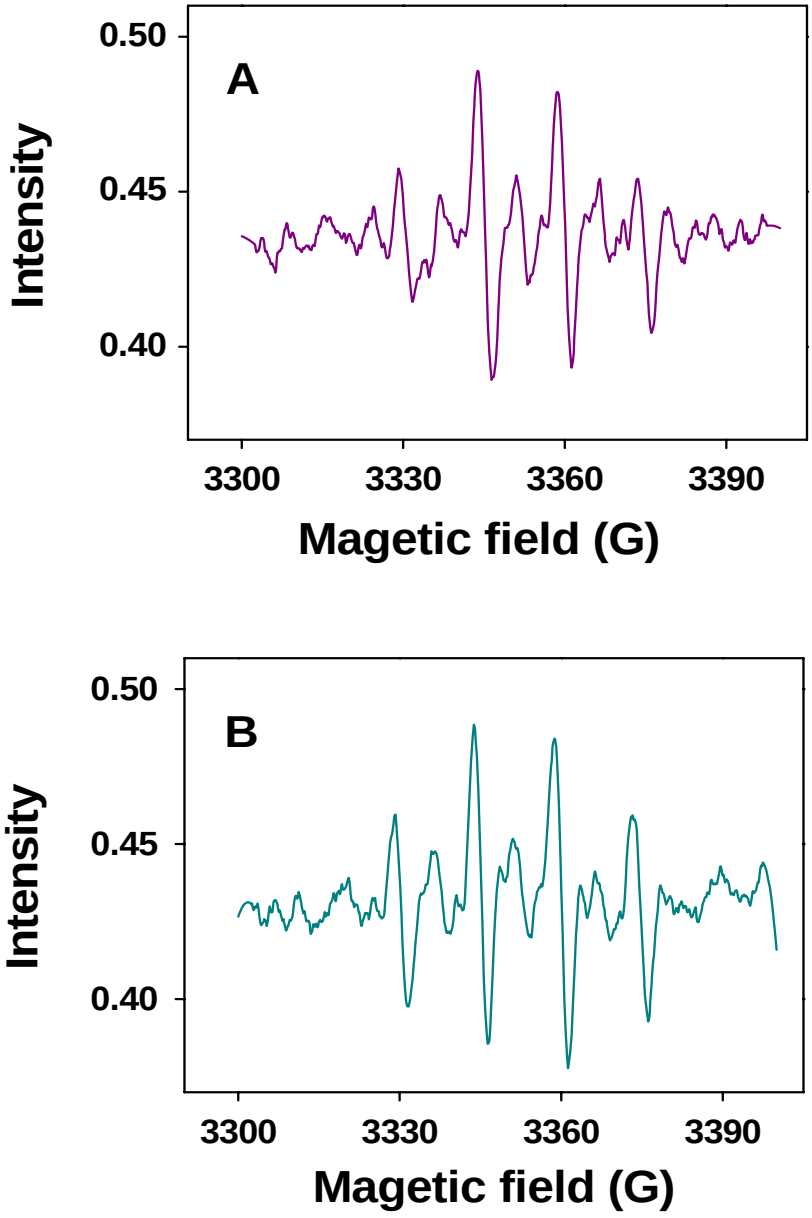

Fig. S9. EPR signals of (A) DMPO-H_2O_2-PAM-4@AuNPs and (B) DMPO-H_2O_2-PAM-4@AuNPs-CIP. The concentrations of DMPO, PAM-4@AuNPs, H_2O_2 and CIP were 0.5 M, 0.1 mM, 0.3 M and 25.0 μM, respectively.

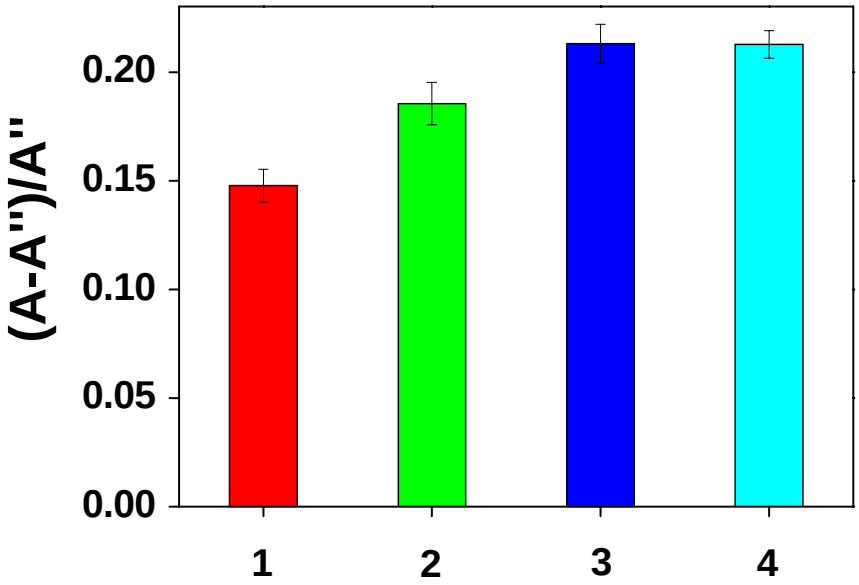

Fig. S10. Effect of ROS inhibitor (0.4 mM benzoquinone) on the UV-*vis* absorbance in different systems: (1) PAM-4@AuNPs-TMB-H_2O_2-CIP; (2) PAM-4@AuNPs-TMB-H_2O_2-STR; (3) PAM-4@AuNPs-TMB-H_2O_2-Mg^{2+} and (4) PAM-4@AuNPs-TMB-H_2O_2-CTAC. A'' and A refer to the UV-*vis* absorption of the systems in the absence and presence of the benzoquinone, respectively.

Table S4. Recovery of the proposed method*

Serums	Added (μM)	Found (μM)	Recovery (%)	RSD (%)
1	3.0	2.97	98.8	0.7
	6.0	5.98	99.5	1.1
	9.0	8.91	98.9	1.9
2	3.0	3.17	105.8	2.0
	6.0	6.25	104.2	2.2
	9.0	9.02	100.2	2.2
3	3.0	3.13	104.1	0.3
	6.0	6.24	103.9	2.2
	9.0	8.53	94.7	1.3

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