Electronic Supplementary Material (ESI) for Analyst.

Supporting information

Colorimetric monitoring of serum dopamine with promotion activity of gold nanoclusters-based nanozymes

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Experiments

Materials and chemicals

Dopamine (DA) was supplied Alfa Aesar Chemicals Co. Ltd. (Shanghai, China). Papain was gotten from Sangon Biotech Co., Ltd. (Shanghai, China). $HAuCl_4$ 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 ($ZnSO_4$) and magnesium chloride ($MgCl_2$) were obtained from Aladdin Chemistry Company (Shanghai, China). L-Amino acids (L-AAs) were purchased from TCI Shanghai Co. Ltd. (Shanghai, China). Hydrogen peroxide (H_2O_2, 30.0%, w/w), 3, 3', 5, 5'-tetramethylbenzidine (TMB), 5.5'-dimethyl pyrroline *N*-oxide (DMPO) 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.

The fluorescence measurements were performed using an F-4600 fluorescence spectrophotometer (Hitachi, Japan).

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).

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 0.1 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 P@AuNCs

All of the glasswares were washed with aqua regia (HCl:HNO_3 volume ratio = 3:1) and rinsed with ultrapure water. The P@AuNCs was prepared with papain as the reducing and capping agent. Simply, in a 20.0 mL-glass flask, 2.5 mL of $HAuCl_4$ (10.0 mM) and 2.5 mL of papain (2.0 mM) aqueous solutions were added and mixed under gentle stirring at 100 ^oC for 10 min. The P@AuNCs solution was centrifuged to remove the larger particles at 10,000 rpm for 10 min. Finally, the P@AuNCs supernatant was collected and stored at 4 ^oC for further use.

DA testing

DA standard solutions (0.2-2.5 mM) were prepared. DA solution (30.0 μL, 1.0 mM), P@AuNCs solution (150.0 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 (2.72 mL, 12.0 mM, pH 3.0). The mixture was incubated at 25 ^oC for 20 min before conducting the UV-*vis* absorption measurements.

Metabolic assay of DA 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 different serum samples (at 0 h, 0.5 h, 1.0 h, 2.0 h, 4.0 h, 6.0) were collected after 7.6 mg/kg DA 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 water, which was heated in a water-bath to boil for 20 min. Consequently, the samples were centrifuged at 10,000 rpm for 10 min and the supernatant was collected and stored at 4 ^oC for further analysis.

The proposed colorimetric P@AuNCs-TMB-H_2O_2 system was applied to determination of DA in the rat serum samples. 30.0 μL rat serums, P@AuNCs 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 °C for 10 min, the UV-*vis* absorption measurements were conducted.

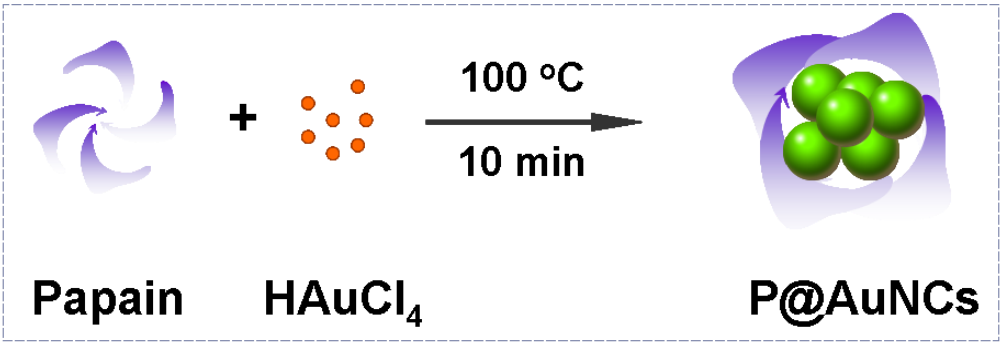

Fig. S1. Schematic diagram of the synthesis process of P@AuNCs.

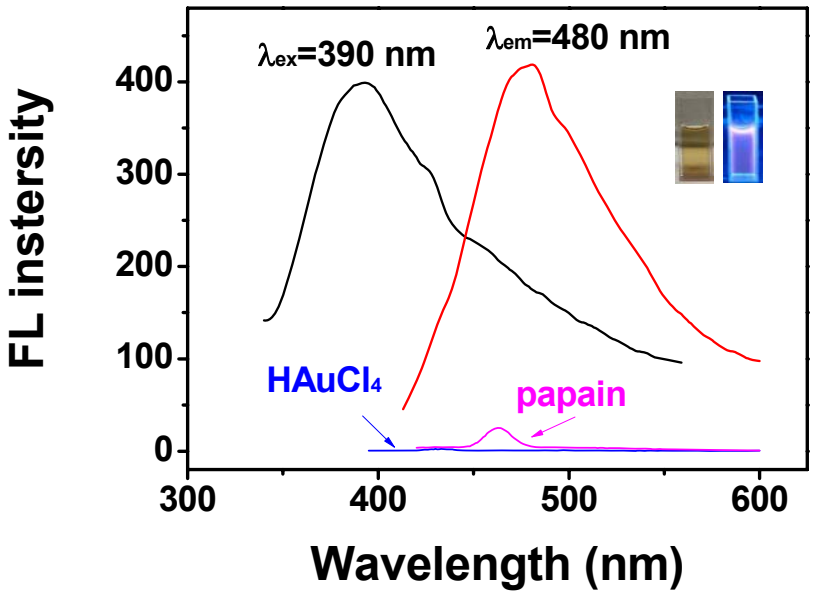

Fig. S2. The fluorescence spectra of the obtained P@AuNCs recorded with an emission wavelength at 480 nm under excitation at 390 nm. Inset: photograph of the P@AuNCs under visible light (left) and UV light (right).

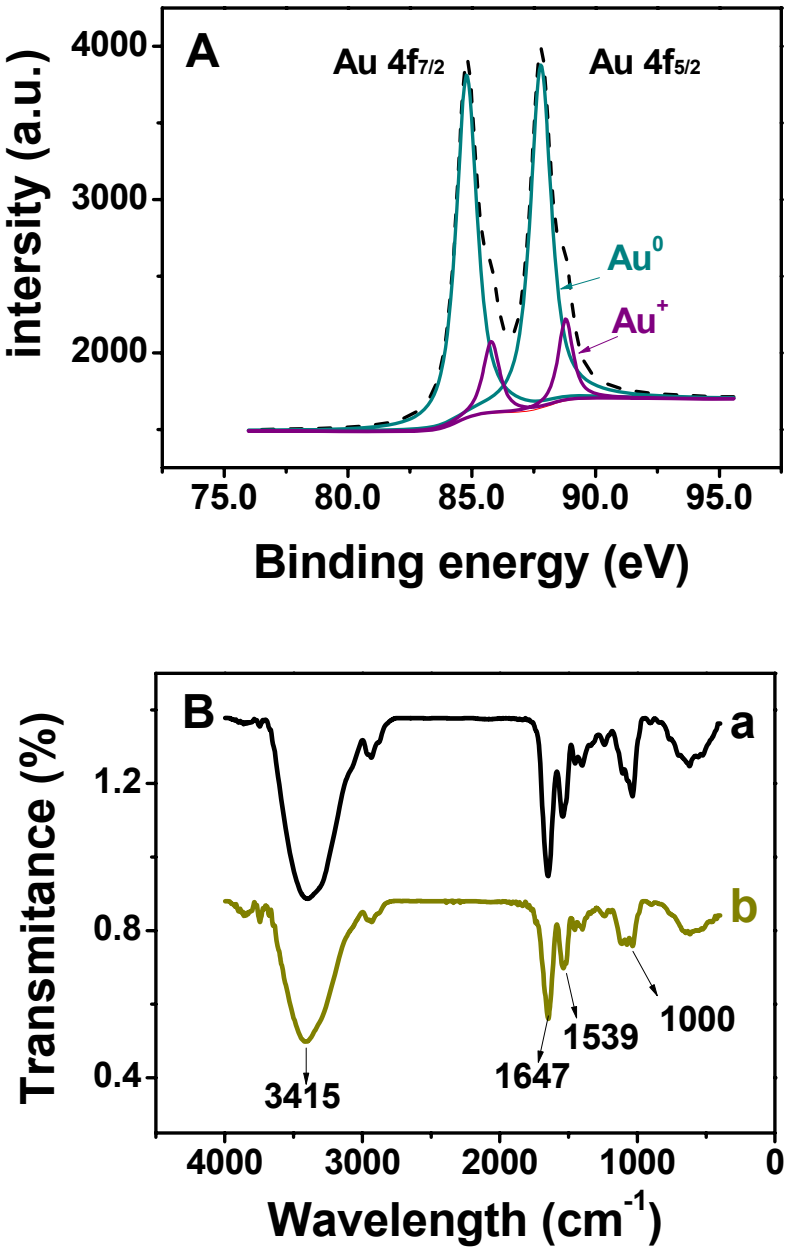

Fig. S3. (A) XPS spectra of Au 4f orbitals of P@AuNCs; (B) FT-IR spectra of papain (a) and P@AuNC (b).

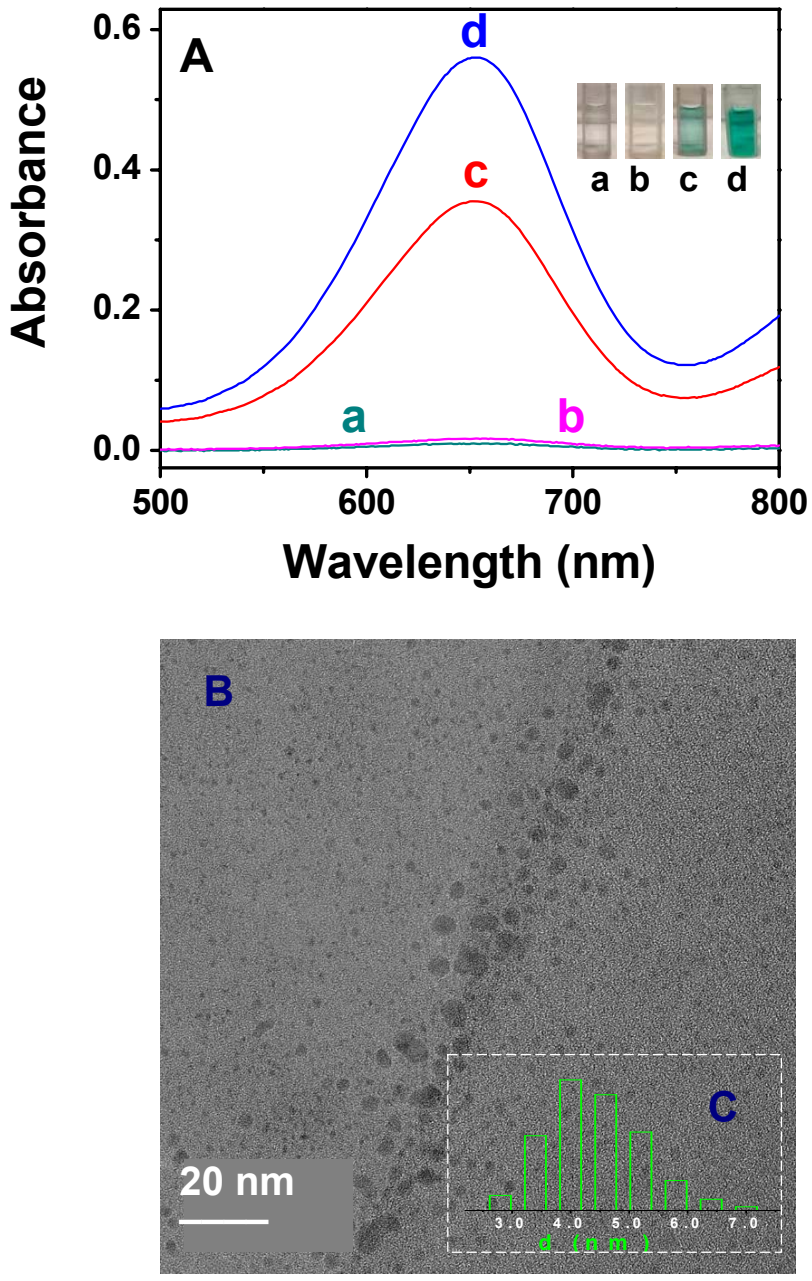

Fig. S4. (A) The UV-*vis* absorption spectra and photos of different systems: (a) TMB-H_2O_2; (b) DA-TMB-H_2O_2; (c) P@AuNC-TMB-H_2O_2; (d) P@AuNC-TMB-H_2O_2-DA. (B) TEM image and (C) size distribution of P@AuNCs-DA.

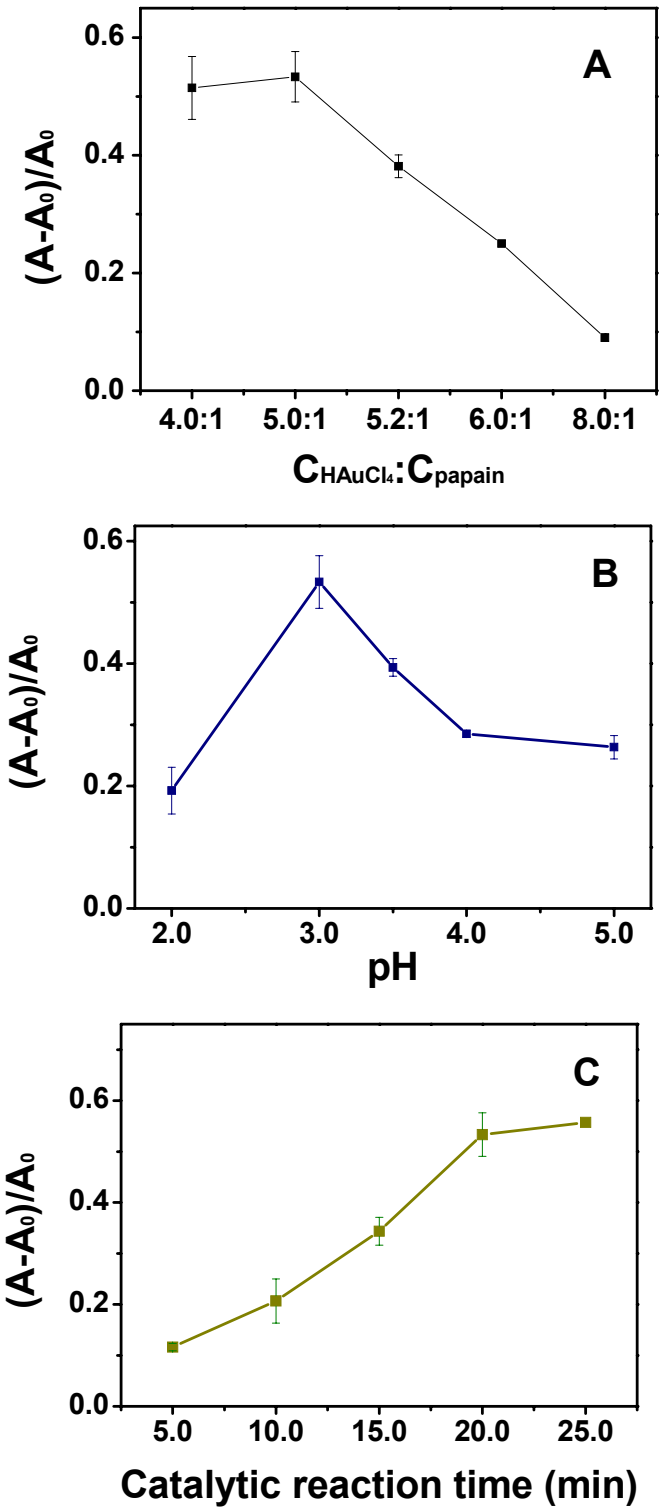

Fig. S5. Dependence of the peroxidase-like activity of P@AuNCs on (A) concentration ratio of $HAuCl_4$ to papain; (B) buffer pH and (C) catalytic reaction time.

Table S1. Kinetic parameters of the nanozymes with TMB as the substrate in the presence of H_2O_2

Catalyst	K_m (mM)	V_{max} (10^{-8} M·s^{-1})
P@AuNCs	0.41	0.99
P@AuNCs-DA	4.08	18.4

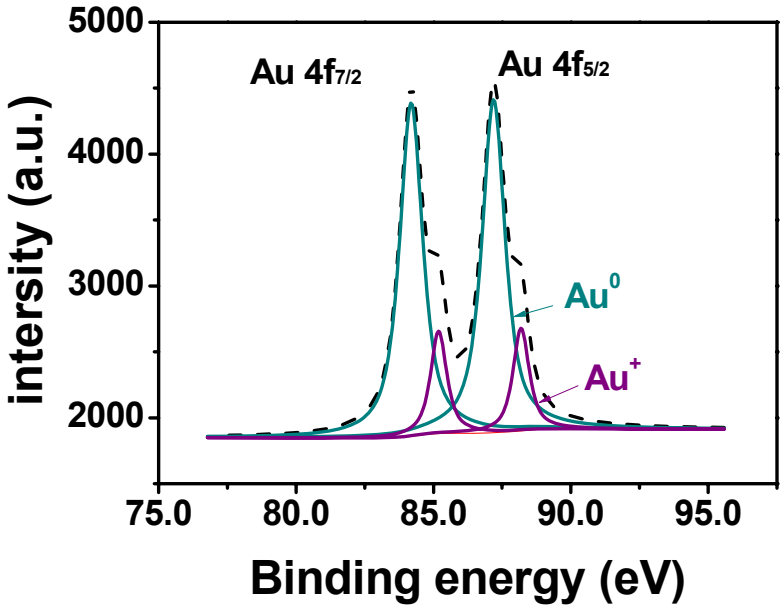

Fig. S6. XPS spectra of Au 4f orbitals of P@AuNCs-DA.

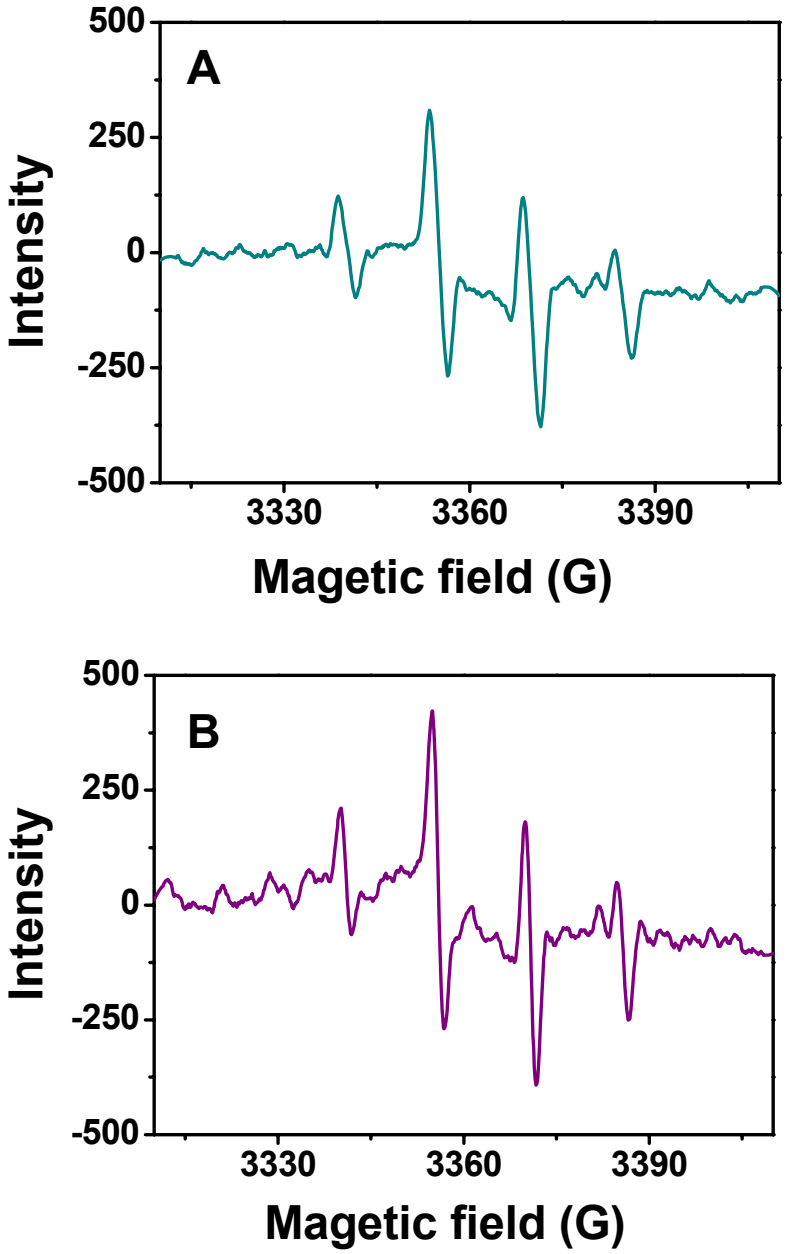

Fig. S7. EPR signals of (A) DMPO-H_2O_2-P@AuNCs and (B) DMPO-H_2O_2-P@AuNCs-DA. The concentrations of DMPO, P@AuNCs, H_2O_2 and DA were 0.1 M, 0.1 mM, 0.3 M and 25.0 μM, respectively.

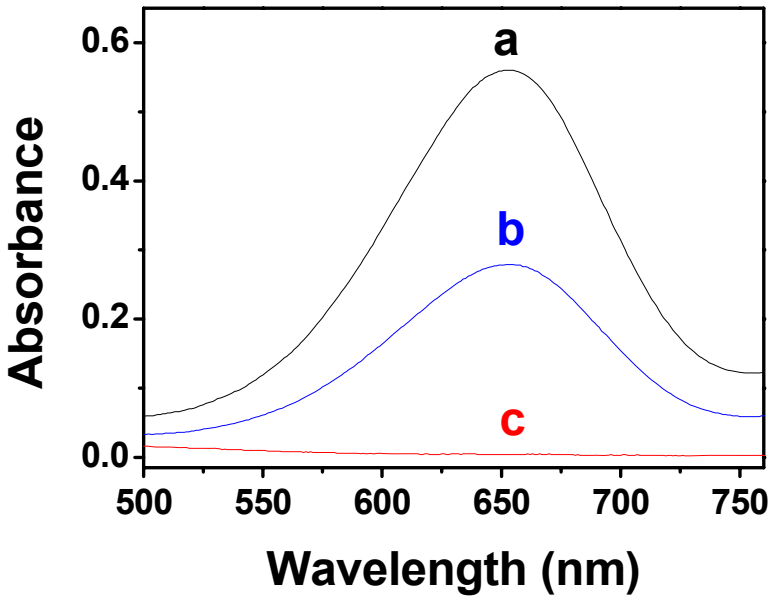

Fig. S8. Effect of ROS inhibitors on the P@AuNCs-TMB-H_2O_2-DA absorbance in the absence (a) and presence of (b) 0.8 mM t-tubyl alcohol or (c) 0.4 mM benzoquinone.

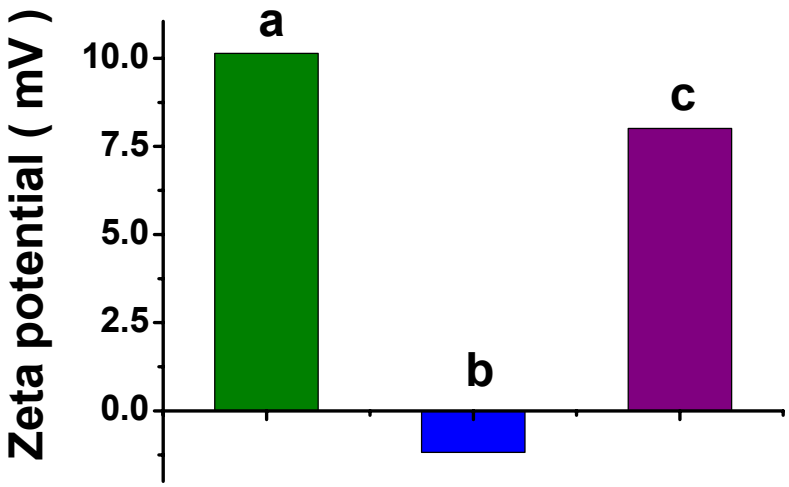

Fig. S9. The apparent zeta potentials of (a) P@AuNCs; (b) DA and (c) P@AuNCs-DA, respectively.

Table S2. Comparison with the reported nanozymes for detection of DA

Nanozymes	Synthesis conditions	Catalytic activity	Linear range (μM)	Samples	References
$CuFe_2O_4$@Cu_9S_8@PPy NTs	25 °C 12.0 h	decrease	2.0-20.0	DA solution	Z. Yang, et al. *Dalton Trans.* 2017, 46, 11171.
Co_3O_4@NiO NTs	80 °C 1.0 h	decrease	1.0-20.0	DA solution	Y. Zhu, et al. *Talanta* 2018, 181, 431.
Ag_2S@CeO_2 NPs	25 °C 5.0 h	decrease	0.5-4.0	DA solution	J. Lian, et al. *Colloid. Surf. A* 2019, 565, 1.
Pt@hBNNS NPs	80 °C 20.0 h	decrease	2.0-50.0	human serum	M.N. Ivanova, et al. ACS Appl. Mater. Interfaces 2019, 11, 22102.
Pt@$CoFe_2O_4$ NPs	60 °C 6.0 h	decrease	20.0-80.0	human serum	F. He, et al. *Microchem. J.* 2020, 158, 105264.
ZIF-67@Co_3O_4 HNCs	25 °C 24.0 h	decrease	4.8-90.0	human serum	H. Wang, et al. *Spectrochim. Acta A* 2021, 246, 119006
P@AuNCs	100 °C 0.17 h	increase	2.0-25.0	rat serum	**This work**

Table S3. Recovery of the proposed method*

Serums	Added (μM)	Found (μM)	Recovery (%)	RSD (%)
1	5.0	4.8	97.7	0.5
	10.0	9.9	99.0	3.0
	20.0	19.3	96.8	3.2
2	5.0	5.4	108.0	0.9
	10.0	10.8	107.9	3.3
	20.0	19.3	96.5	3.0
3	5.0	5.1	102.0	1.6
	10.0	10.0	100.2	1.7
	20.0	19.0	95.1	1.5

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