Electronic Supplementary Material (ESI) for Analyst.


# Supporting information

## Boosting the peroxidase-like activity of gold nanoclusters for colorimetric detection of oxytetracycline in rat serum

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## Experiments

### *Materials and chemicals*

Oxytetracycline (OTC) was supplied by Titan Scientific Co., Ltd. (Shanghai, China). Streptomycin was obtained from MedChemexpress Company (Shanghai, China). L-Trptophanonitrile (LTN) and other L-amino acids (L-AAs) were purchased from TCI Shanghai 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). Glucose, vitamin C, zinc sulfate ($ZnSO_4$) and magnesium chloride ($MgCl_2$) were obtained from Aladdin Chemistry Company (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 fluorescence measurements were performed using an F-4600 fluorescence spectrophotometer (Hitachi, Japan).

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).

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, $1\times10^5$; 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 LTN@AuNCs***

All of the glasswares were washed with aqua regia ($HCl:HNO_3$ volume ratio = 3:1) and rinsed with ultrapure water. The LTN@AuNCs was prepared with LTN as the reducing and capping agent. Simply, in a 20.0 mL-glass flask, 4.0 mL of $HAuCl_4$ (6.0 mM) and 2.0 mL of LTN (80.0 mM) aqueous solutions were added and mixed under gentle stirring at 100 $^oC$ for 10 min. Then, the LTN@AuNCs solution was centrifuged to remove the larger particles at 10,000 rpm for 10 min. Finally, the LTN@AuNCs supernatant was collected and stored at 4 $^oC$ for further use.

***OTC testing***

OTC standard solutions (0.05-1.5 mM) were prepared. OTC solution (30.0 μL, 1.0 mM), LTN@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, 15.0 mM, pH 3.0). The mixture was incubated at 25 $^oC$ for 20 min before conducting the UV-*vis* absorption measurements.

***Metabolic assay of OTC in rat serum***

Three male-Sprague-Dawley-rats (about 250 g) were gotten from Beijing Vital River Laboratory Animal Technology Co. Ltd. (Beijing, China). All experiments using rat samples were performed in accordance with the institutional animal care and use guidelines of China (GB/T 27416-2014) and approved by the ethics committee at Institute of Chemistry, Chinese Academy of Sciences.

The controlled blank serum samples and five different serum samples (at 0.5 h, 1.0 h, 2.0 h, 4.0 h, 6.0 h) were collected after 18.4 mg/kg OTC 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 LTN@AuNCs-TMB-$H_2O_2$ system was applied to determination of OTC in the rat serum samples. 30.0 μL rat serums, LTN@AuNCs solution (150.0 μL), TMB (36.0 μL, 25.0 mM), $H_2O_2$ (90.0 μL, 10.0 M) and acetate buffer (2.7 mL, 0.20 M, 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.

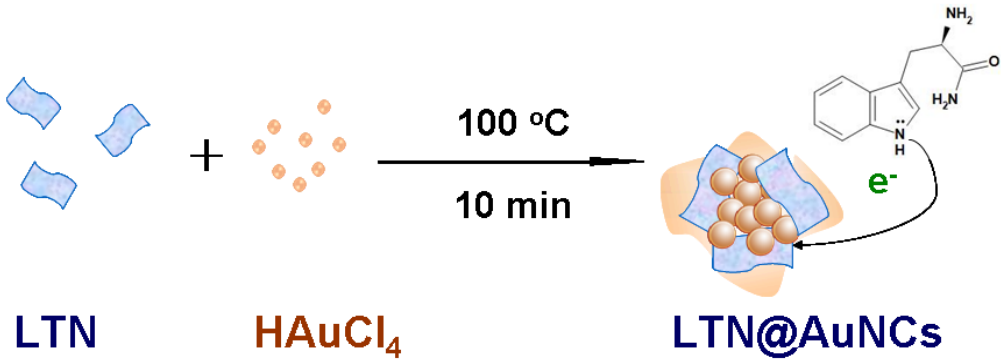


**Fig. S1.** Schematic diagram of the synthesis process of LTN@AuNCs.

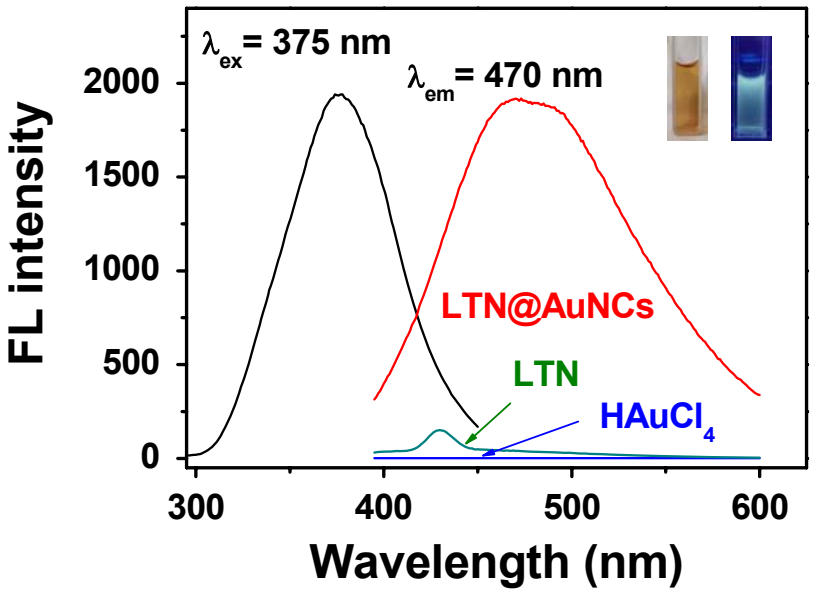


**Fig. S2.** The fluorescence spectra of the obtained LTN@AuNCs recorded with an emission wavelength at 470 nm under excitation at 375 nm. Inset: photograph of the LTN@AuNCs under visible light (left) and UV light (right).

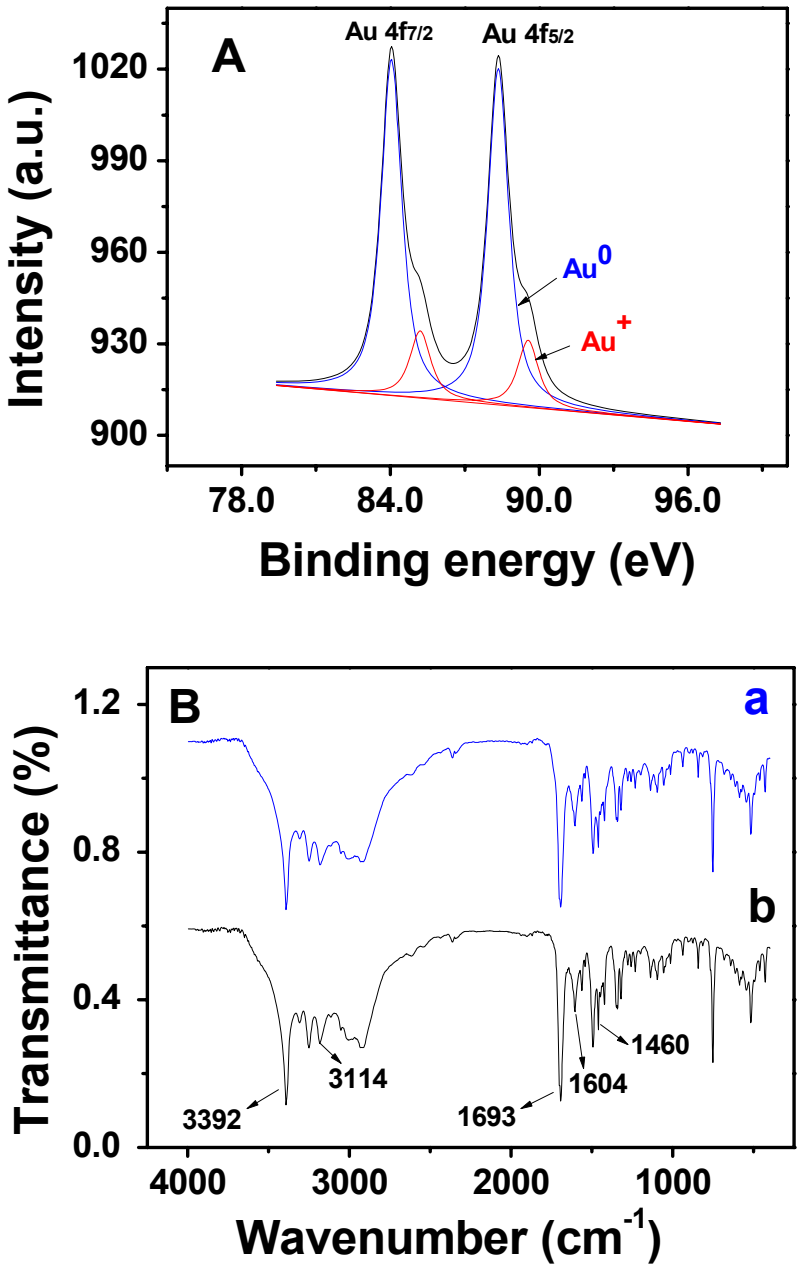


**Fig. S3.** (A) XPS spectra of Au 4f orbitals of LTN@AuNCs; (B) FT-IR spectra of LTN (a) and LTN@AuNCs (b).

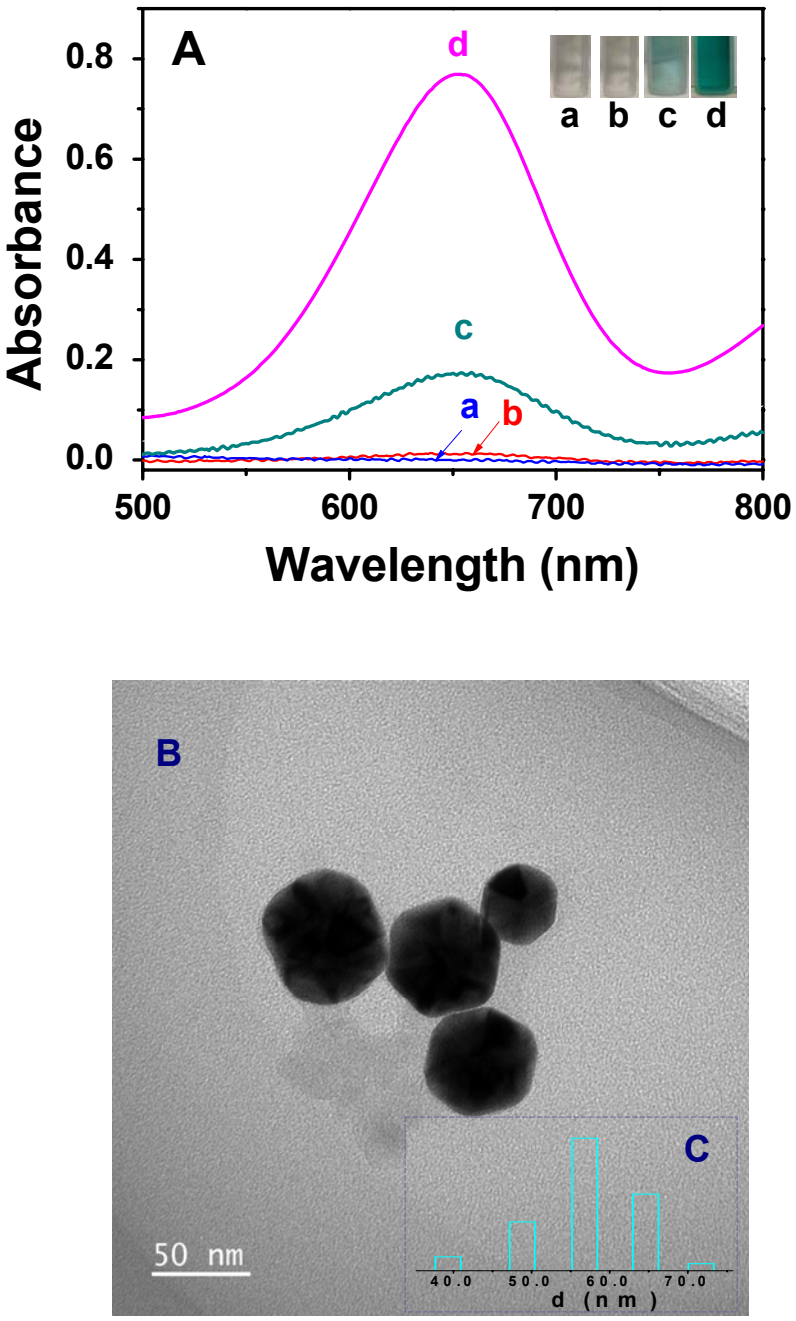


**Fig. S4.** (A) The UV-*vis* absorption spectra and photos of different systems: (a) TMB-$H_2O_2$; (b) LTN-TMB-$H_2O_2$; (c) LTN@AuNCs-TMB-$H_2O_2$; (d) LTN@AuNCs-TMB-$H_2O_2$-OTC. (B) TEM image and (C) size distribution of LTN@AuNCs-OTC.

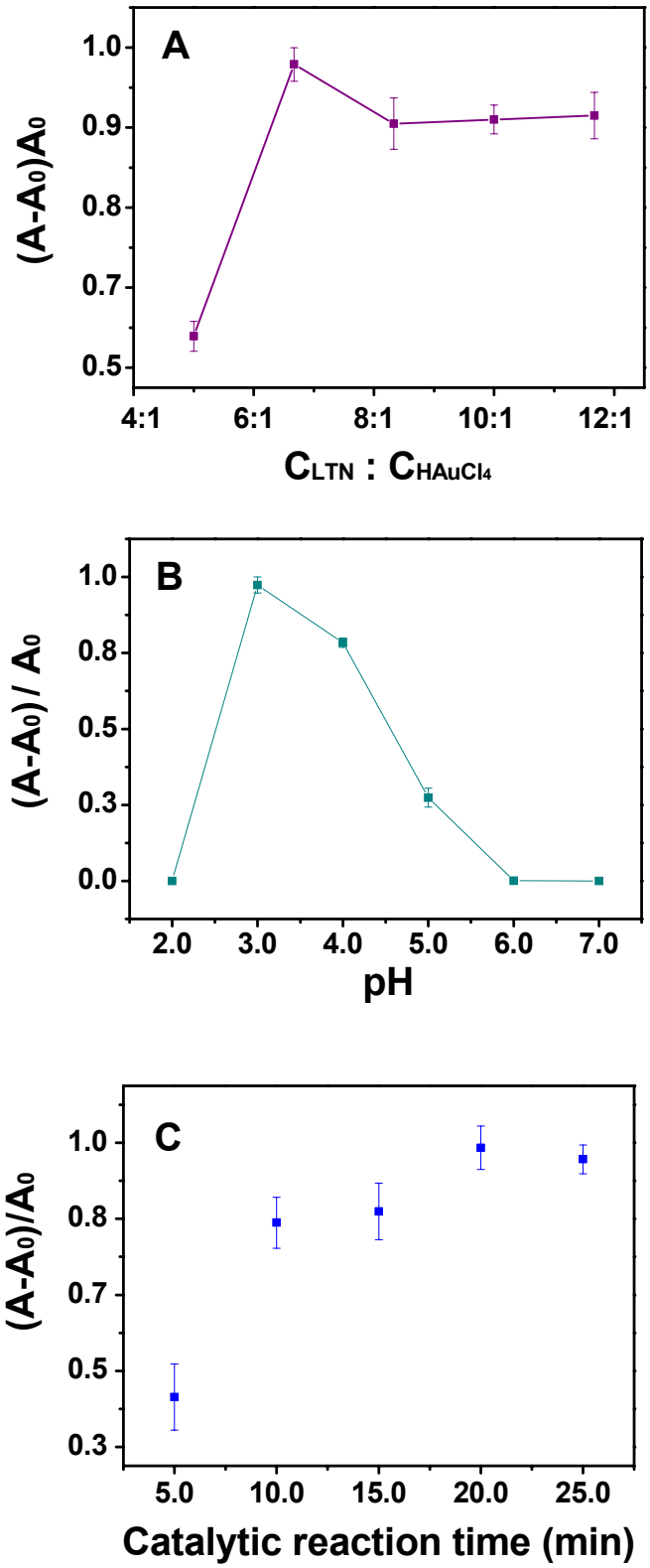


**Fig. S5.** Dependence of the peroxidase-like activity of LTN@AuNCs on (A) concentration ratio of LTN to $HAuCl_4$; (B) buffer pH and (C) catalytic reaction time.

**Table S1.** Kinetic parameters ($K_m$ and $V_{max}$) of the nanozymes

| Catalyst | Substrate | $K_m$ (mM) | $V_{max}$ ($10^{-8}$ M·s$^{-1}$) |
|---|---|---|---|
| LTN@AuNCs | TMB | 0.66 | 1.47 |
| | $H_2O_2$ | 0.036 | 0.24 |
| LTN@AuNCs-OTC | TMB | 1.23 | 10.99 |
| | $H_2O_2$ | 0.24 | 5.00 |

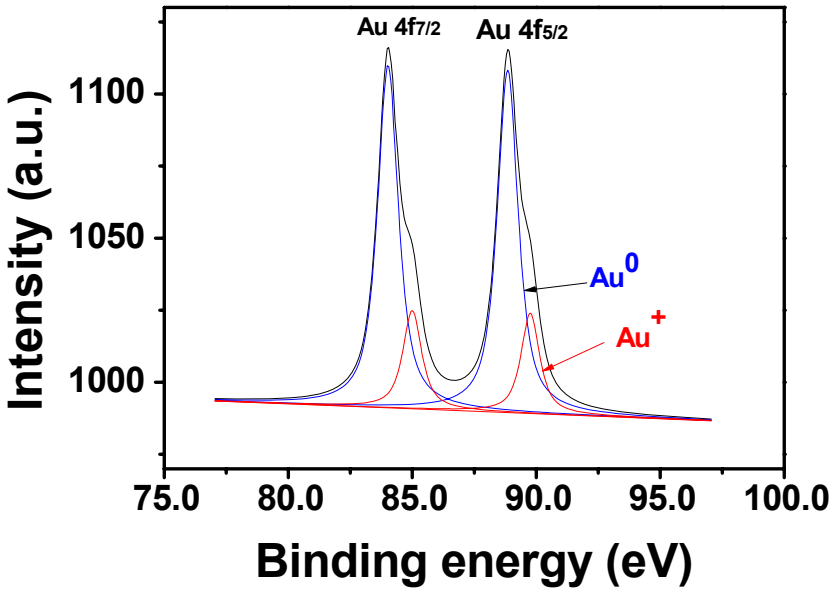


**Fig. S6.** XPS spectra of Au 4f orbitals of LTN@AuNCs-OTC.

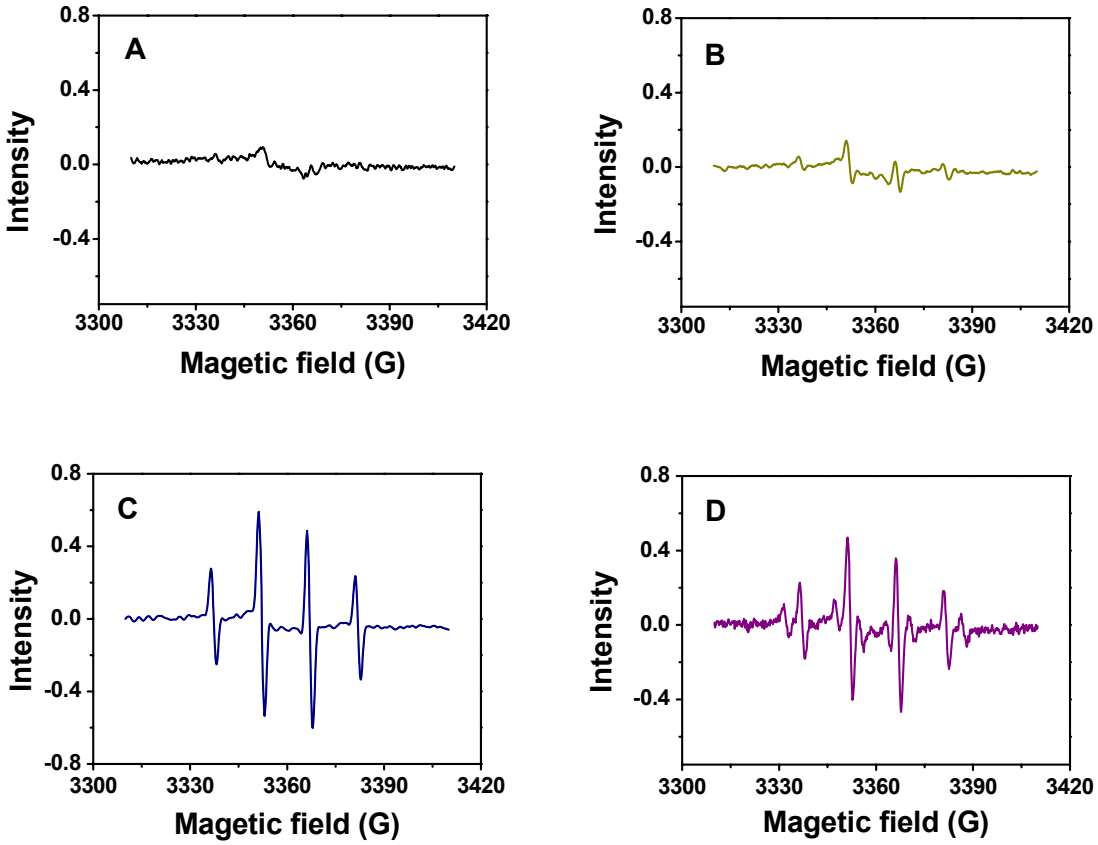


**Fig. S7.** EPR signals of (A) DMPO; (B) DMPO-$H_2O_2$; (C) DMPO-$H_2O_2$-LTN@AuNCs and (D) DMPO-$H_2O_2$-LTN@AuNCs-OTC, respectively. The concentrations of DMPO, LTN@AuNCs, $H_2O_2$ and OTC were 0.1 M, 0.17 mM, 0.3 M and 30.0 μM, respectively.

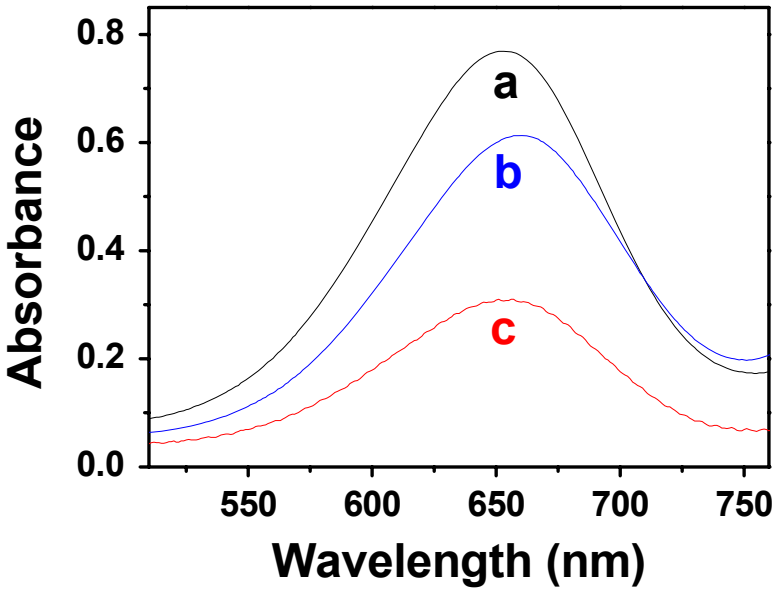


**Fig. S8.** Effect of ROS inhibitors on the LTN@AuNCs-TMB-$H_2O_2$-OTC absorbance in the absence (a) and presence of (b) 1.6 mM t-tubyl alcohol or (c) 0.4 mM benzoquinone.

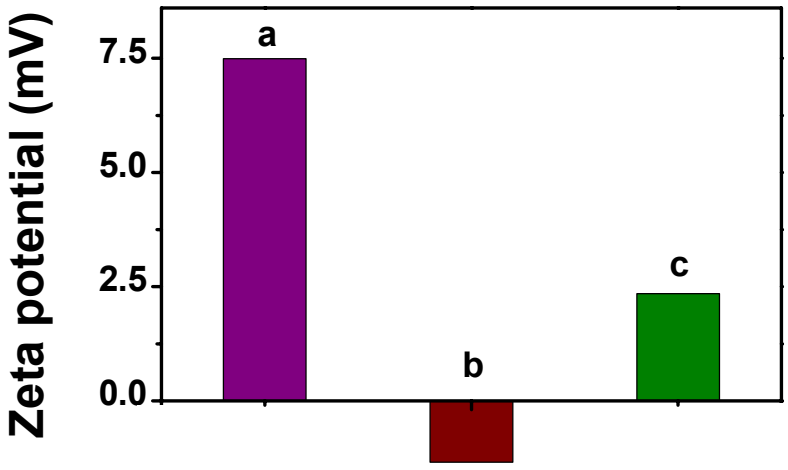


**Fig. S9.** The apparent zeta potentials of (a) LTN@AuNCs; (b) OTC and (c) LTN@AuNCs-OTC, respectively.

**Table S2.** Comparison with the reported nanozymes for detection of OTC

| Nanozymes | Synthesis conditions | Catalytic activity | Linear range (μM) | Samples | References |
|---|---|---|---|---|---|
| **FCCN composites** | 200 °C 5.0 h | increase | 21.7-43.4 | waste water | M. Zhang, et al. Chem. Eng. J. 2020, 396, 125343. |
| **3DBC@$C_3N_4$-ssDNA** | 55 °C 8.0 h | decrease | 0.1-4.3 | cells | Y. Fan, et al. ACS Appl. Mater. Interfaces 2019, 11, 17467. |
| **DNA@AuNPs@MOFs** | 150 °C 6.0 h | decrease | 0.05-0.1 | tap water | J. Li, et al. Environ. Int. 2019, 125, 135. |
| **DNA@AuNPs** | 37 °C 1.0 h | increase | 0.02-1.7 | milk | Y. Xu, et al. Microchim Acta 2018, 185, 1. |
| **$Fe_3O_4$@NPs** | 25 °C 0.5 h | increase | 0.05-1.0 | tablets | Y. Wang, et al. Sens. Actuators B 2016, 236, 621. |
| **LTN@AuNCs** | 100 °C 0.17 h | increase | 0.5-15.0 | rat serum | **This work** |

**Table S3.** Recovery of the proposed assay*

| Serums | Added (μM) | Found (μM) | Recovery (%) | RSD (%) |
|---|---|---|---|---|
| **1** | 2.0 | 2.1 | 104.5 | 1.6 |
| | 6.0 | 6.2 | 102.8 | 2.5 |
| | 10.0 | 9.8 | 98.3 | 4.3 |
| **2** | 2.0 | 2.1 | 106.8 | 3.3 |
| | 6.0 | 5.8 | 96.2 | 2.7 |
| | 10.0 | 9.7 | 96.8 | 4.7 |
| **3** | 2.0 | 1.9 | 95.0 | 4.2 |
| | 6.0 | 5.7 | 94.8 | 1.9 |
| | 10.0 | 9.7 | 97.3 | 1.3 |

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