Supplementary Material (ESI) for Analyst
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Highly catalytic and stable Au@AuPt nanoparticles for visual and quantitative detection of *E. coli* O157:H7

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Experimental details

Material and Chemicals. Chloroauric acid tetrahydrate ($HAuCl_4 \cdot 3H_2O$), chloroplatinic acid hexahydrate($H_2PtCl_6 \cdot 6H_2O$), tris-(2-carboxyethyl)-phosphine hydrochloride (TCEP), 3,3',5,5'-tetramethylbenzidine(TMB), and hydroxylamine hydrochloride were purchased from Adamas (Shanghai, China). Bovine serum albumin was obtained from Beyotime Biotechnology Co. (Shanxi, China). Ascorbic acid, Trisodium citrate dehydrate, sodium chloride, magnesium chloride, Phosphoric acid, and dibasic sodium phosphate were received from Aladdin Industrial Co. (Shanghai, China). Horseradish Peroxidase (HRP) was purchased from Solarbio Co. (Beijing, China). Sodium borohydride was purchased from Sino Pharm Co. (Shanghai, China). Hydrogen peroxide and sulfuric acid were purchased from Xilong Scientific Co., Ltd (Guangzhou, China). *E. coli* O157:H7 was provided by BNCC Co. (Beijing, China). The DNAs were synthesized by Tsingke Biotechnology Co., Ltd. (Beijing, China). The sequences are listed below in Table S1.

Table S1 Sequence of the DNA strands.

Name	Sequence
aptamer[1]	5'-ATCCGTCACACCTGCTCTGTCTGCGAGCGGGGCG CGGGCCCGGCGGGGGATGCGTGGTGTTGGCTCCCGTAT-3'
DNA1	5'-SH-ATACGGGAGCCAACACCACGCATC-3'
DNA2	5'-SH-GAGCCAACACCACGCATCCAAAGTGATGCGTG GTGTTGGCTCCCGTAT-3'

Preparation of microplates

To form a uniform gold film on the microplate for DNA modification, the methoxypoly (ethylene glycol) thiol (mPEG-SH) facilitating method was used. Briefly, 100 μL of 16 nm AuNPs (2.5 nM) and 5 μL of mPEG-SH (1 μM) were added into the microplate and dried at 80 °C for vaporization. Next, 50 μL of 10 mM $HAuCl_4$ and 50 μL of 20 mM hydroxylamine

hydrochloride mixture were added at 25°C for 30 minutes to deposit gold atoms and form a uniform film and then washed three times with ultrapure water. Subsequently, 100 μL of 200 nM DNA1 was added and incubated at 25 °C for 4 hours to anchor on the microplate and washed with Tris buffer (10 mM Tris-HCl, 300 mM NaCl, 5 mM $MgCl_2$). After that, 100 μL of 200 nM aptamer was added to form a DNA1-aptamer duplex on the microplate. Finally, 100 μL 2% BSA solution was added and incubated at 25 °C for 2 hours to block the unmodified sites and rewashed with Tris buffer.

Synthesis of gold nanoseeds

Gold nanoseeds were synthesized based on previously reported method[2]. Briefly, all used glass devices were dipped in aqua regia overnight and then rinsed and dried with plenty of ultrapure water before use. One mL $HAuCl_4$ (10 mM) was added to 1mL of $NaBH_4$ (100 mM) and 36 mL of ultrapure water to form Au nanoseeds with vigorously stirring at 25 °C. Then, the prepared colloidal solution was aged for 4 hours to hydrolyze the unreacted $NaBH_4$. Finally, the gold nanoseeds were characterized by an ultraviolet-visible (UV-Vis) spectrophotometer and transmission electron microscope.

Preparation and catalytic ability of Au@AuPtNPs

Au@AuPtNPs were synthesized according to the seed growth method[3]. After the addition of 10 mL mixture of chloroplatinic acid (0.4-3.2 mM) and chloroauric acid (2-16 mM) into 100 mL of 5 nm gold nanoparticle solution with stirring, 5 mL of ascorbic acid (80 mM) was dropped at uniform speed and set at room temperature for 5 h to form Au@AuPtNPs. Finally, 100 μL solution containing 1 mM TMB, 2 mM H_3PO_4, 10 mM H_2O_2 was added, reacted at 37 °C for 20 min, and stopped by 20 μL of 2 M H_2SO_4 to form yellow products. The absorbance at 450 nm was measured for catalytic ability comparison.

The catalytic stability assessment

The HRP-like catalytic stability of Au@AuPtNPs and HRP were assessed by the catalytic reaction of TMB oxidation by H_2O_2, and the procedure is described as follows: 10 μL of Au@AuPtNPs (0.021 mg/mL, 62 pM) or HRP (0.10 mg/mL, 25 μM), 10 μL of phosphate-citrate buffer (20 mM, pH 7.0), 10 μL of H_2O_2 (10 mM), and 10 μL of TMB (1 mM) was added to ultrapure water to make a total volume of 100 μL. The mixture was reacted at room temperature for 20 minutes before stopped by 20 μL of 2 M H_2SO_4, and absorbance was measured at 450 nm. Similar assays were performed to investigate the effects of pH, storage time, and temperature on the catalytic stability of Au@AuPtNPs and HRP by the variance of corresponding parameters, such as pH (2.0-9.0), temperature (4 °C-85 °C) and storage time (1-7 day) .

Enzymatic kinetics evaluation of Au@AuPtNPs and HRP

The kinetic assays were conducted similarly at room temperature as mentioned above, except for the concentrations of substrate, TMB or H_2O_2. The kinetic parameters of the HRP-like activity were calculated using a Lineweaver-Burk plot of the double reciprocal of the Michaelis-Menten equation: $1/V = K_m/V_{max}\ (1/[S] + 1/K_m)$, where V is the velocity of the reaction, V_{max} is the maximal reaction velocity, [S] is the concentration of substrate, and K_m is the Michaelis-Menten constant.

DNA-Au@AuPtNPs preparation

Ten μL different concentrations of DNA2 (40 nM - 400 nM) were mixed firstly with 50 μL of Au@AuPtNPs and 170 μL phosphate buffer (5 mM, pH 7.0) and then 20 μL of 1 M NaCl was added, and incubated at 25 °C for 2 h. After the reaction, the mixture was centrifuged to remove free DNA2 and redispersed in 100 μL buffer solution to form DNA2-Au@AuPtNPs and stored at

4 °C before use.

Catalytic reaction conditions optimization for Au@AuPtNPs

To obtain excellent HRP-like catalysis of Au@AuPtNPs, the concentrations of TMB substrate and H_2O_2 were studied. To optimize the concentration of TMB, 10 μL of Au@AuPtNPs (62 pM), 10 μL of TMB substrate (0-1.2 mM), and 10 μL of H_2O_2 (10 mM) were added together in a total volume of 100 μL phosphate buffer (2 mM, pH 4.0) and incubated at 37 °C for 20 minutes, and then stopped by 20 μL H_2SO_4 (2 mM). The absorbance at 450 nm was then measured. To gain optimal concentration of H_2O_2, 10 μL of Au@AuPtNPs (62 pM), 10 μL of TMB substrate (1 mM), and 10 μL of H_2O_2 (0-5 mM) were mixed to a total volume of 100 μL phosphate buffer (2 mM, pH 4.0) to perform the measurements as mentioned above.

To further optimize the reaction conditions of Au@AuPtNPs on microplates, the concentration of DNA1 (0-600 nM) and reaction time (0-120 minutes) were optimized. Different concentrations of DNA1 were added into microplates for 4 h and washed with wash buffer (10 mM Tris-HCl, 300 mM NaCl, 5 mM $MgCl_2$, 0.05% Tween-20) to remove unbound DNA1. Then, Au@AuPt-DNA2 was hybridized with DNA1 at 37 °C for 30 min and washed thrice with wash buffer. The absorbance measurements were then performed as mentioned, except for catalytic time.

Sensitivity and specificity of the detection method for bacteria

Before the bacterial detection, the concentration of O157:H7 bacteria was primarily counted by the standard plate counting method. The colorimetric bacterial detection process is described as follows: the diluted *E. coli* O157:H7 solutions (10^2-10^7 CFU/mL) were transferred to the DNA1-aptamer modified microplate and reacted at 25 °C for 30 min. After the recognition reaction, the microplate was washed three times with the wash buffer. Afterwards, 50 μL of Au@AuPt NPs-DNA2 was added to hybridize with the exposed DNA1 for 30 min at 37 °C and then rinsed with

wash buffer thrice. Finally, the absorbance measurements were performed as mentioned, and the yellow products were visualized and recorded by the camera.

To test the specificity of this method, 100 μL of different solutions (*S. aureus*, *E. coli* K12, *B. subtilis*, PBS, *E. coli* O157:H7), where the concentration of O157:H7 was 1×10^5 CFU/mL, and the concentration of other bacteria was 5×10^5 CFU/mL, were reacted in the microplate at 25 °C for 30 min and then rinsed three times with buffer. Adding 50 μL of Au@AuPt-DNA2 and incubating at 37 °C for 30 min was followed by three times washes. The absorbance measurements and color recording were then performed as mentioned.

Detection in spiked samples

The applicability of the method was evaluated by the detection of O157:H7 spiked tap water and milk tea samples. Briefly, 100 μL of spiked samples without further treatment was added to the microplate well, incubated at 25 °C for 30 min, and then washed thrice. Afterwards, Au@AuPt-DNA2 was added to set at 37 °C for 30 min and washed thrice. Finally, the absorbance measurements were performed and the recovery rates were calculated.

Supplementary Figures

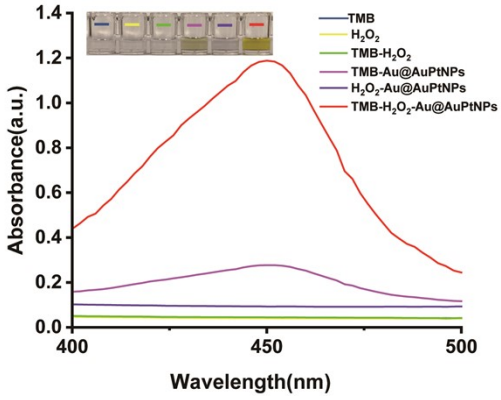

Fig. S1 Validation of the HRP-like activity of Au@AuPtNPs with TMB and H_2O_2.

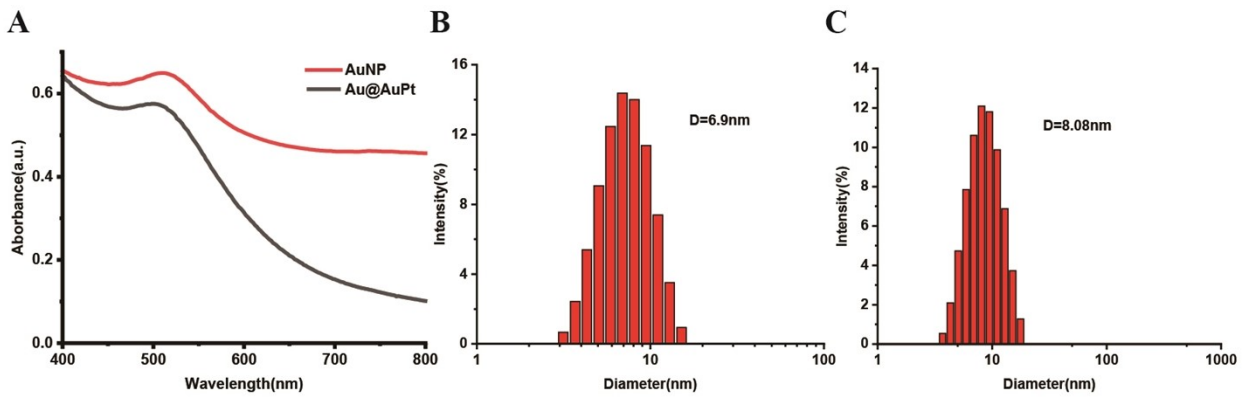

Fig. S2 Characterization of Au@AuPtNPs. (A) UV-Vis spectra of AuNPs and Au@AuPtNPs, (B) DLS images of AuNPs, (C) DLS images of Au@AuPtNPs.

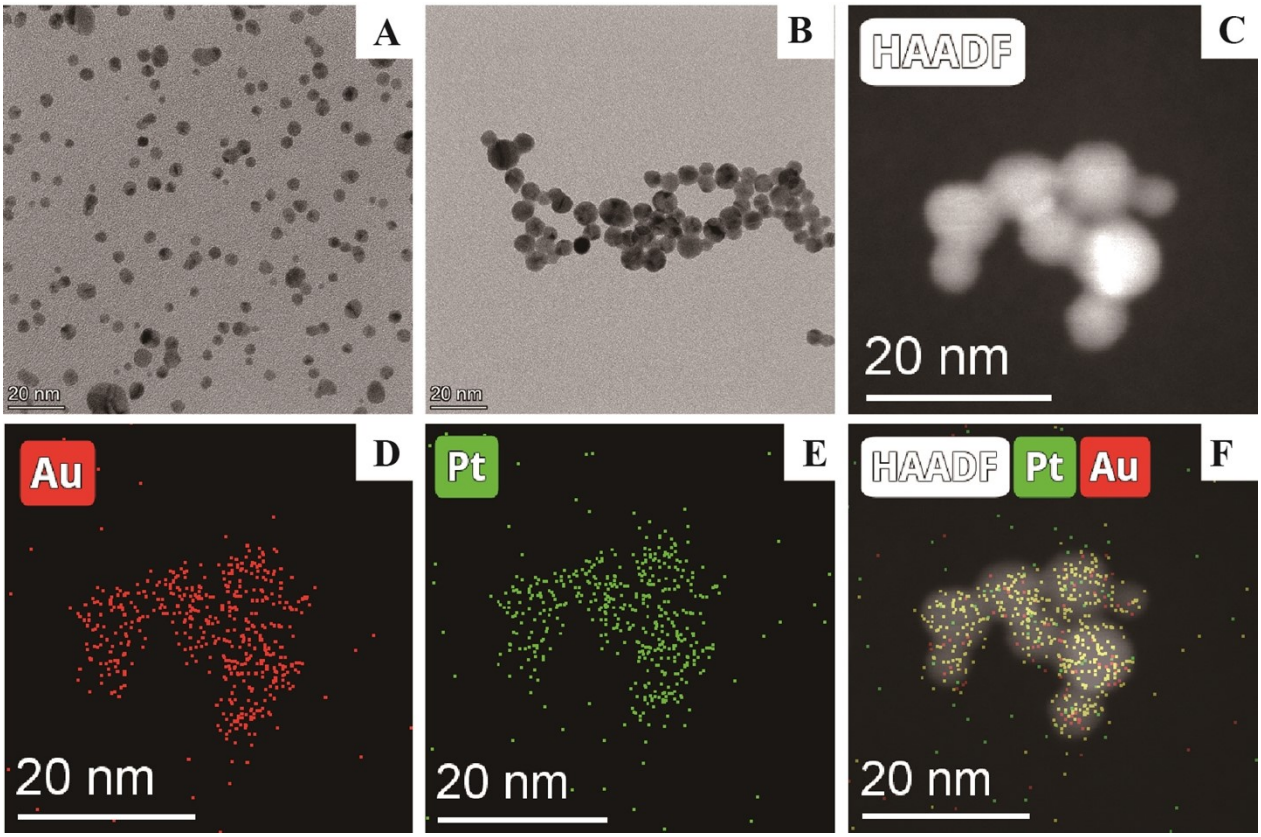

Fig. S3 TEM characterization of Au@AuPtNPs. TEM image of gold nanoseeds (A) and Au@AuPtNPs (B). HAADF-STEM images and the corresponding elemental mapping of Au@AuPtNPs (C-F).

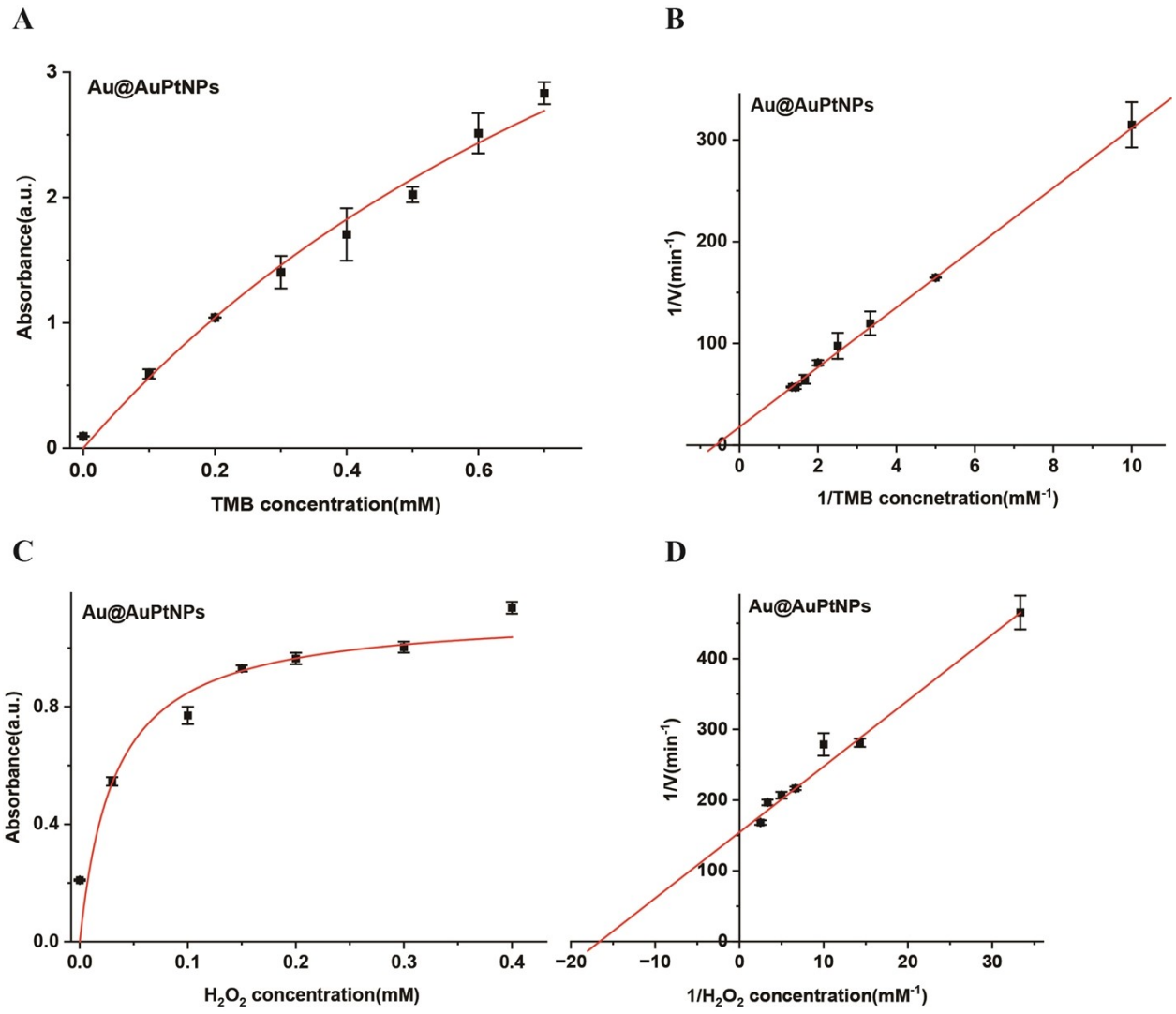

Fig. S4 Steady-state kinetics assays of Au@AuPtNPs. (A, C) TMB concentration was varied at a fixed H_2O_2 concentration. (B, D) H_2O_2 concentration was varied at a fixed TMB concentration.

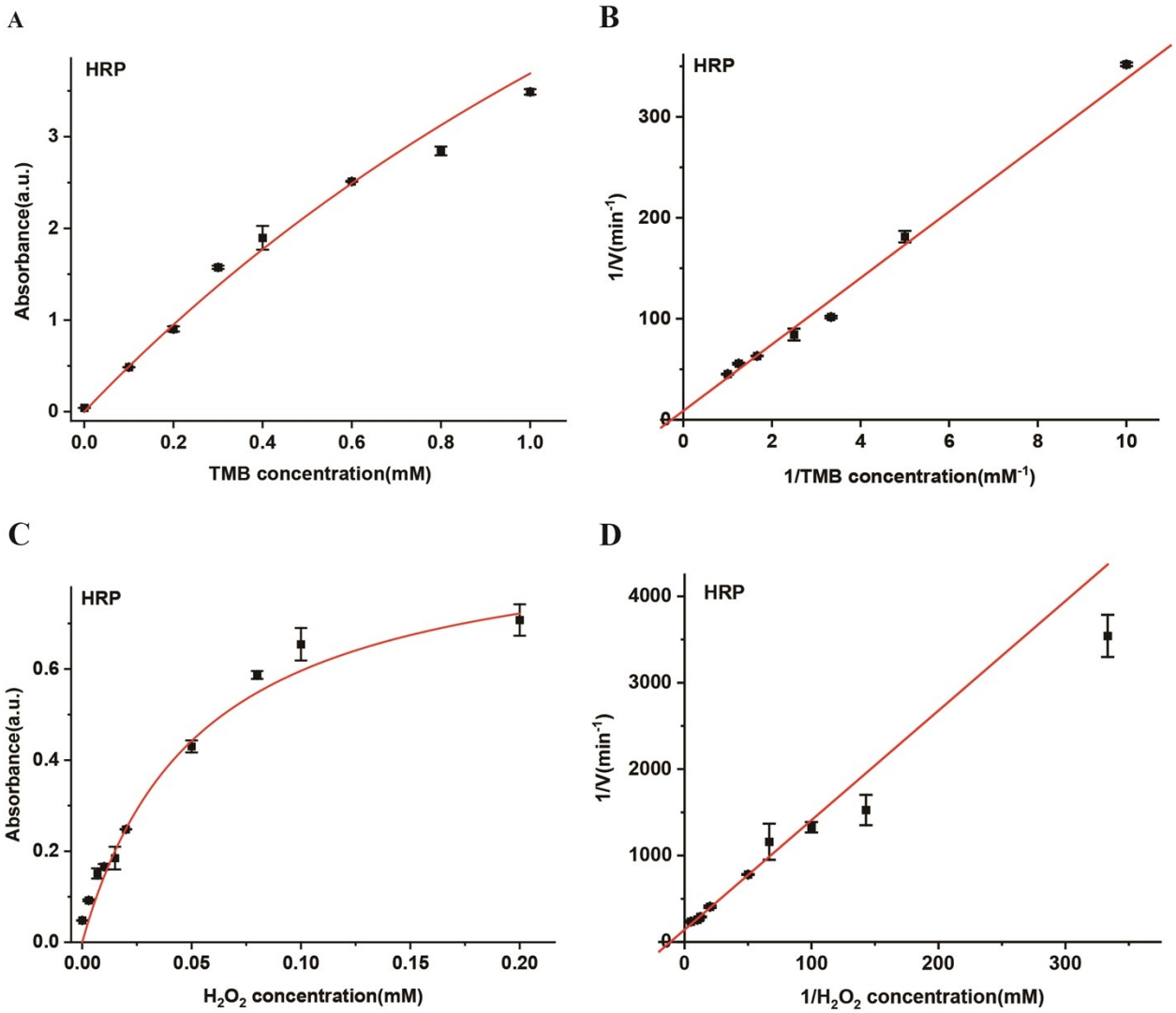

Fig. S5 Steady-state kinetics assays of HRP. (A, C) TMB concentration was varied at a fixed H_2O_2 concentration. (B, D) H_2O_2 concentration was varied at a fixed TMB concentration.

Table S2 Michaelis-Menten kinetics parameters of Au@AuPtNPs and HRP.

Catalyst	Substrate	$V_{max}(min^{-1})$	K_m
Au@AuPtNPs	TMB	0.0556	1.61
Au@AuPtNPs	H_2O_2	0.00654	0.06
HRP	TMB	0.111	4
HRP	H_2O_2	0.00735	0.1

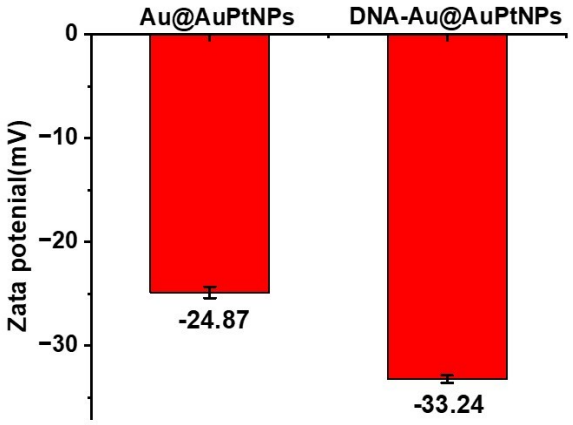

Fig. S6 The zeta potential of nanozyme with or without DNA modification.

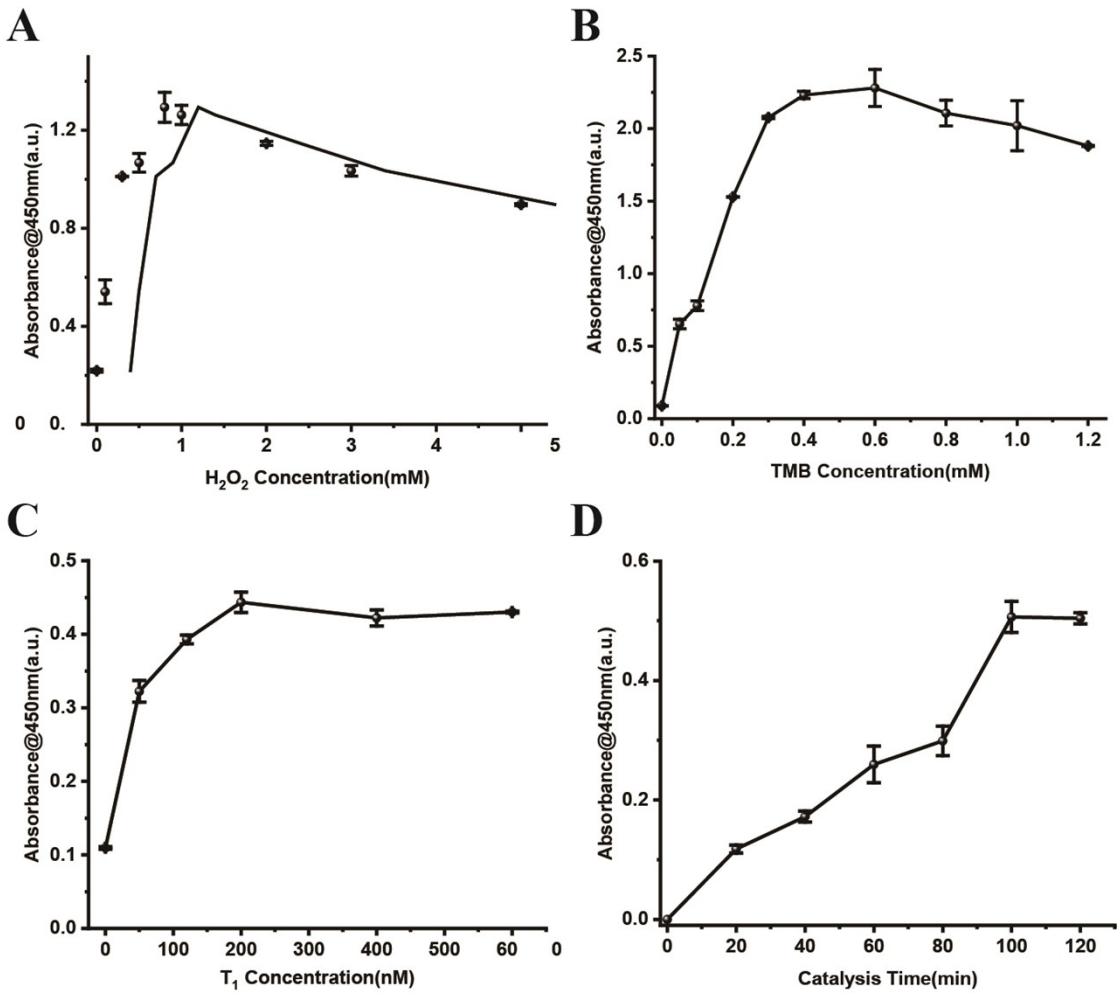

Fig. S7 Condition optimization for TMB oxidation reaction. A. Optimization of H_2O_2 concentration. B.

Optimization of TMB concentration. C. Optimization of T1 concentration on the microplate. D. Optimization of catalytic time.

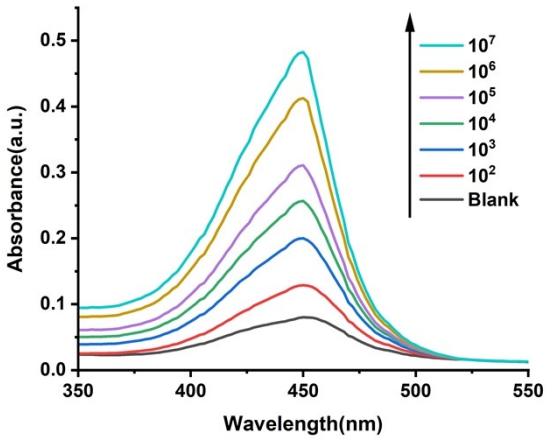

Fig. S8 UV-vis absorption spectra of *E. coli.* O157:H7 concentration ranged from 10^2 to 10^7 CFU/mL.

Table S3 Comparison of the analytical methods for *E. coli O157:H7*

Analytical Method	Detection range（CFU/mL）	Detection Limit（CFU/mL）	Visible	Reference
PCR	10^2–10^7	2×10^2	No	Ref[4]
Fluorescence	10^4-10^6	3.75×10^3	No	Ref[5]
Electrochemical	3×10^1-3×10^7	30	No	Ref[6]
Absorbance	10^2-10^7	59	Yes	This work

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