

Supporting Information

Galvanic replacement synthesis of PtPdAu hollow nanorods as
peroxidase mimic with high specific activity for colorimetric detection

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Materials

Tellurium dioxide powder (TeO_2 , 99.99%), and selenous acid (H_2SeO_3 , 99.99%) were purchased from Aladdin Chemistry Co., Ltd. Hydrazine monohydrate ($\text{N}_2\text{H}_4 \cdot \text{H}_2\text{O}$, 85%, AR), sodium dodecyl sulfate (SDS, 99%), tetrachloroauric (III) acid hydrate (HAuCl_4 , AR), Palladium (II) chloride (PdCl_2 , AR), Chloroplatinic acid ($\text{H}_2\text{PtCl}_6 \cdot 6\text{H}_2\text{O}$, AR), polyvinylpyrrolidone (PVP Average molecular weight 58000 AR), hydrogenperoxide (H_2O_2 , 30%, AR) were provided by Sinopharm Chemical Reagent Co., Ltd. 3,3',5,5' tetramethylbenzidine (TMB), Citric acid/sodium was obtained from McLean chemical reagent Co., Ltd. The three vitamin C tablets were provided by Xinxiang ChangLe, Heilongjiang Dina and Yangshengtang Pharmaceutical Co., Ltd, respectively.

Material Characterization

Transmission electron microscopy (TEM) imaging was conducted on a FEI TECNAI F30 microscope operated at 200 kV and copper grids were used to load the samples. All values of the material sizes were measured through TEM images. X-ray spectroscopy (EDS) was carried out under the high-angle annular dark field (HAADF) mode with an EDAX attachment. Inductively coupled plasma-mass spectrometry (ICP-MS) measurements were performed on NexION 300Q (PerkinElmer, USA). X-ray diffraction (XRD) was tested on a Bruker D8 Advance X-ray diffractometer with $\text{Cu K}\alpha$ radiation. Samples were prepared by depositing nanostructures on glass. The scanning speed was set as 15 degrees/min. X-ray photoelectron spectra (XPS) were collected on an ESCALAB 250Xi spectrophotometer (Thermo Fisher) with $\text{Al K}\alpha$ X-ray radiation and calibrated using the C 1s peak (284.8 eV). UV-vis absorption spectra were measured by a Macy UV-1800 spectrophotometer (Macylab, China).

Measurement of the Specific Activity

Specific activity (SA) was measured according to the protocol reported in former reports.¹ Specifically, at 20 °C, 0.1 M citric acid/sodium citrate was selected as buffer (pH=4.0). H₂O₂, nanozyme material, TMB (50 μL 10 mg/mL) were added successively. The final volume is controlled at 1 mL, in which the concentration of H₂O₂ is 1.0 M, and the quality of nanozyme material added each time is controlled. The absorbance of the reaction solution at λ_{max}=653 nm was measured by UV-vis spectrophotometer at the interval of 1 second immediately after the addition of all substances for 50 s. The absorbance-time curve is then obtained and SA is calculated by the following equation.

$$b_{nanozyme} = \frac{V \cdot \Delta A}{\epsilon l \cdot \Delta t}$$

where $b_{nanozyme}$ is the nanozyme activity (U), V is the total volume of reaction solution (μL), ϵ is the molar absorption coefficient of the TMB substrate (39,000 M⁻¹ cm⁻¹ at 653 nm), l is the optical path length through reaction solution (cm), and $\Delta A/\Delta t$ is the initial rate of the absorbance change (min⁻¹). When using different amounts of the nanozyme to measure the peroxidase-like activity, the specific activity of the nanozyme was determined using the following equation:

$$SA = \frac{b_{nanozyme}}{m}$$

where SA is the specific activity of the nanozyme (U mg⁻¹), and m is the nanozyme amount (mg).

Kinetic analysis

Peroxidase-like activities of the nanozyme material were evaluated by the steady-state kinetic assays, according to the previous report.² Specifically, 0.1 M citric acid/sodium citrate were added successively as buffer (pH=4.0). H₂O₂, nanozyme material (50 μL, 10 mg/L) and TMB were added in the cuvette (path length, l=1.0 cm) at 20 °C. The final volume is controlled at 1

mL, in which the concentration of H₂O₂ is 2.0 M, and TMB is controlled as the variable. After adding all substances, the absorbance of the reaction solution at $\lambda_{\max} = 653$ nm was measured by UV-vis spectrophotometer at an interval of 2 seconds for 50 s. Then, the absorbance and time curve is obtained, from which the initial reaction rate is calculated and the maximum reaction rate V_{\max} and the Michaelis constant (K_m) are accessed by the Michaelis-Menten equation.

$$V = \frac{V_{\max}[S]}{K_m + [S]}$$

where V_{\max} is the maximal reaction velocity, $[S]$ is the concentration of TMB, and K_m is the Michaelis constant. The values of K_m and V_{\max} can be obtained from the double reciprocal plots.

Isopropanol quenching hydroxyl radical test process

In simple terms, 50 μ L isopropanol, 50 μ L TMB (10 mg/mL), 50 μ L H₂O₂ with different concentrations and 50 μ L PtPdAu HNRs (10 mg/L) were added to sodium citrate (0.1 M, pH=4.0). The final volume is controlled at 1 mL, After adding all substances, the absorbance of the reaction solution at $\lambda_{\max} = 653$ nm was measured by UV-vis spectrophotometer at an interval of 1 seconds for 100 s.

Colorimetric Determination of H₂O₂ and AA

The steps determination of H₂O₂ and AA are based on the previously reported methods.³ In simple terms, for H₂O₂ detection, 50 μ L TMB (10 mg/mL), PtPdAu HNRs (50 μ L ,95 mg/L) and 50 μ L H₂O₂ with different concentrations were successively added to 850 μ L sodium citrate (0.1 M, pH=4). The reaction system was tested by UV-vis spectroscopy after incubation for 1 min. For AA detection, 50 μ L TMB (10 mg/mL), PtPdAu HNRs (50 μ L), H₂O₂ (10 mM, 50 μ L), 50 μ L ascorbic acid with different concentrations were successively added in 800 μ L sodium citrate (0.1 M, pH=4.0). The reaction system was tested by UV-vis spectroscopy after incubation for 1 min.

Determination of vitamin C tablets

We first diluted the actual sample into the linear range of the constructed AA sensing platform based on PtPdAu nanozyme, and used 0.025 mM AA as the test concentration. The test procedure was similar to that of the AA solution except for replacing AA solution with the diluted solution of actual samples. Finally, the AA contents of the actual samples was calculated through absorbance value at 653 nm and the AA standard curve.

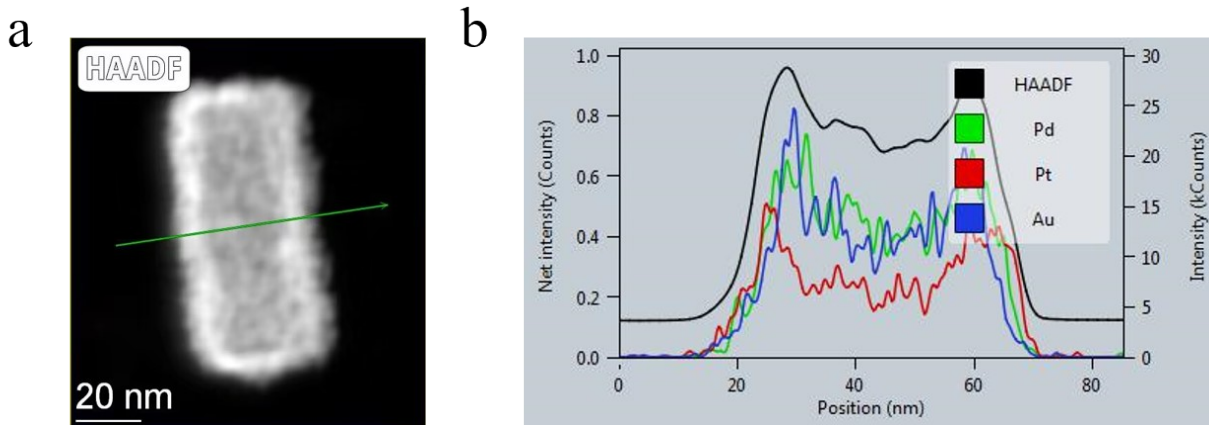


Fig. S1 (a) HAADF-STEM image of PtPdAu HNRs. (b) EDS line scanning of the PtPdAu HNRs in the left figure.

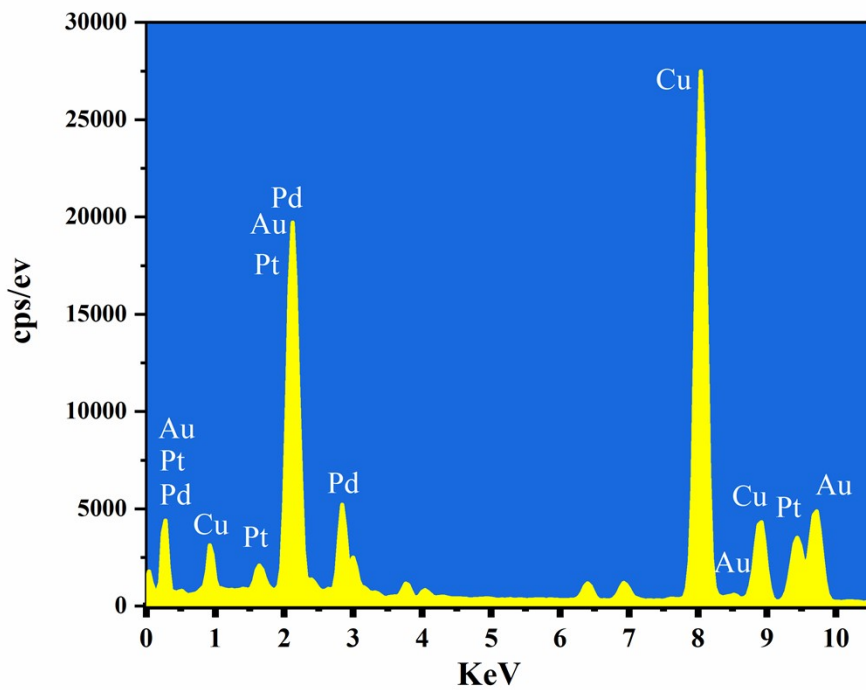


Fig. S2 EDS of PtPdAu HNRs.

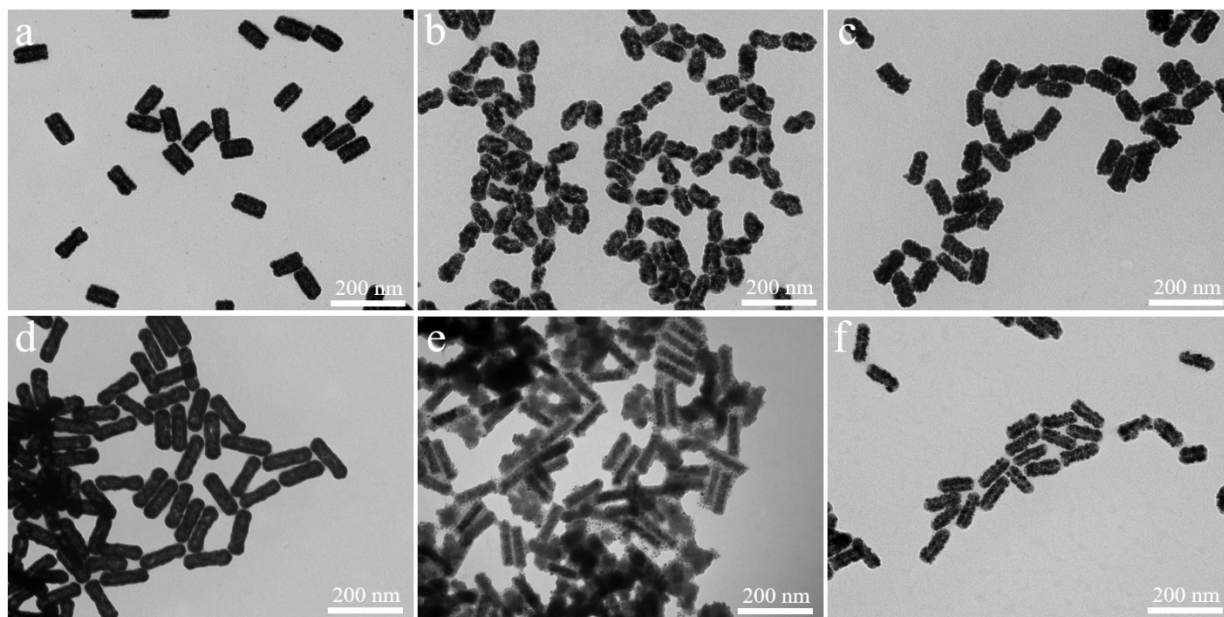


Fig. S3 TEM image of the PtAu nanorods (a), PtPd nanorods (b), PdAu nanorods (c), Au nanorods (d), Pd nanorods (e), Pt nanorods (f).

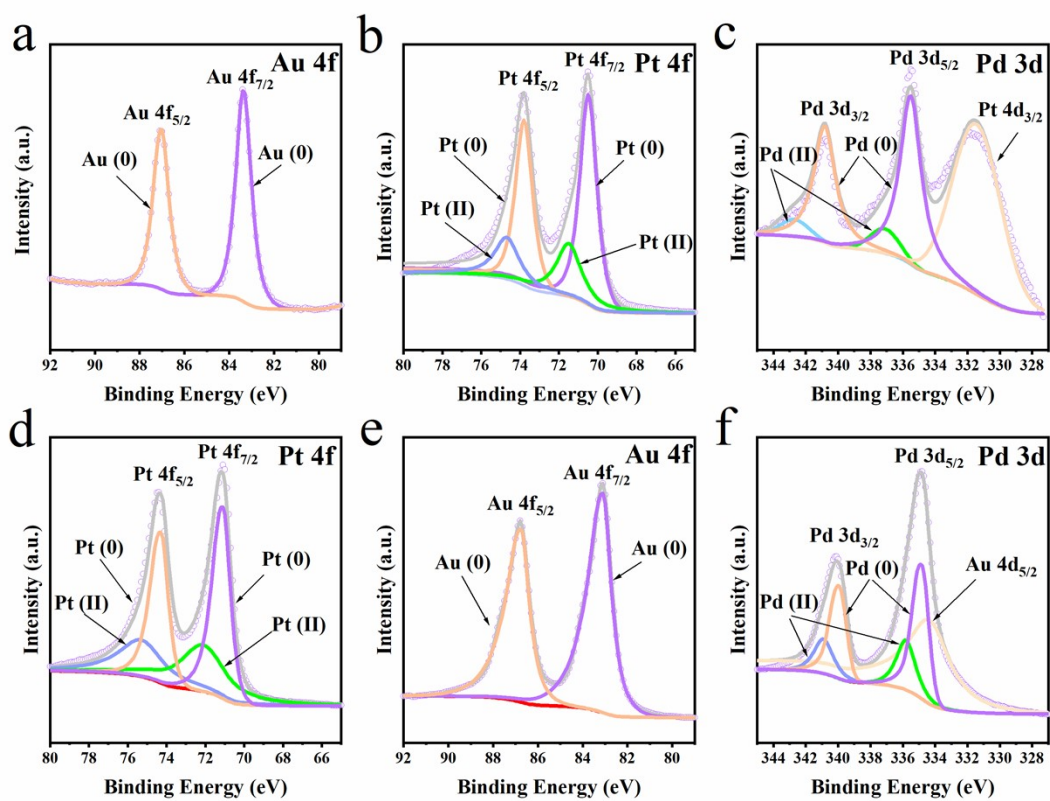


Fig. S4 XPS spectra of Au 4f (a), Pt 4f (b) for PtAu nanorod; Pd 3d (c), Pt 4f (d) for PtPd nanorod; Au 4f (e), Pd 3d (f) for PdAu nanorod.

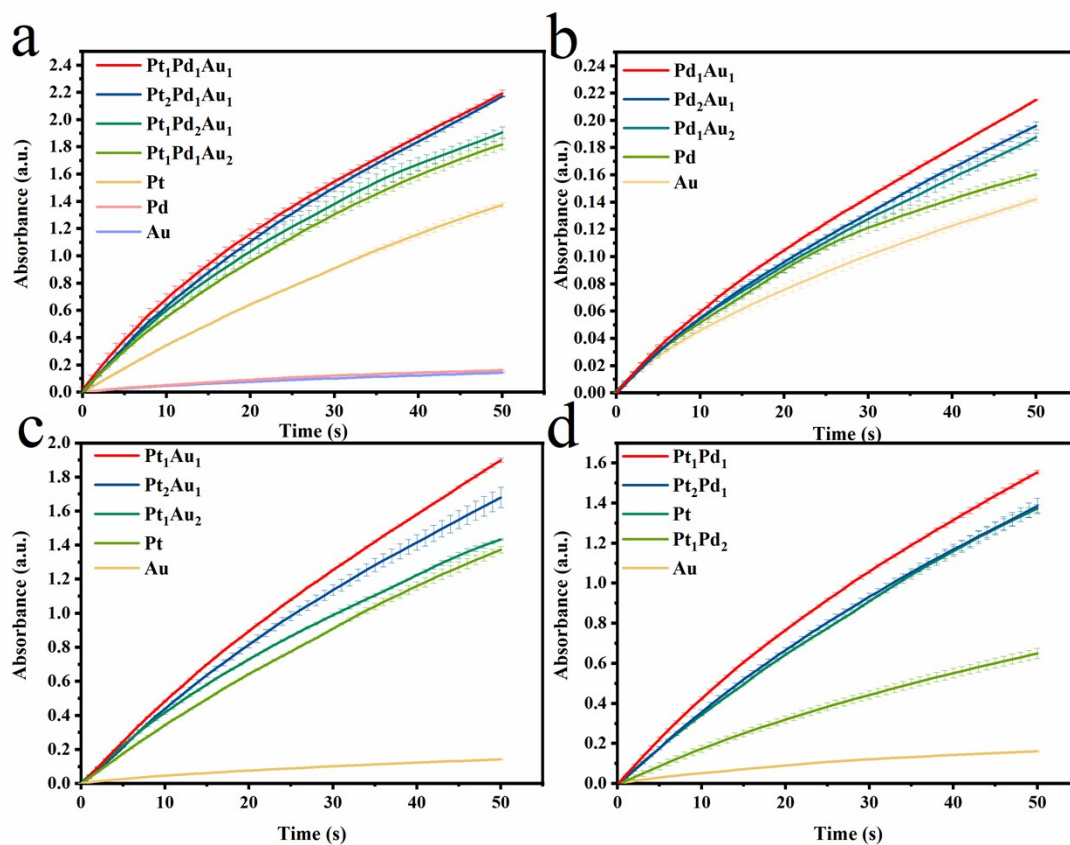


Fig. S5 Time-absorbance curves for different metal contents of HNRs. (a) PtPdAu, (b) PdAu, (c) PtAu, (d) PtPd.

Table S1 The elements ratio of PtPdAu, PtAu, PtPd, PdAu HNRs.

	Au (mg/L)	Pd (mg/L)	Pt (mg/L)
PtPdAu	0.1992	0.1025	0.1983
PtAu	0.2434	/	0.2530
PtPd	/	0.1609	0.2907
PdAu	0.3037	0.1566	/

Table S2 The specific activities of nanozymes.

	Substrate	SA(U mg ⁻¹)	ref
H-Pt ₃ Sn	TMB	345.32	4
FeSA-PtC	TMB	87.7	5
PdPtAu alloy	TMB	81.245	6
Pyrite	TMB	58	7
FeN ₃ P-SAzyme	TMB	316	8
USPBNPS	TMB	465.8	9
AuPtCo	TMB	27.1	10
Natural HRP	TMB	327	11
PtPdAu	TMB	563.71	
PtAu	TMB	528.75	This work
PtPd	TMB	387.43	
PdAu	TMB	6.58	

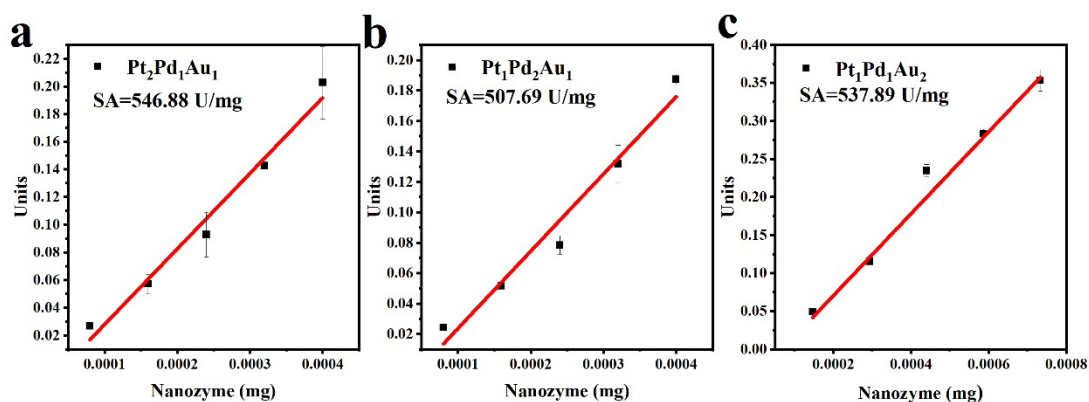


Fig. S6 The specific activity of PtPdAu HNRs with different metal ratios. (a) $\text{Pt}_2\text{Pd}_1\text{Au}_1$, (b) $\text{Pt}_1\text{Pd}_2\text{Au}_1$, (c) $\text{Pt}_1\text{Pd}_1\text{Au}_2$.

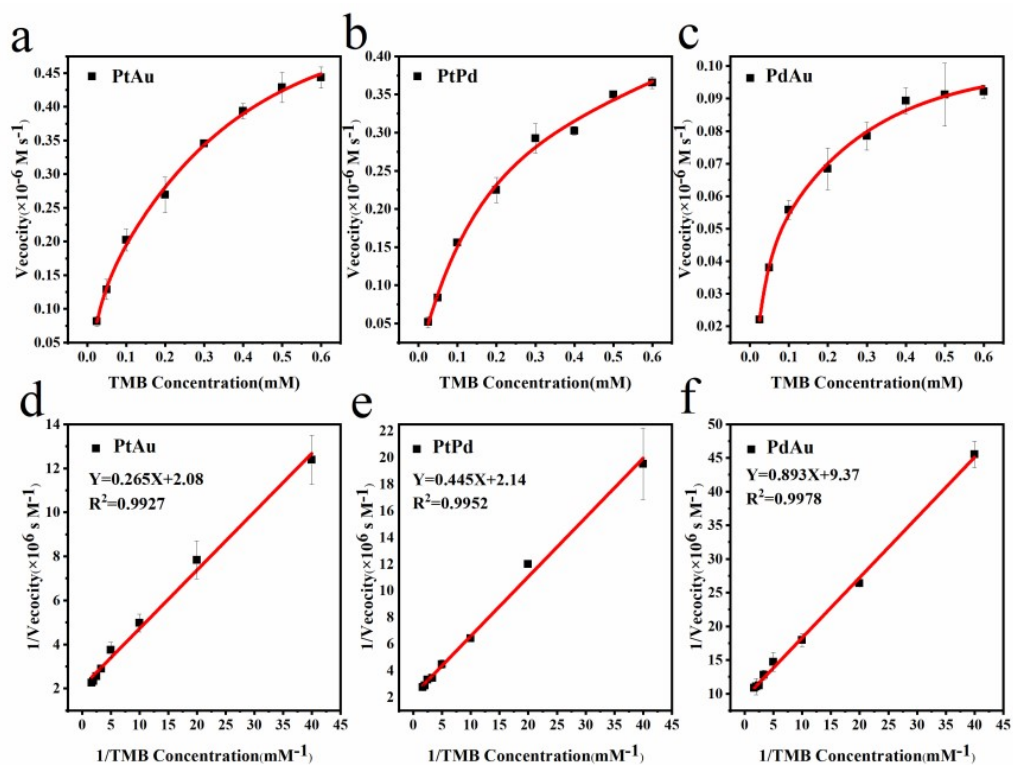


Fig. S7 Initial reaction velocity against TMB concentration and double-reciprocal plots for different nanozymes: PtAu nanozyme (a, d), PtPd nanozyme (b, e), PdAu nanozyme (c, f).

Table S3 Kinetic parameters of different nanozymes.

Nanozymes	Substrate	$V_{\max}(\times 10^{-6} \text{ M S}^{-1})$	$K_m(\text{mM})$
PtPdAu	TMB	0.49	0.11
PtAu	TMB	0.48	0.127
PtPd	TMB	0.47	0.208
PdAu	TMB	0.11	0.095

Table S4 Comparison of limit of detection of H_2O_2 using different nanozymes.

Nanozymes	Signal type	Detection range(μM)	Limit of detection(μM)	Reference
MIL-88	Colorimetry	2-20.3	0.562	12
PdIr aerogels	Colorimetry	1-500	0.8	3
Co_3O_4 NPs	Colorimetry	50-25000	10	13
Hemin@MOF	Colorimetry	5-200	2	14
GO-COOH	Colorimetry	0.05-1	0.05	15
C-Dots	Colorimetry	1-100	0.2	16
WS_2	Colorimetry	10-100	1.2	17
Hemin/ WS_2 -NSs	Colorimetry	5-140	1.0	18
MIL-53(Fe)	Colorimetry	0.95-19	0.13	19
PtPdAu	Colorimetry	1-500	1.8	This work

Table S5 Comparison of limits of detection of AA using different nanozymes.

Nanozymes	Signal type	Detection range	Limit of detection	Reference
Au/RGO	Electrochemistry	0.24-1.5 mM	0.05 mM	20
Pt–Ni alloy	Electrochemistry	0.57-5.7 mM	0.33 mM	21
PPF	Electrochemistry	0.4-6 mM	0.12 mM	22
ERGO	Electrochemistry	0.5-2 mM	0.3 mM	23
SNC-900	Colorimetry	0.1-5 mM	80 μ M	24
Au/Cu NRs	Colorimetry	0-2 mM	25 μ M	25
MIL-88	Colorimetry	2.57-10.1 μ M	1 μ M	12
PdIr aerogels	Colorimetry	0.5-250 μ M	0.22 μ M	3
PtPdAu	Colorimetry	1-50 μ M	0.068 μ M	This work

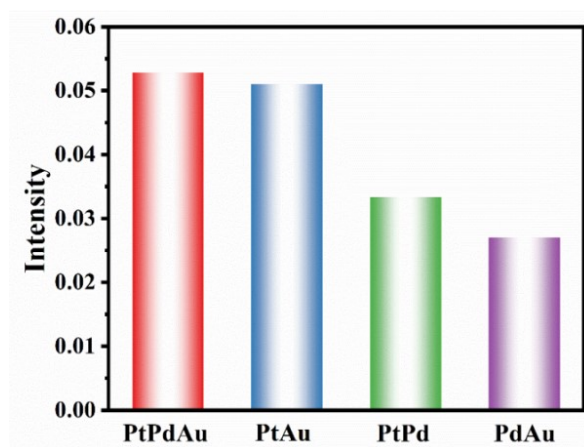


Fig. S8 the signal intensities of hydroxyl radical generated by different nanozymes under EPR test.

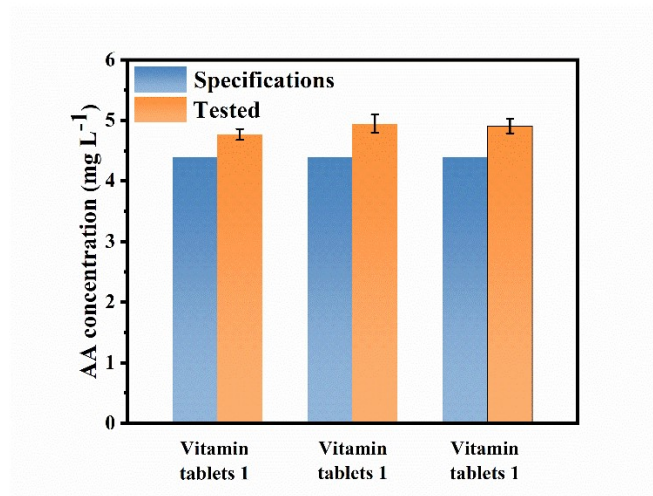


Fig. S9 AA concentration of vitamin C tablets tested by colorimetric detection in comparison with their specifications.

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