Electronic Supplementary Material (ESI) for Analyst.

Supporting information

Engineering Gold Nanoparticles-Carbon Dots Nanocomposite with pH-Flexibility for Monitoring Hydrogen Peroxide Released from Living Cells

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Materials and Reagents

The horseradish peroxidase (HRP), β-cyclodextrin (β-CDs), 3,3′,5,5′-tetramethylbenzidine (TMB), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), o-phenylenediamine (OPD), sodium hydroxide, phosphate buffer saline (PBS), and phorbol 12-myristate 13-acetate (PMA) were all purchased from Aladdin. The H_2O_2 solution, manganese chloride, ethylene diamine tetraacetic acid (EDTA) and terephthalic acid (PTA) were supplied by Sinopharm Chemical Reagent Co., Ltd. The singlet oxygen sensor green reagents (SOSG) were obtained from Sigma Aldrich. All other regents were in the analytical grade, and all solutions were prepared with ultrapure deionized water (18.2 MΩ·cm, Millipore).

Preparation of CDs

The CDs were prepared by a facile one-step hydrothermal method. In detail, 0.3 g of β-cyclodextrin was dissolved in 15 mL of aqueous solution, which was transferred to a 50 mL Teflon-lined autoclave for heating at 160 °C for 4 h. The obtained CDs were dialyzed with a dialysis membrane (MWCO~1000) for 24 h and then collected by freeze-drying. The concentration of CDs was fixed at 0.4 mg/mL, which was the same as the amount of AuNPs and Au-CDs nanocomposite in colorimetric assay.

Characterization of AuNPs and Au-CDs nanocomposite

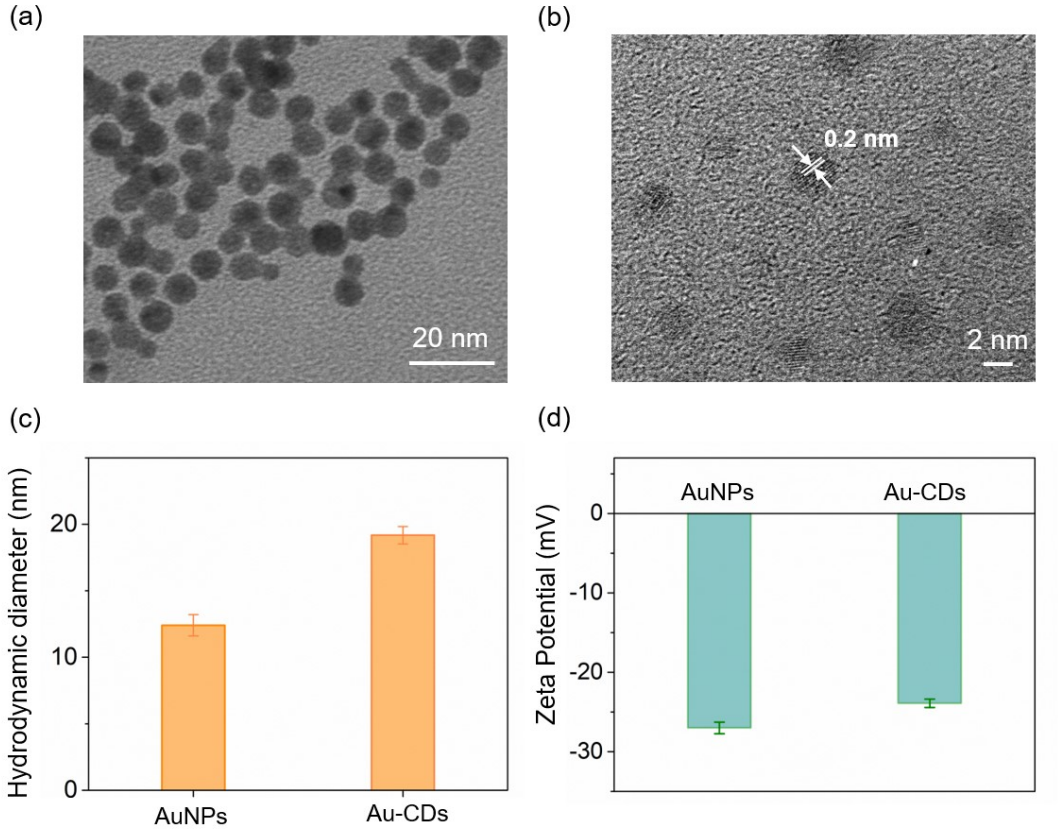

Figure S1 (a, b) TEM image of AuNPs (a) and CDs (b). (c, d) Variation of hydrodynamic size distribution (c) and zeta potential (d) in the construction of Au-CDs nanocomposite.

Elemental mapping images of Au-CDs nanocomposite

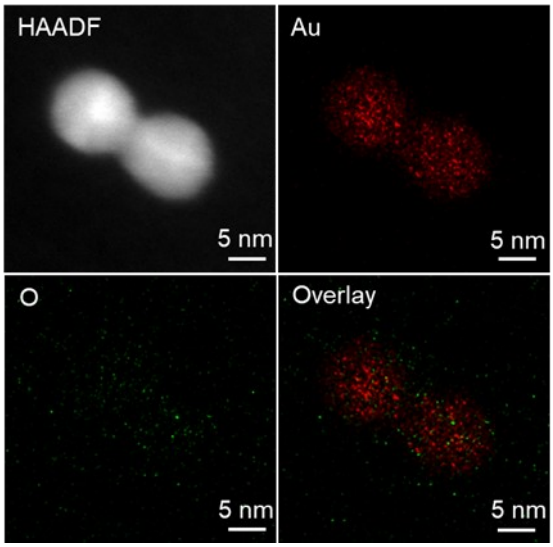

Figure S2 HAADF-STEM, Au, O, and overlay elemental mapping images of Au-CDs nanocomposite.

XPS spectra of AuNPs

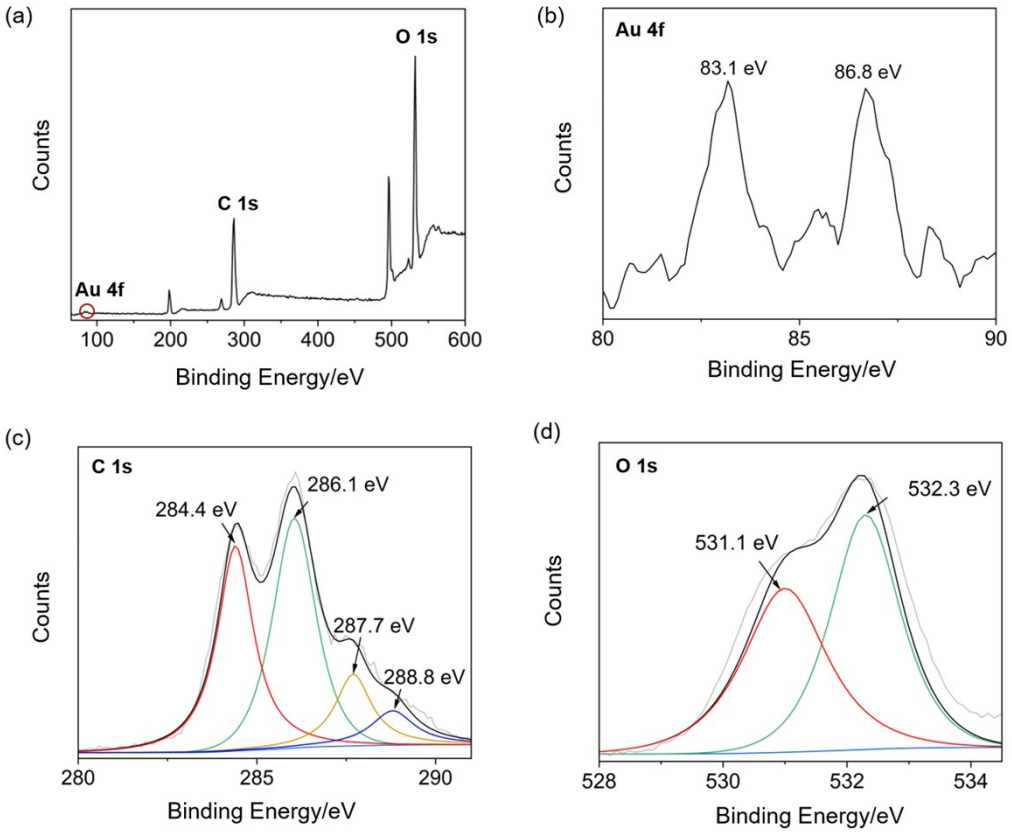

Figure S3 XPS survey spectrum of AuNPs (a) and high-resolution XPS spectra of Au 4f (b), C 1s (c) and O 1s (d).

XPS spectra of CDs

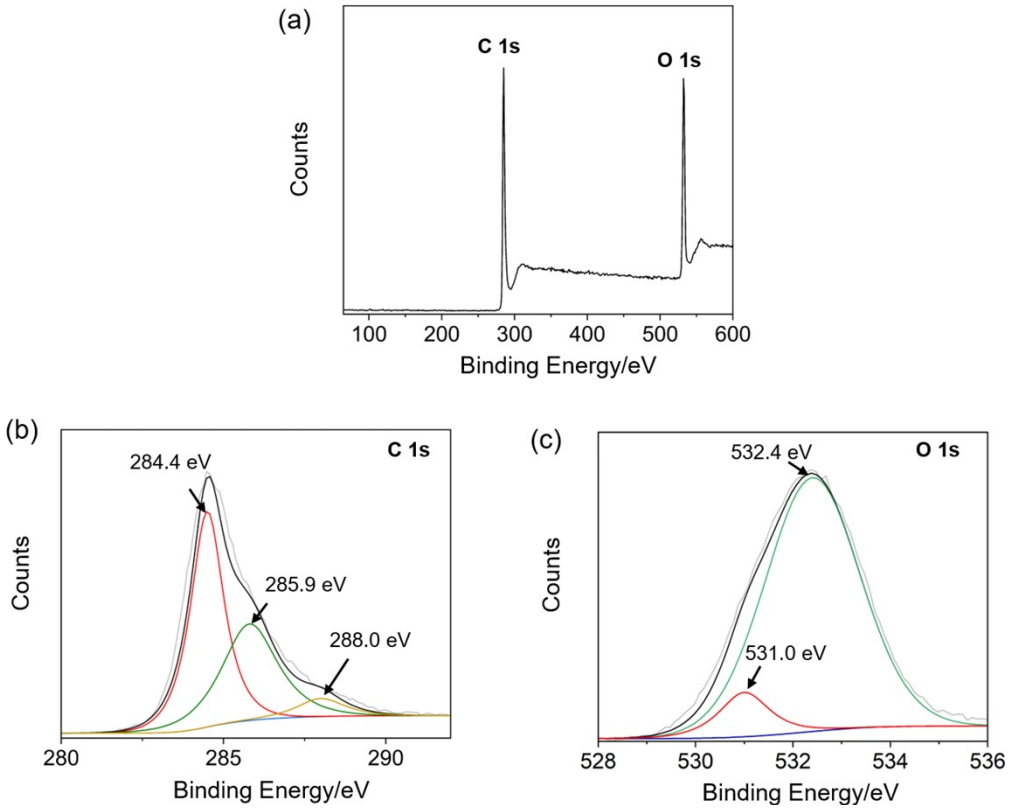

Figure S4 XPS survey spectrum of carbon dots (a) and high-resolution XPS spectra of C 1s (b) and O 1s (c).

Optimization of reaction parameters in the construction of Au-CDs nanocomposite

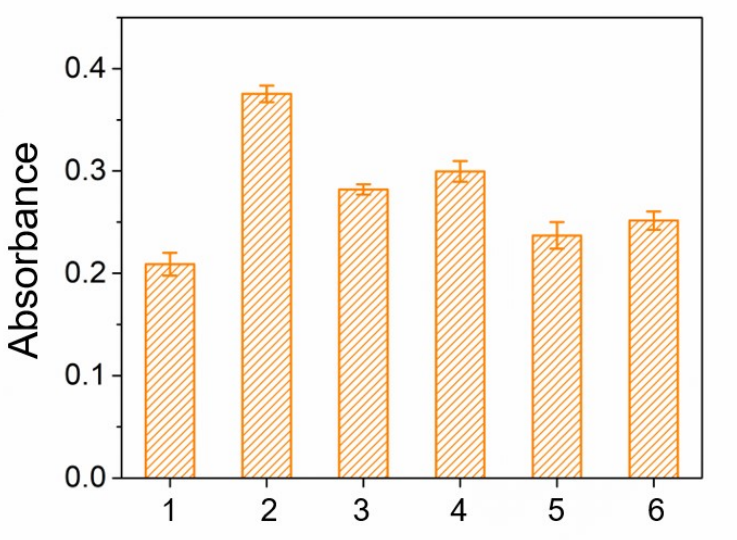

Figure S5 The absorbance of oxTMB at 652 nm varied with different reaction parameters in the construction of Au-CDs nanocomposite. The reaction conditions were as follows: 1: calcination at 160°C for 2 h; 2: calcination at 160°C for 4 h; 3: calcination at 160°C for 6 h; 4: calcination at 180°C for 2 h; 5: calcination at 180°C for 4 h; 6: calcination at 180°C for 6 h.

Optimization of reaction parameters in the Au-CDs nanocomposite mediated colorimetric assay

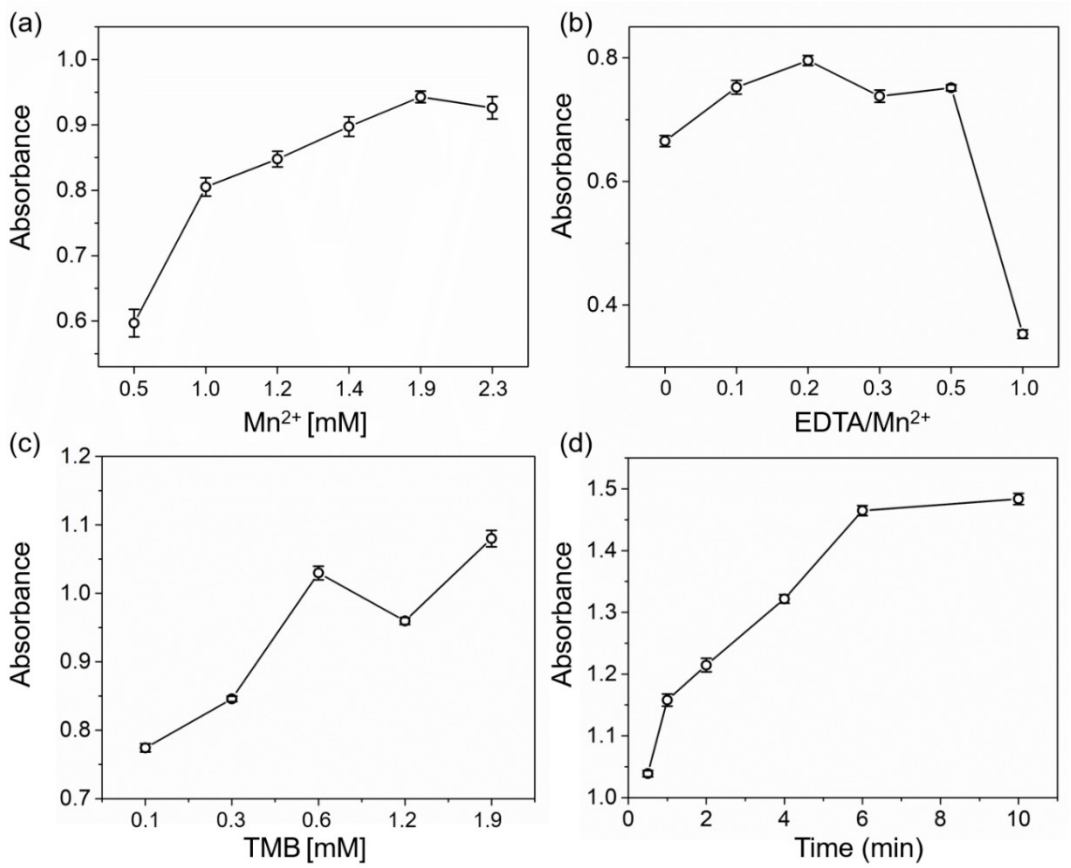

Figure S6 The absorbance of oxTMB at 652 nm varied with the concentrations of Mn^{2+} ions (a), different concentration ratios of EDTA to Mn^{2+} ions (b), the concentrations of TMB substrate (c) and the light illumination time (d).

Photo-oxidation performance of Au-CDs nanocomposite

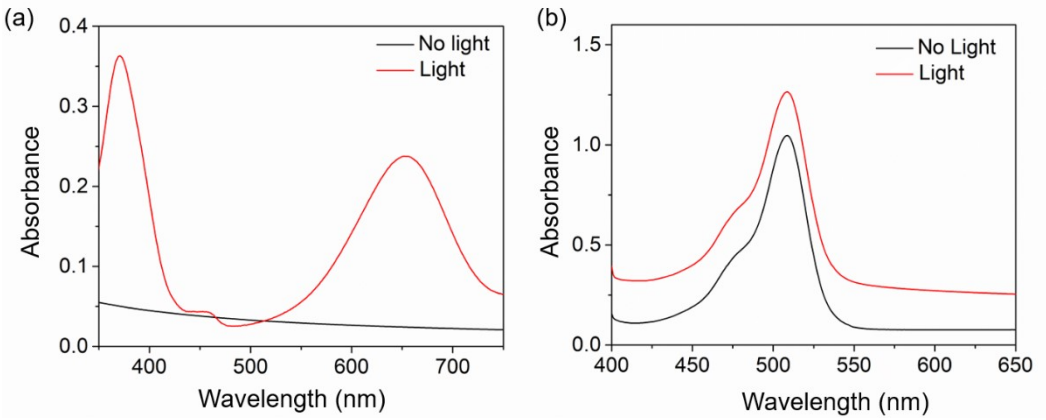

Figure S7 (a) The UV-vis spectra of TMB oxidized by Au-CDs nanocomposite with and without light irradiation in PBS buffer (10 mM, pH=7.4). (b) UV-vis spectra of SOSG incubated with Au-CDs nanocomposite with and without light irradiation in PBS buffer (10 mM, pH=7.4).

Verification of the hydroxyl radical during light irradiation

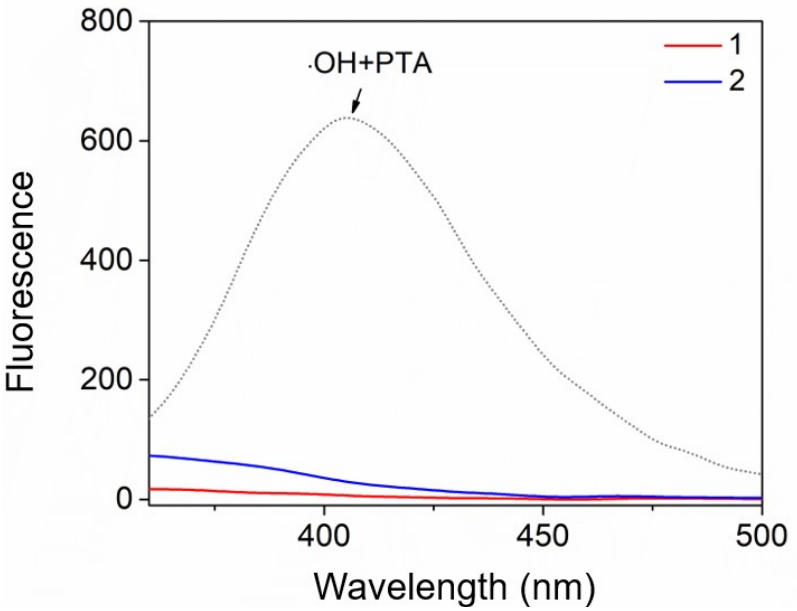

Figure S8 The fluorescent spectra of 2-hydroxy terephthalic acid (HTA) in the reaction buffer (10 mM PBS, pH=7.4) containing Au-CDs nanocomposite without (line 1) or with (line 2) light irradiation. In theory, the ·OH in the solution can react with PTA to generate 2-hydroxy terephthalic acid (HTA) emitting blue fluorescence (dot line) under excitation by 315 nm light.

The specificality of Mn^{2+} and EDTA ligand for improvement of catalytic performance

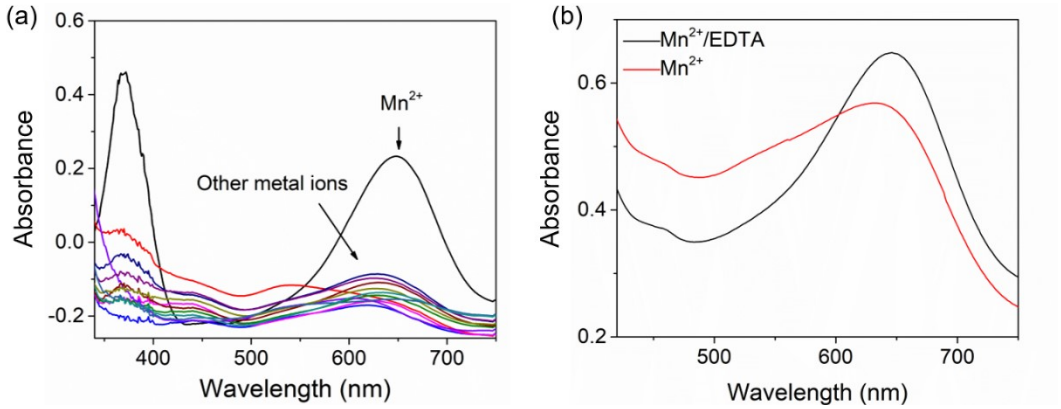

Figure S9 (a) The UV-vis spectra of TMB substrate oxidized by Au-CDs nanocomposite in the presence of various metal ions and EDTA ligands. The concentrations of metal ions including Mn^{2+}, Cr^{3+}, Co^{2+}, Ca^{2+}, Ba^{2+}, Zn^{2+}, Fe^{3+}, Mg^{2+}, Cd^{2+}, Ni^{2+}, Hg^{2+} and Cu^{2+} were 1.9 mM. (b) The UV-vis spectra of TMB substrate oxidized by Au-CDs nanocomposite with and without addition of EDTA ligands in the presence of Mn^{2+}.

Photo-oxidation capability of different nanozymes

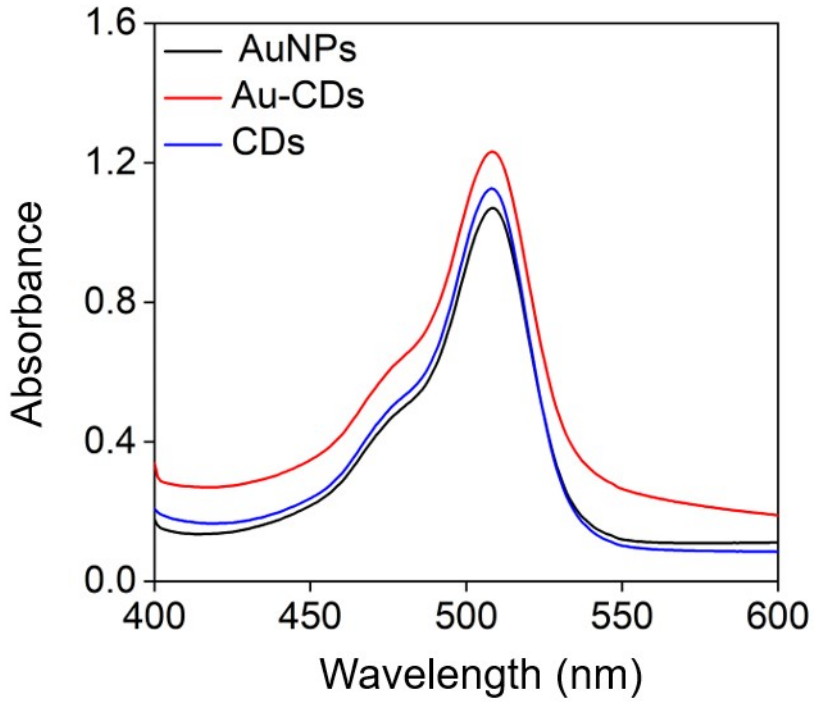

Figure S10 UV-vis absorption spectra of SOSG incubated with different nanoprobes under light irradiation.

Kinetics assay of Au-CDs nanocomposite

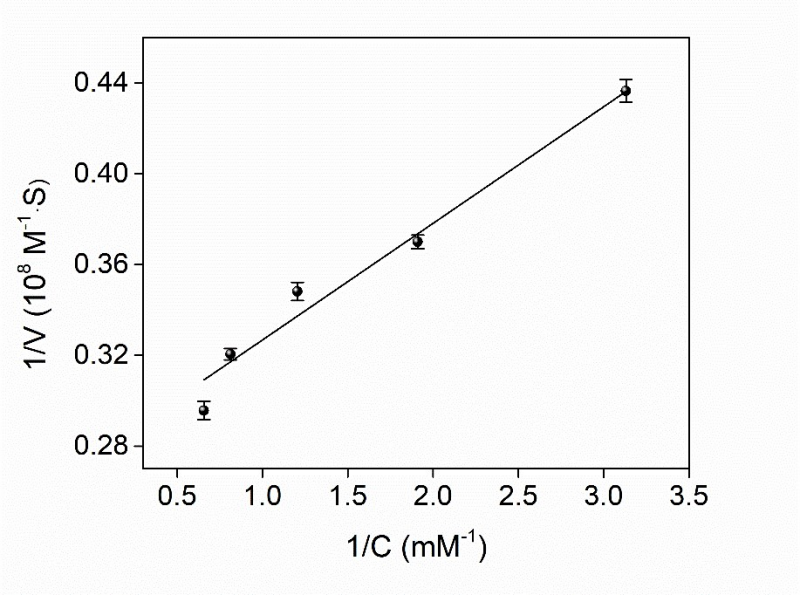

Figure S11 The double-reciprocal plots of the initial reaction velocity and the concentrations of TMB substrate in PBS buffer (10 mM, pH 7.4). The relative low K_m values (0.188 mM) of Au-CDs nanocomposite demonstrated the strong binding affinity compared to HRP.

Calculation of Limit of Detection (LOD)

The LOD was calculated according to the literature.

According to the calibration curve:

$$Y = a + bX \quad (1)$$

When b>0,

$$LOD = 10^{\frac{(y_0 + 3SD) - a}{b}} \quad (2)$$

When b<0,

$$LOD = 10^{\frac{(y_0 - 3SD) - a}{b}} \quad (3)$$

where *SD* and y_0 are the standard deviation and relative absorbance of the blank control, respectively.

The linear regression equation was expressed as:

$$Y = 0.059 + 0.521X \quad (4)$$

Since y_0 and *SD* of blank sample are 0 and 0.002 respectively, the LOD is calculated to be 1.86 nM.

The UV-vis absorption spectrum of MnO_2

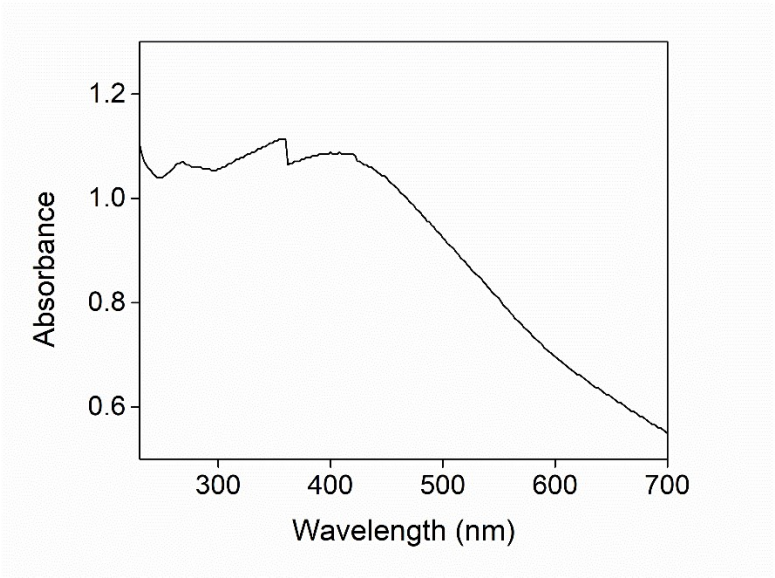

Figure S12 The UV-vis absorption spectrum of MnO_2 nanoparticles.

Fluorescent assay for the verification of hydroxyl radical with addition of H_2O_2

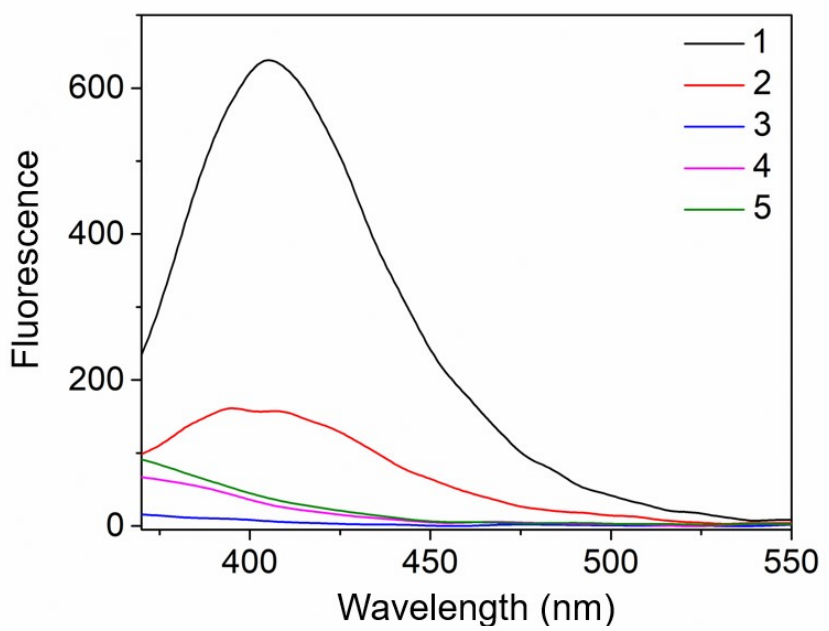

Figure S13 The fluorescence spectrum of terephthalic acid incubated with different reaction solution. 1: 10 mM PBS (pH=4.5) buffer containing Mn^{2+} ions, EDTA, H_2O_2 under light irradiation. 2: 10 mM PBS (pH=4.5) buffer containing Mn^{2+} ions and EDTA under light irradiation. 3: 10 mM PBS (pH=7.4) buffer containing Au-CDs nanocomposite under light irradiation. 4: 10 mM PBS (pH=7.4) buffer containing Au-CDs nanocomposite, Mn^{2+} ions and EDTA under light irradiation. 5: 10 mM PBS (pH=7.4) buffer containing Au-CDs nanocomposite, Mn^{2+} ions, EDTA and H_2O_2 under light irradiation.

Cytotoxicity of the Au-CDs nanocomposite

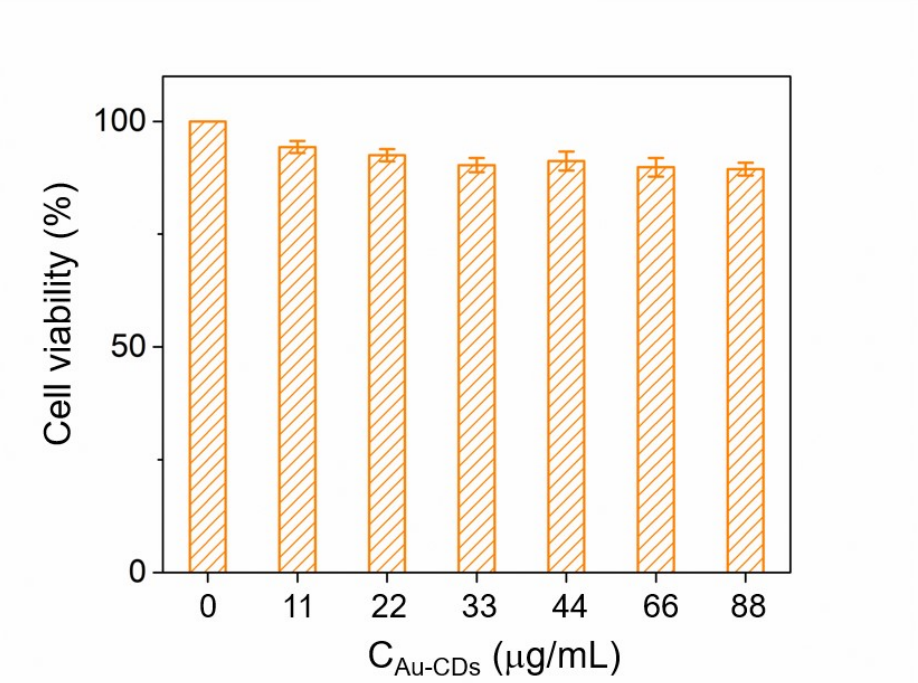

Figure S14 Cell viability of SW480 cells incubated with different concentrations of Au-CDs nanocomposite for 12 h.

Table S1 Comparison of the kinetic parameters of Au-CDs nanocomposite with HRP and other nanozymes in catalyzing TMB substrate

Catalyst	V_{max} **(10^{-8} M s^{-1})**	K_m **(mM)**	**Optimal pH**	**Ref.**
HRP	10.0	0.434	acid	17
AuNPs	12.15	0.74	acid	S1
Fe_3O_4	17.66	0.233	acid	17
Au-PtNCs	13.67	0.362	acid	S2
Au-CDs	3.51	0.188	neutral	This work

Table S2 Comparation of the performance of Au-CDs based colorimetric assay with previous methods for assaying H_2O_2

Material	**Analytical technique**	**Detection range**	**LOD**	**Ref.**
AuCuNWs	Electrochemical	3-360 nM	2 nM	S3
AuNCs-MnO_2	Fluorometric	0.06-2 μM	53 nM	4
ZIF-8	SERS	1 nM-1 mM	0.357 nM	10
CPP nanoflare	Colorimetric	5 μM-5 mM	1.2 μM	12
Au@MnO_2	Colorimetric	60-200 μM	6.54 μM	13
Au-CDs	Colorimetric	2.3 nM-0.94 mM	1.86 nM	This work

References

1 X. Chen, X. Tian, B. Su, Z. Huang, X. Chen and M. Oyama, *Dalton Trans.*,2014, **43**, 7449-7454.

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3 N. Wang, Y. Han, Y. Xu, C. Gao and X. Cao, *Anal. Chem.*, 2015, **87**, 457-463.