Electronic Supporting Information

Double Rare Earth Doped CDs Nanoplatform for Nanocatalytic/Starving-like Synergistic Therapy with GSH-Depletion and Enhanced Reactive Oxygen Species Generation

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1.1. Chemicals and reagents

Cerium nitrate hexahydrate ($Ce(NO_3)_3 \cdot 6H_2O$, 99.5%), gadolinium nitrate hexahydrate ($Gd(NO_3)_3 \cdot 6H_2O$, 99.5%), and hydrogen peroxide (H_2O_2, 35 wt%) were purchased from Innochem Science & Technology Co., Ltd (Beijing, China). Citric acid (CA) was acquired from Fengchuan Chemical Reagent Co., Ltd (Tianjin, China). 1,2-ethylenediamine (EDA, >99%) was obtained from Aladdin Bio-Chem Technology Co., Ltd (Shanghai, China). 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) was obtained from Sigma-Aldrich. N-hydroxysuccinimide (NHS) was acquired from Shanghai Aladdin Biochemical Technology Co., Ltd. H_2O_2 assay kit was obtained from Beijing Solarbio Science & Technology Co., Ltd. cell counting kit-8 (CCK-8) assay kit was obtained from Sigma-Aldrich (USA). 4T1 cells (mouse breast cancer cells) and L929 cells (mouse fibroblast cells) were attained from the Chinese National Infrastructure of Cell Line Resource. All chemicals were used as received without any further purification. Deionized water was prepared from the Milli-Q water purification system.

1.2. Instrumentation

The particle images were obtained on the transmission electron microscope (TEM) (JEOLJEM–1011, Japan) at the accelerating voltage of 100 kV. The morphology and particle size distribution of CDs were observed on a high-resolution transmission electron microscope (HR-TEM, Talos F200C). The size distribution of CDs was performed by dynamic light scattering (DLS, Malvern). The X-ray diffraction (XRD) spectrum of CDs were collected on a diffractometer (Smart Lab-9 kW) with the scanning range from 10° to 80°. Under dark conditions, the Raman spectra of CDs were analyzed on a Raman spectrometer (LABRAM HR Evolution). Fluorescence emission spectra were carried out on a LS-55 fluorophotometer. UV–Vis absorption spectra were conducted on a Shimadzu UV-2450 spectrophotometer. Fourier transform infrared (FT-IR) spectra of CDs were recorded on a Bruker Vertex 70

spectrometer from 4000 to 500 cm^{-1}. X-ray photoelectron spectra (XPS) were carried out on a Thermo Scientifific ESCALAB 250 Multitechnique Surface Analysis. Zeta potential was determined by using a Malvern Zeta-sizer Nano. The prepared CDs were freeze-dried using an FDU-2110 machine. The concentration of Gd^{3+}/Ce^{3+} in Ce-Gd@CDs-GOx solution was determined by using inductively coupled plasma-optical emission spectrometry (ICP-OES, PerkinElmer Optima 8000). The cell images were observed on inverted fluorescence microscopy (Leica DMI4000B).

2.5 Detection of the generation of H_2O_2

To test the ability of GOx in Ce-Gd@CDs-GOx nanozymes to produce H_2O_2, we evaluated it with Titanium (IV) oxysulfate (Ti(IV)OSO_4). Ce-Gd@CDs-GOx nanozymes solution was mixed with glucose solution (100 μg mL^{-1}, 10 mL) in a 37 ℃ constant temperature bath. 100 μL of solution was removed at different time points and mixed with 500 μL of acetone, then 50 μL Ti (IV) OSO_4 solution (30 mM), 100 μL $NH_3 \cdot H_2O$ and 500 μL 1M H_2SO_4 were added. The absorbance at 405 nm was measured and the concentration of H_2O_2 was determined according to the fitted standard curve. At different time points, the solutions were collected and their pH values were measured by a pH meter.

2.6 Oxygen generation

The generated oxygen was detected by a dissolved oxygen meter. Typically, 300 μL of H_2O_2 aqueous solution (100 mM) was added into 29.7 mL of Ce-Gd@CDs-GOx (2 mg mL^{-1}) PBS solutions at different pH values (5.5, 6.5, and 7.4) under vigorous stirring condition. The variation of dissolved oxygen was automatically recorded every 3 min.

2.7 The detection of ·OH

Detection of ·OH generation of Ce-Gd@CDs-GOx nanozymes was performed using

TMB as substrates in the presence of glucose in PBS solution (pH 6.5). The absorbance of the color reactions was recorded after a certain reaction time using a UV-Vis spectrophotometer. The steady-state kinetic assays were performed in 3 mL PBS solution (pH 6.5) with Ce-Gd@CDs-GOx (100 μg mL^{-1}) as catalyst in the presence of glucose and TMB.

For each glucose concentration, the initial reaction rates (v_0) of the generation of ·OH were calculated from the absorbance variation using the Beer-Lambert Law (Equation (1)) (with an ε of 39000 m^{-1} cm^{-1} for oxTMB). The reaction rates were then plotted against their corresponding glucose concentration and then fitted with the Michaelis-Menten curves (Equation (2)). Furthermore, a linear double-reciprocal plot (Lineweaver-Burk plot, Equation (3)) was used to determine the maximum velocity (V_{max}) and Michaelis-Menten constant (K_m). The Km reveals the affinity of the nanozyme for the substrate.

$$A = \varepsilon lc \tag{1}$$

$$V_0 = \frac{V_{max} \cdot [S]}{K_m + [S]} \tag{2}$$

$$\frac{1}{V_0} = \frac{K_m}{V_{max}} \cdot \frac{1}{[S]} + \frac{1}{V_{max}} \tag{3}$$

2.10 Cell culture

Mouse mammary cancer 4T1 cells and mouse fibroblasts cells (L929 cells) were purchased from Changchun Yiyuan Biotechnology Co., LTD.. 4T1 cells and L929 cells were cultured in RPMI 1640 medium supplemented with 10.0% fetal bovine serum (FBS) and 1.0% penicillin/streptomycin at 37°C under 5% CO_2.

2.10 Cytotoxicity assay

The standard CCK-8 assay was applied to ascertain cytotoxicity for 4T1 cells. Briefly, 4T1 cells were incubated in 96-well plate (5×10^3 cells per well) for 24 h at 37℃ under 5% CO_2. The cells were treated by RPMI-1640 medium containing different concentrations (0-1.0 μg mL^{-1}) of Ce-Gd@CDs and Ce-Gd@CDs-GOx nanozymes and incubated for 24 h. Next, the cells of each well were handled with 10.0 μL CCK-8 and cultivated for 4 h. Finally, the optical density (OD) was quantified at 450 nm using the microplate reader, and the activity of cells was assessed as a percentage.

$$\text{Cell viability (100\%)} = \frac{\text{the absorbance of samples - the absorbance of blank}}{\text{the absorbance of control group - the absorbance of blank}}\times 100\% \quad (1)$$

2.12 Statistical analysis

All experimental data in this paper were expressed as mean ± standard deviation (SD). Meanwhile, the statistical analyses were performed with Origin 2019.

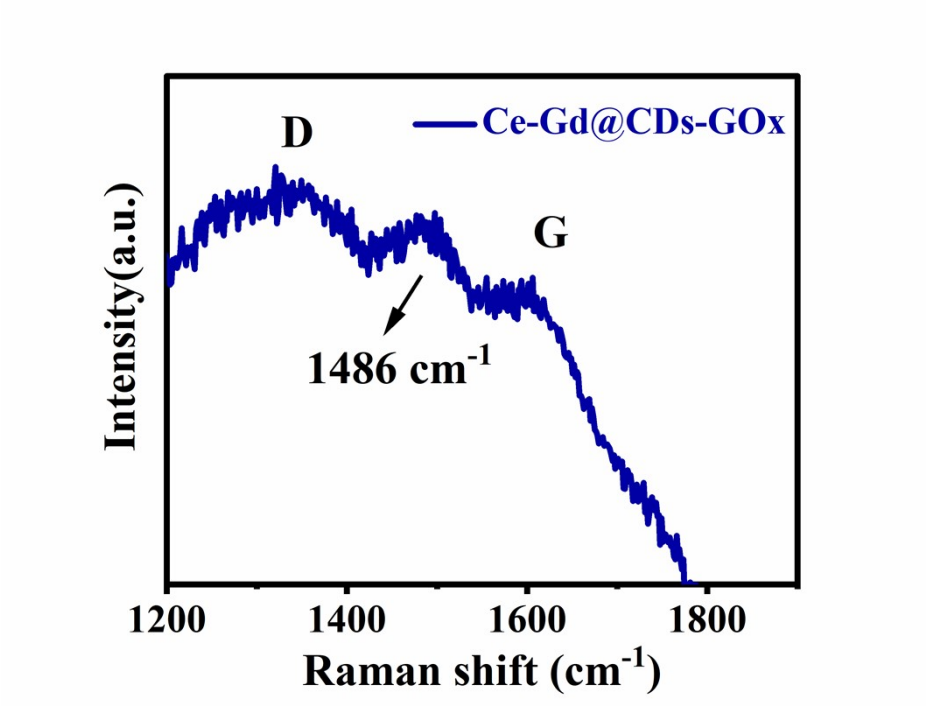

Fig. S1 Raman spectrum of Ce-Gd@CDs-GOx

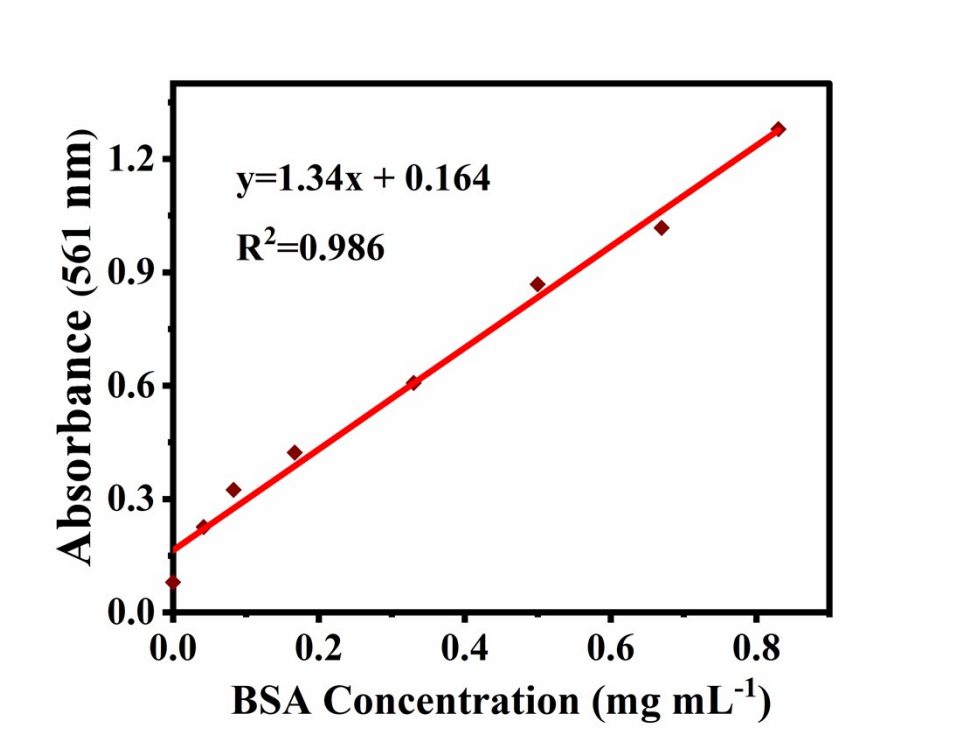

Fig. S2 The standard curve of BSA for BCA protein assay kit.

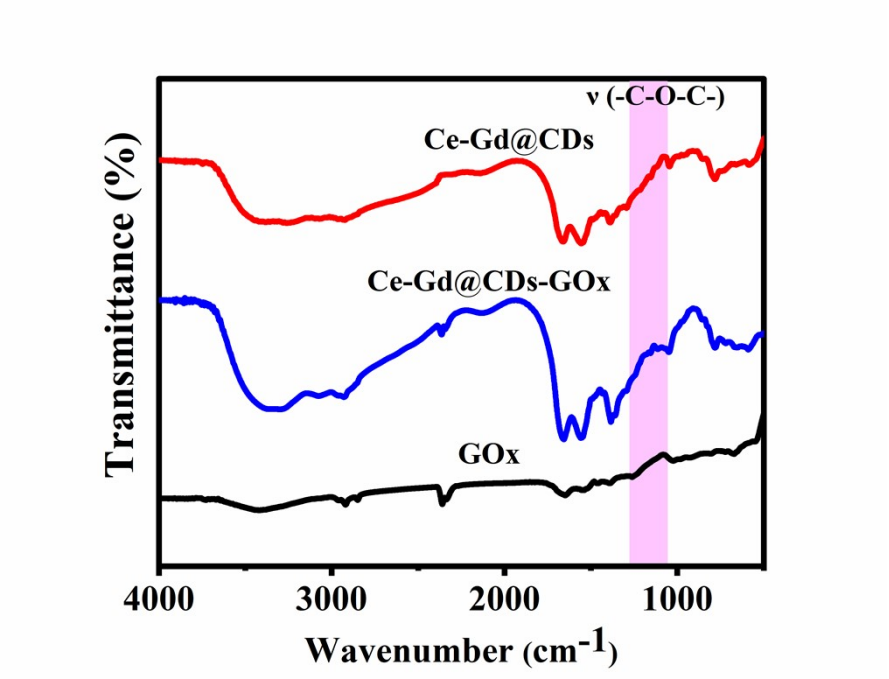

Fig. S3 FT-IR spectra of Ce-Gd@CDs, Ce-Gd@CDs-GOx, and Free GOx

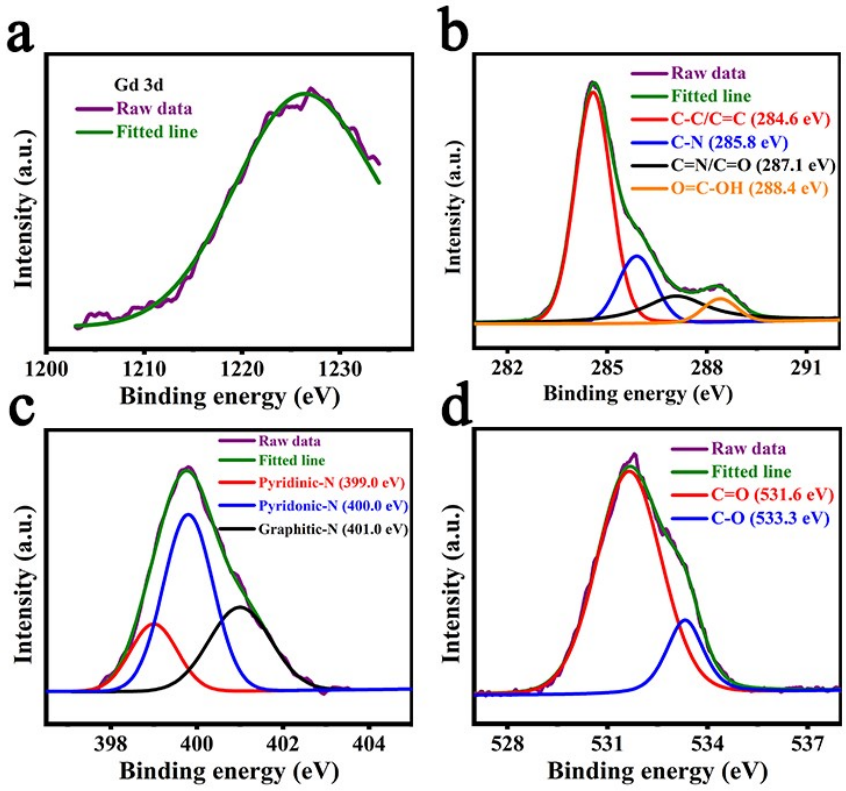

Fig. S4 High-resolution XPS spectra of Gd3d(a), C1s(b), N1s(c), and O1s(d)

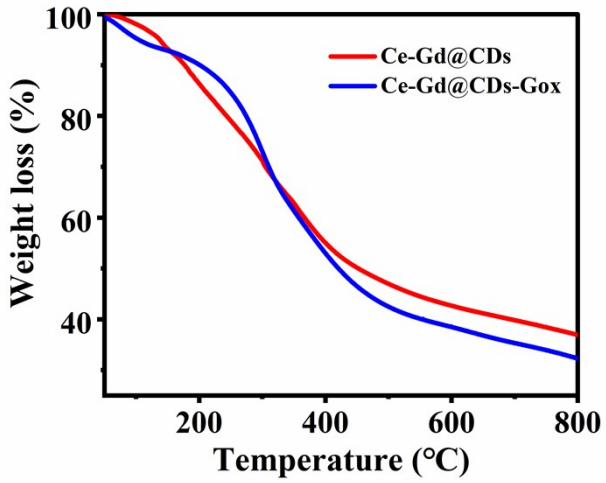

Fig. S5 The thermogravimetric curves of Ce-Gd@CDs and Ce-Gd@CDs-GOx

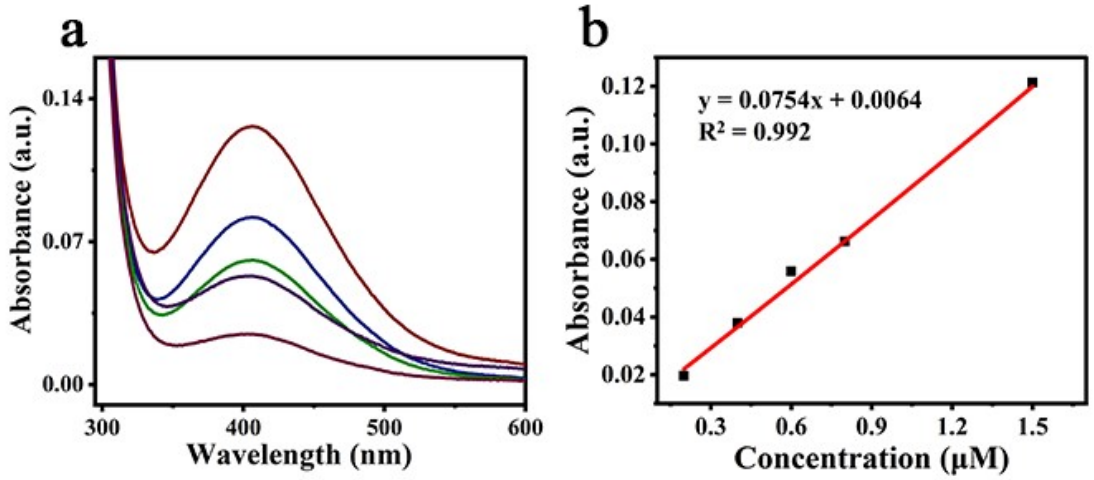

Fig. S6 (a) UV-Vis absorption spectra of $Ti(IV)O_2SO_4$ at 405 nm. (b)The standard curve of H_2O_2 concentration derived from the of $Ti(IV)O_2SO_4$ at 405 nm.