Supplementary Information for

Co_3O_4 Cored Carbon Dots for Chemiluminescence Analysis of Intracellular Hydrogen Peroxide

Junyu Zhou [a], Jiangjiang Gu [b], Chunxiu Tian [b], Dechen Jiang [b], Yun Chen [a]*, Kai Xi [b]*

[a] School of Pharmacy, Nanjing Medical University, Jiangsu, 210000, China

[b] School of Chemistry and Chemical Engineering, Nanjing University, Jiangsu, 210093, China

Email: ychen@njmu.edu.cn (Y.C); xikai@nju.edu.cn (K.X)

Phone: 086-25- 025-86862764 (Y.C); 86-25-83597080 (K.X)

Fax: 086-25-86868477 (Y.C); 86-25-83597080 (K.X)

Experimental section.

Synthesis of Co_3O_4 nanoparticles and Co_3O_4@CDs complex.

0.5 g $Co(CH_3COO)_2 \cdot 4H_2O$ was dissolved in 25 mL pure water and stirred for 15 min. 2.5 mL ammonia solution (25~28%) was dropped and stirred for the other 10 min to form a homogeneous suspension liquid. The mixture was transferred into a 50 mL Teflon equipped stainless steel autoclave and maintained in an oven at 150°C for 3 h. Then, the autoclave was cooled down at room temperature. The obtained black solid was washed using pure water via centrifugation–redispersion process and dried at 60°C for 4 h to get the final dry Co_3O_4 nanoparticles.

For the preparation of Co_3O_4@CDs complex, 0.1 g Co_3O_4 nanoparticles and 4 g polyacrylamide solution (50%) were diluted in 20 mL pure water and processed with ultrasonic disruptor for 30 min. The mixture was transferred into a 50 mL Teflon equipped stainless steel autoclave and placed at 260°C for 24 h. Then, the autoclave was cooled to room temperature naturally. Finally, the product was washed with pure water via centrifugation–redispersion process and dried at 60 °C for 4 h to obtain the final Co_3O_4@CDs complex.

Characterization of Co_3O_4@CDs complex.

JEM-2100 electron microscope (JEOL, Japan) at an accelerating voltage of 200 kV was used to obtain transmission electron microscopy (TEM) images. Fourier transform infrared (FTIR) spectroscopy were performed on an Vector-22 spectrometer (Bruker) in the KBr pellet，ranging from 500 to 4000 cm^{-1}. The X-ray diffraction (XRD) patterns were measured by a D8 Advance X-ray diffractometer (Bruker) with Cu Ka radiation (40 kV, 20 mA, λ =1.54051 Å). Elemental analysis was measured with a CHN-O-Rapid elemental analyzer (Heraeus, Germany). All fluorescence spectra of the CNPs were measured with a F-4500 fluorescence spectrometer (Hitachi, Japan) with slit width of 5 nm for both excitation and emission.

Loading of the complex into the cells.

MCF 7 and Hela cells were seeded in Dulbecco's modified Eagle's medium (DMEM)/high-glucose medium supplemented with 10% fetal bovine serum (FBS) and 1% antibiotics (penicillin/streptomycin). Cultures were maintained at 37 °C under a humidified atmosphere containing 5% CO_2. For the loading of the complex into the cells, Co_3O_4@CDs complex was attempted to be loaded into MCF 7 or Hela cells through the incubation in the medium with

Co_3O_4@CDs complex (0.1 mg/mL) overnight. After washing by PBS for three time, the bright-field and fluorescence images of the cells were recorded using confocal microscopy (Leica, TCS SP5) with the excitation wavelength of 488 nm.

MTT assay

The cells (10^5 cells/ml) with Co_3O_4@CDs complex complex or Co_3O_4 nanoparticles were cultured in the medium in a 96-well microplate (200 μl/well) overnight. After washing the cells using 10 mM PBS, 5 mg/ml 3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide (MTT) solution was introduced into the wells for 4 h. Then, the remaining MTT solution was removed and 150 μl of DMSO was added into the wells to dissolve the formazan crystals. The absorbance was recorded at 490 nm in the En-Spire multimode plate reader. In the control group, the complex was not loaded into the cells.

More figures.

Figure S1: XRD spectrum of Co_3O_4@CDs complex and Co_3O_4 nanoparticles

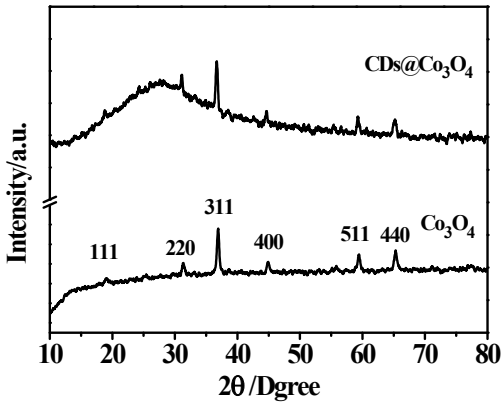

Figure S2: The luminescence spectrum from Co_3O_4@CDs complex (0.1 mg/ml) and 1 mM hydrogen peroxide collected through the filters

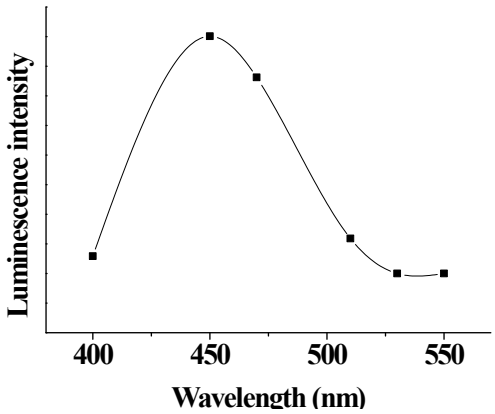

Figure S3: Correlation of luminescence intensity with hydrogen peroxide concentration from the concentration of 10 μM to 1 mM. The concentration of the complex was 0.1 mg/ml. The error bar presented the standard deviation (n = 3). The linear regression was 0.96.

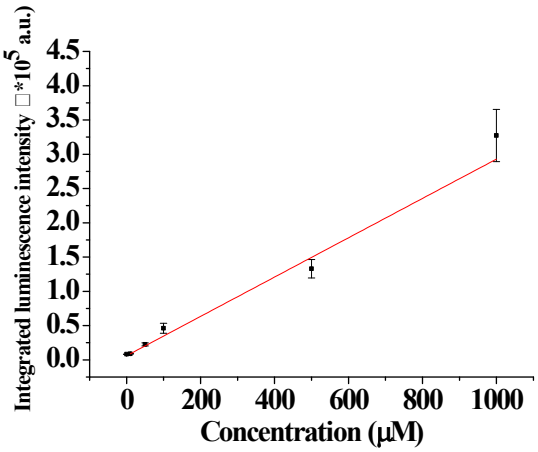

Figure S4: The lifetime of the luminescence from Co_3O_4@CDs complex (0.1 mg/ml) and 1 mM hydrogen peroxide.

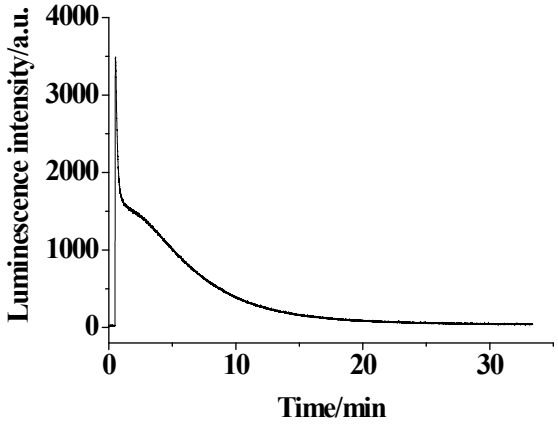

Figure S5: (A) MTT assay of Hela cells loaded with Co_3O_4@CDs and Co_3O_4 nanoparticles; (B) the typical luminescence traces collected from the Co_3O_4@CDs loaded cells exposure to 10 mM hydrogen peroxide.