Supporting Information

Facile and Green Synthesis of Carbon Nanodots from Environmental Pollutant for Cell Imaging and Fe^{3+} detection

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1. Chemical reagents and synthesis of CNDs

All commercial materials were used without further purification, unless indicated. The deionized water was prepared in the laboratory. Acid orange 7 (AO7) was purchased from Shanghai Macklin Biochemical Co., Ltd. The CNDs were synthesized by using the hydrothermal method, in a typical procedure. Briefly, 0.15 g of AO7 and 34 mL of deionized water were added into a 50 mL Teflon-lined autoclave and heated at 200 ˚C for 14 hours. After cooled naturally, the solution was centrifuged at 10000 r/min for 30 min to remove the sediments. Next, the solution was filtered by the 0.22 μm membrane filter. Finally, the CNDs solution was obtained and the yield was calculated to be 90.7 %.

2. Characterization of CNDs

Transmission electron microscope (TEM) and High-resolution TEM (HRTEM) were utilized to observe the synthesized CNDs on JEOL JEM-2100 F. Atomic force microscopy (AFM) images were obtained on BRUKER Dimension Icon. Raman spectrum was measured on Thermo Scientific DXR. Powder X-ray diffraction (XRD) was performed on a Rigaku Ultima IV (Cu Kα radiation, 3kW). X-ray photoelectron spectroscope (XPS) was performed on a Thermo ESCALAB 250XI. Fourier transform Infrared spectrum (FT-IR) was acquired on PE Fourier Transform. UV-vis. was obtained on a UV-1900 (Shimadzu, Japan). PL emission spectra were measured by using the RF-6000 (Shimadzu, Japan).

3. Cell culture and cell viability assay

The human cervical cancer cells (HeLa) were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with FBS (10%) and 1% Gibco® antibiotic-antimycotic solution in 5% CO_2 at

37 °C. A colorimetric MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, Sigma Aldrich) assay was performed to detect cell viability as previously described [59]. Specifically, Hela cells were plated as 2×10^3 cells / well in 96-well plates. After treatment with gradient concentrations of AO7 or CNDs (0, 100, 250, 750, 1000, 1500 mg/L) for 24 hours, cells were washed with PBS and incubated with 0.5 mg/mL MTT in DMEM medium at 37°C for 3 hours. Subsequently, the medium was removed and DMSO was used to dissolve crystals. After 5 min incubation at room temperature the cell mortality was assessed with an automatic microplate reader (Tecan Switzerland), using a test wavelength of 450 nm and a reference wavelength of 490 nm. The cell viability was calculated using the following equation: Cell viability (%) = (optical density of the cells treated with AO7 or CNDs / optical density of the control) × 100%.

4. Cell imaging

The potential for bioimaging with CNDs was tested by using HeLa cells. CNDs were initially dissolved in ultrapure water at a concentration of 40 mg/mL before use. Approximately 2×10^3 cells were deposited on each confocal petri dish (14 mm glass center) to form a sparsely distributed layer of cells to ensure good exposure to the CNDs. HeLa cells were cultured in DMEM growth medium with fetal bovine plasma (10%) at 37 °C under 5% CO_2. After 24 hrs incubation of 100 mg/L CNDs, the cells were washed three times with PBS (pH = 7.4) and then fluorescence images were obtained by using a confocal fluorescence microscope (LSM 880, Carl Zeiss ZEISS).

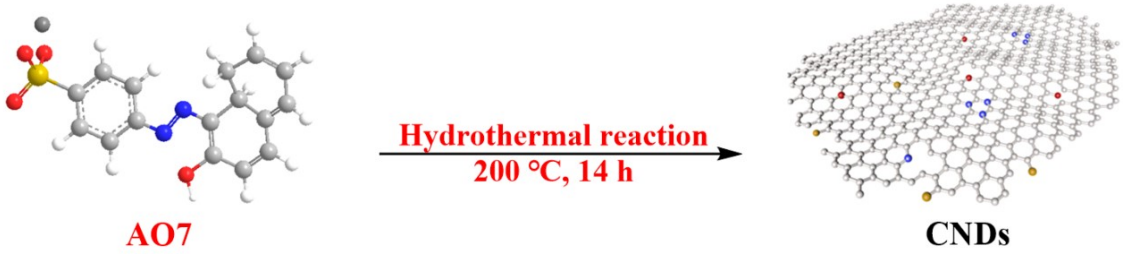
5. CNDs selective detection of Fe^{3+}

A variety of metal ions (Fe^{3+}, Fe^{2+}, Ni^{2+}, Co^{2+}, Cr^{3+}, Ca^{2+}, Al^{3+}, Na^{+}, Cu^{2+}, K^{+} and Zn^{2+}) were detected

by AO7-CNDs solution. Typically, for the detection of Fe^{3+}, 0.1 mL CNDs solution (4.0 mg/mL) and 3.6 mL DI water were added into flask. Next, 0.3 mL Fe^{3+} solution was added into the above solution until the final concentration of Fe^{3+} fixed at 8 mmol/L. Finally, PL emission spectra were measured at an excitation wavelength of 320 nm.

6. Statistical analysis

Data were analyzed using GraphPad Prism (Version 7.0) or OriginPro (Version 8). All the numerical results were expressed as means ± SEM. Analysis of variance (ANOVA) was used to determine significant differences followed by Sidak's multiple comparison post-test. *P* values < 0.05 were considered statistically significant. Statistical differences of $p < 0.05$, $p < 0.01$, $p < 0.001$ are represented by *, ** or ***, respectively.



Scheme S1. The synthesis of CNDs from Acid Orange 7 (AO7).

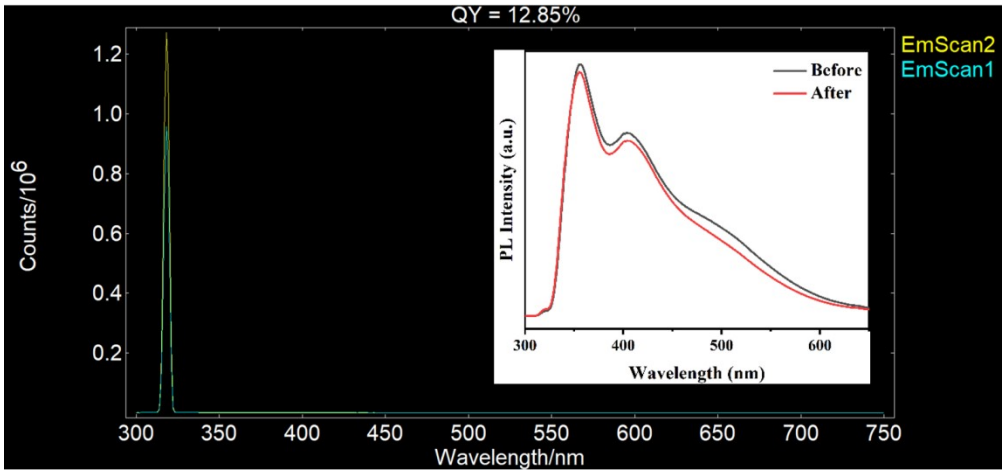


Figure S1. The PLQY measurement of CNDs using FS5 spectrometer (The illustration shows the fluorescence emission spectra of CNDs at 320 nm excitation around 12 hours of continuous irradiation of a 365 nm UV lamp).

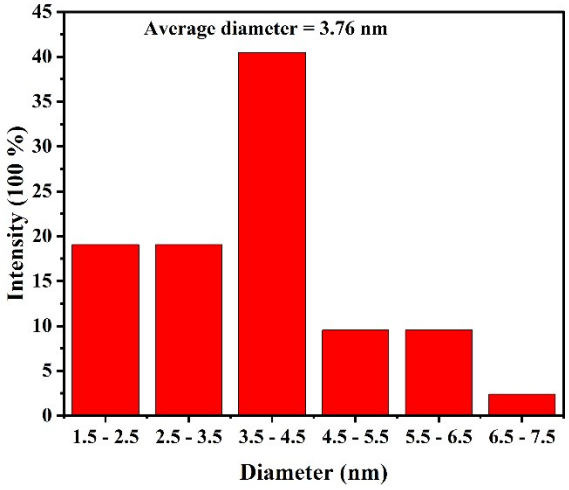


Figure S2. The Diameter picture of CNDs.

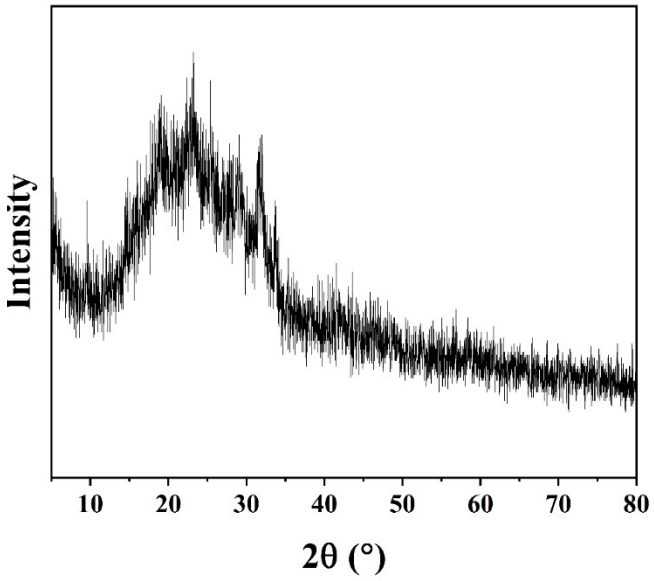


Figure S3. The XRD of CNDs.

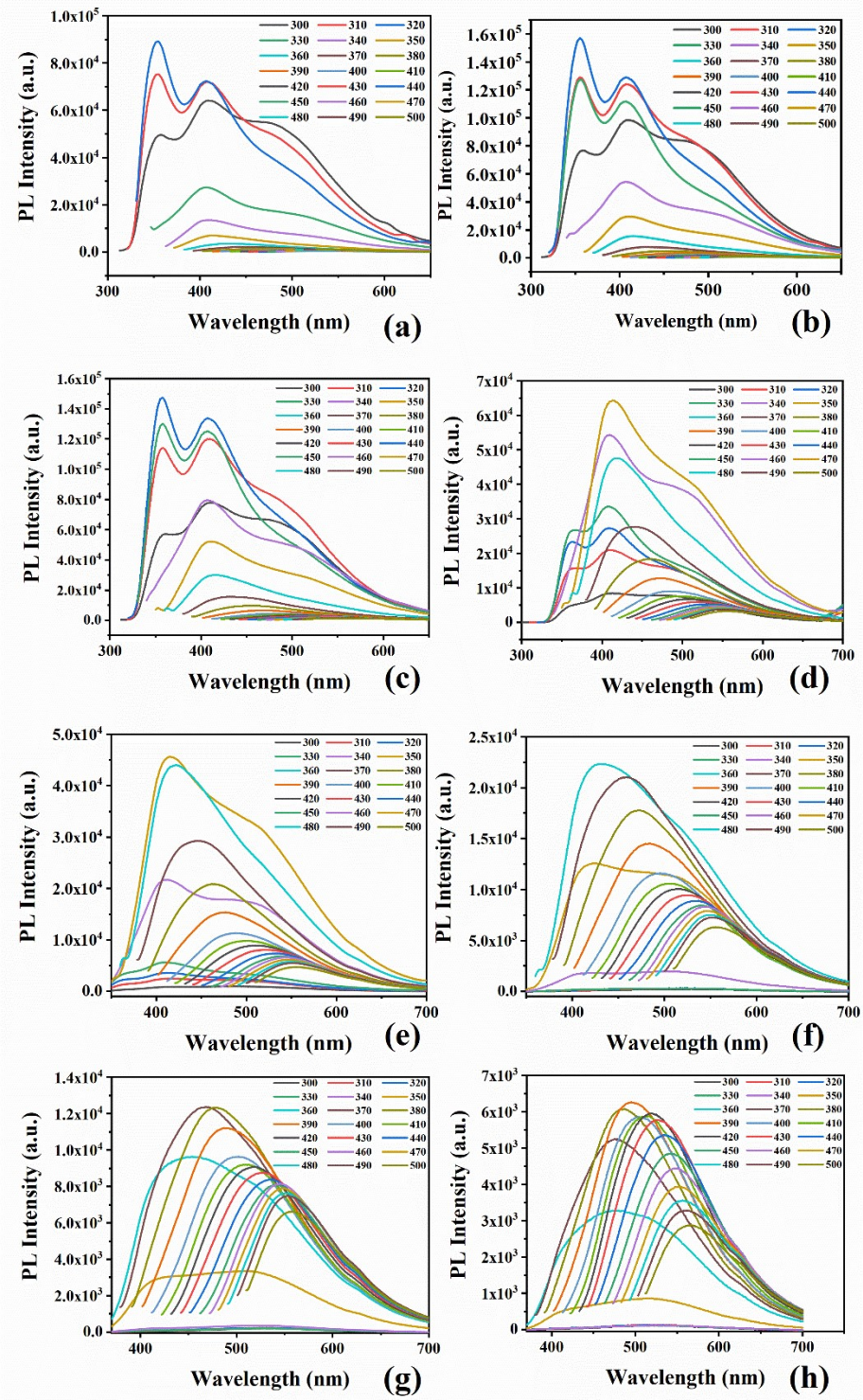


Figure S4. The PL emission spectrum of CNDs solution. **(a)** 0.05 mg/mL, **(b)** 0.10 mg/mL, **(c)** 0.20 mg/mL, **(d)** 0.60 mg/mL, **(e)** 1.00 mg/mL, **(f)** 2.00 mg/mL, **(g)** 3.00 mg / mL, and **(h)** 4.00 mg/mL (Ex wavelength: from 300 nm to 500 nm).

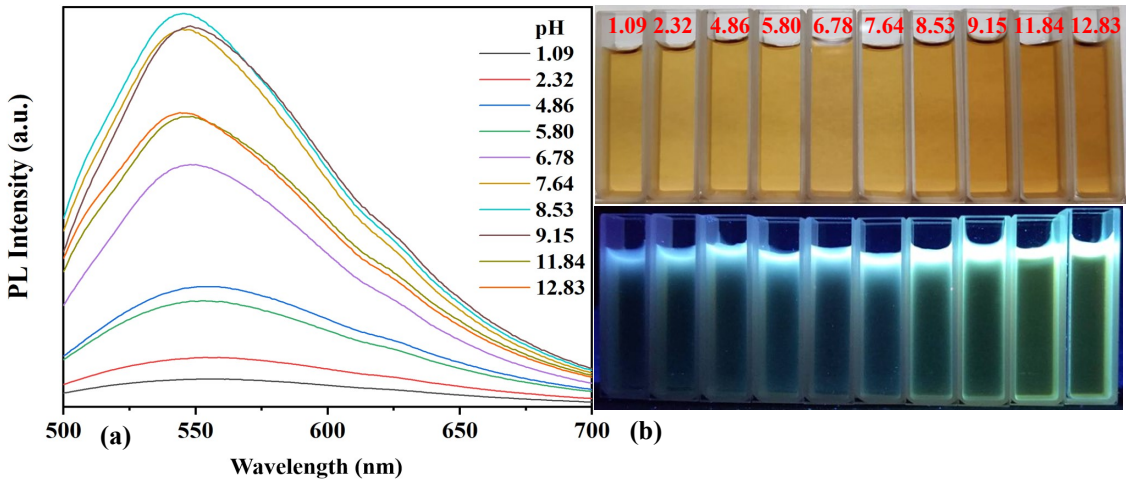


Figure S5. (a) The PL emission spectra of CNDs solution at different pH. (Concentration: 4 mg/mL, Ex=470 nm); **(b)** The photographs of corresponding CNDs solution at different pH.

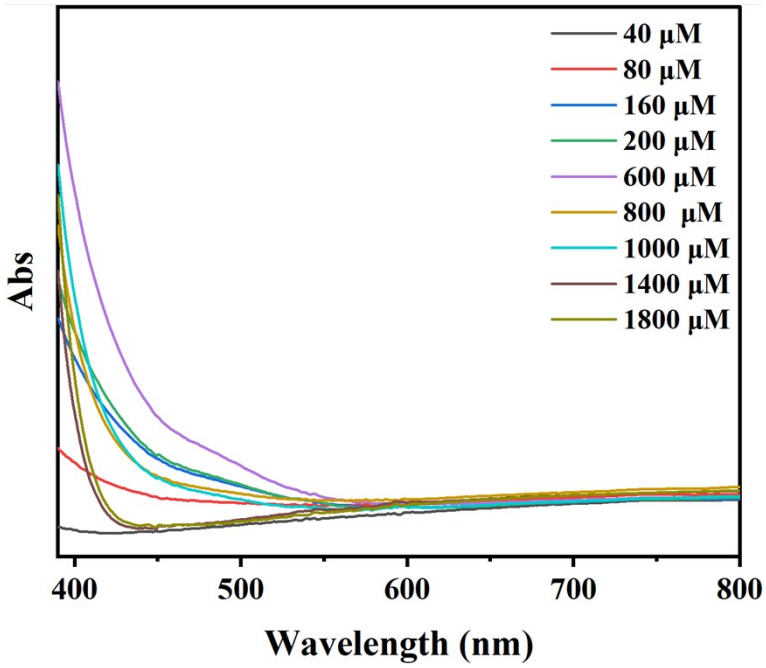


Figure S6. UV.vis of Fe^{3+} solutions (the concentrations of Fe^{3+} solutions is 40 μM, 80 μM, 160 μM, 200 μM, 600 μM, 800 μM, 1000 μM, 1400 μM, 1800 μM).

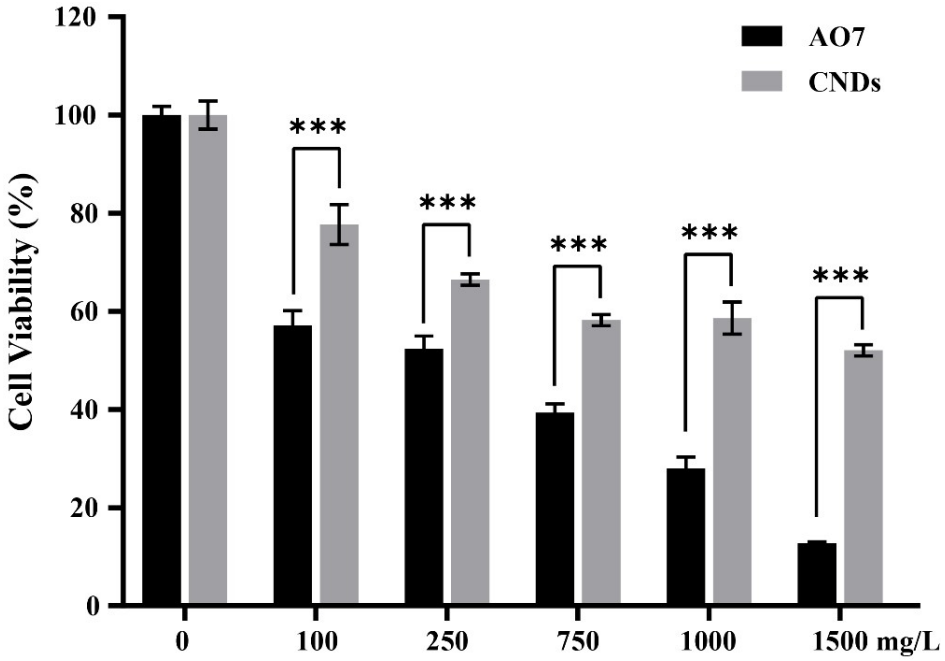


Figure S7. Cytotoxicity assessment. MTT cell viability assay was performed in HeLa cells after AO7 and CNDs exposure for 24 hours. Concentrations are plotted on the X axes, cell viability (%) ± SEM with respect to untreated cells is plotted on the Y axes. Significant differences are indicated by asterisks (***$p < 0.001$).