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Electronic Supplementary Information

Rapid Microwave-Assisted Synthesis of Ultra-bright Fluorescent Carbon Dots for Live Cell Staining, Cell-Specific Targeting and in Vivo Imaging

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1. Experimental Section

1.1. Synthesis and characterization of CDs

Citric acid (4.2g) and ethylenediamine (5.5 mL) was dissolved in 100 mL ultra-pure water (18.2 MΩ). 30 mL of the solution was then transferred to a sealed digestion vessel and placed into the microwave digestion furnace. The microwave digestion system (WX-4000, Shanghai Yi-Yao Instruments) was equipped with controllable temperature units within variation of ± 1 ^{o}C at the set temperature. The system can be operated at 2450 MHz frequency, and work at 0-1000 W power. The reaction temperature and time can be programed by users (the temperature decides the pressure). When set at 600 W power, the reaction temperature was rapidly elevated to 200 ^{o}C. The synthesis process could be accomplished in 5 min. The CD samples were taken out from the reaction vessel when the temperature was naturally cooled to lower than 80 ^{o}C. The product, which was brown-yellow and transparent, was subjected to dialysis (500-Da cutoff) in order to obtain the CDs. The CD samples were diluted for optical characterizations. Ultraviolet-visible (UV-Vis) absorption spectra were obtained using a Shimadzu UV-2450 spectrophotometer. Fluorescence spectra were recorded by a Hitachi F-2500 fluorescence spectrophotometer. All optical measurements were performed at room temperature under ambient conditions. The quantum yields (QYs) of CDs was measured using quinine sulfate in 0.1M H_2SO_4 solution (QY = 54%) as a reference standard as described previously.[1,2] Briefly, the absorbance for the standard and the CD samples at the excitation wavelengths and the fluorescence spectra of the same solutions were measured respectively. Six different concentration of quinine sulfate and CD solutions (absorbance at excitation wavelength < 0.1) were used in the measurements. The integrated fluorescence intensity vs. absorbance was plotted. The plot obtained should be a straight line with a gradient M, which was used to calculate the quantum yield according to the following equation:

$$\phi_x = \phi_s \left(\frac{M_x}{M_s} \right) \left(\frac{\eta_x}{\eta_s} \right)^2$$

Where the subscripts s and x denote standard (quinine sulfate) and test samples, respectively, ϕ is QY, and η is the refractive index of the solvent. The excitation wavelength for measurements of QY was set at 360 nm in our experiments. TEM samples were prepared by dropping the CD solution onto carbon-coated copper grids with excess solvent evaporated. TEM images were recorded on a JEM-2100 electron microscope operating at 200 kV.

1.2. Conjugation of RGD to CDs

Integrin $\alpha_v\beta_3$ targeting peptide cyclo(RGDfC) (Arg-Gly-Asp-D-Phe-Cys) were purchased from GL Biochem Ltd. (Shanghai, China). The peptide was conjugated to CDs using the following procedure: 2 mL of CDs (1 mg/mL) in PBS buffer (pH 7.4) was first mixed with 7 mg EDC, 1 mg NHS and 1.25 mg *N*-(2-hydroxyethyl)maleimide, and then gently vortexed for 2.5 h at room temperature. After that, 6 mg cyclo(RGDfC) was added and the mixture was stirred overnight. The solution was repeatedly dialyzed using dialysis membrane tubing with a molecular weight cutoff of 1000-Da against PBS buffer for 24 h. After dialysis, the purified RGD-CD solution was stored at 4 ^{o}C until use.

1.3. Cell imaging

NIH 3T3 cells, human cervical cancer HeLa cells and human breast cancer MCF-7 cells were cultured with high Dulbecco's Modified Eagle's medium supplemented with 10% fetal bovine serum in a humidified incubator at 37 °C in which the CO_2 level was kept constant at 5%. 1 mL solution with approximately 3000 3T3 cells was seeded onto 10-mm sterile coverslip in 24-well plate. After 24 h, 100 μL CDs (8 mg/mL) were added into the medium (1 mL) for 5 min or 24 h incubation at 37 °C under 5% CO_2. To examine quenching effect of Fe^{3+} on CDs, 3T3 cells were first cultured in the medium containing 100 mM Fe^{3+} for 24 h and then incubated with CDs under the same conditions stated above. The cells on the coverslip were rinsed with PBS buffer (pH 7.4) and then directly observed with fluorescence microscopy (DMI3000B, Leica) under a 20× objective by UV light excitation. For RGD-modified CDs, HeLa and MCF-7 cells were incubated with RGD-modified CDs (~8 μg/mL) for about 15 min at 4 ^{o}C. After that, the cells were washed three times with PBS to

remove the unbound particles. The FL images were taken with a Nikon A1 confocal laser scanning system (Nikon, Japan) under a 100× oil objective by 405 nm laser excitation with emission collected at 425-475 nm, and by 488 nm laser excitation with emission collected at 500-530 nm, respectively. 3D FL images were reconstructed by Nikon's NIS-Elements software.

1.4. *In vivo* imaging

All animals were purchased, maintained, and handled using protocols approved by the Institute of Radiation Medicine, Chinese Academy of Medical Sciences (CAMS). The tumor models were generated by subcutaneous injection of 2×10^6 HeLa cells suspended in 50 μL of PBS into the right shoulder of male nude mice. Before the experiments, the mice were anesthetized by chloral hydrate. CDs of 200 μL at the concentration of 8 mg/mL were respectively intravenous injected into three male nude mice (three mice per group) after the tumor inoculation when the tumor volume reached 100-120 mm^3. The mice were then imaged using the *in vivo* fluorescence imaging system (Caliper Inc.). UV-Visible light with a central wavelength of 405 nm was used as the excitation source. The in vivo imaging wavelength range was 450-800 nm with an exposure time of 86 ms. In *ex vivo* imaging, the FL intensity of the samples was measured at the excitation wavelength of 405 nm. The biodistribution of CDs was obtained by *ex vivo* fluorescence.

Figures S1-4 and Video S1

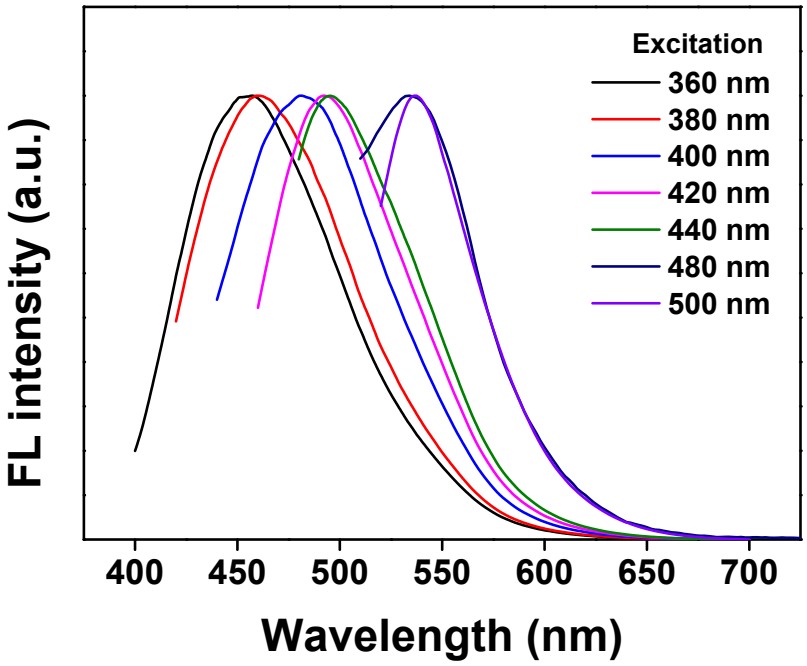

Fig. S1 The normalized excitation-dependent fluorescence emission spectra of the CDs.

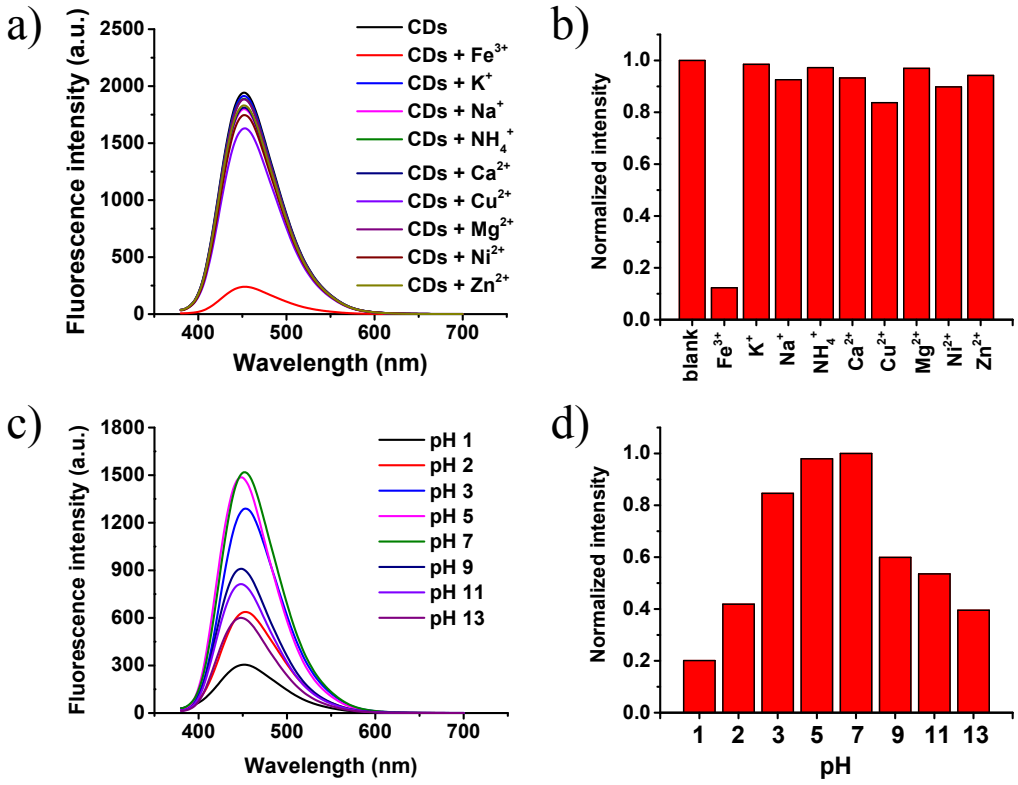

Fig. S2 (a, b) The fluorescence spectra and intensity analysis of CDs after the addition of different metal ions (1 mM). (c, d) The fluorescence spectra and intensity analysis of CDs in the aqueous solutions with different pH values.

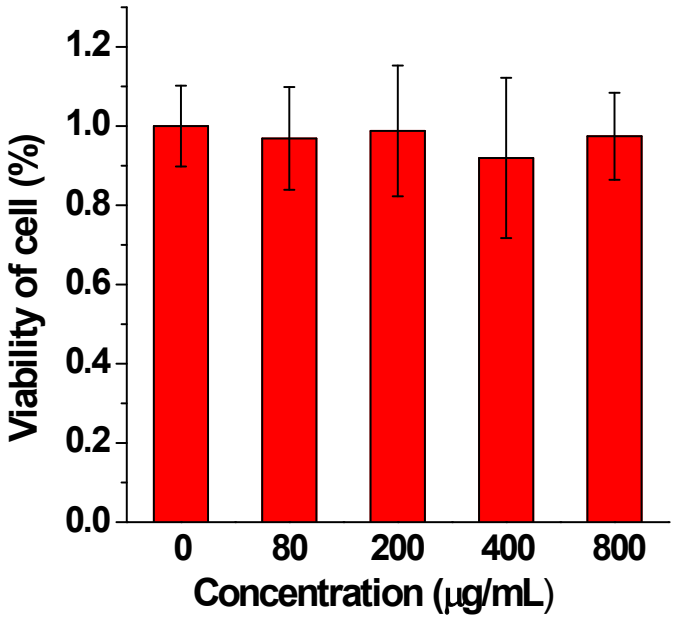

Fig. S3 Cytotoxicity of CDs against NIH 3T3 cells by using MTT assays, illustrating percentage cell viability when compared to non-treated cells being arbitrarily assigned 100% viability upon exposing the cells to different concentrations of CDs for 24 h. The cells were pre-seeded on 96-well plates at a density of 2×10^4 cells/well in 100 μL medium containing 10% FBS. The plates were then incubated for 24 h at 37 ^oC containing 5% CO_2. 100 μL of different concentrations of CDs in PBS solution were added and the cells were incubated for further 24 h. Wells containing cells without CDs served as controls. Subsequently, 20 μL of MTT solution (5 mg/mL) was added to each well and the plates were incubated for further 4 h at 37 ^oC. The precipitated formazan was dissolved in 200 μL of DMSO (Dimethyl sulfoxide). The absorbance at 490 nm was measured using a microplate autoreader (Molecular Devices, M2e). Percent cell survival is expressed as a percent ratio of A490 of cells treated with CDs over control cells.

Fig. S4 Optical photographs of the CD solutions synthesized via microwave irradiation at 120 °C, 140 °C, 160 °C, 180 °C, and 200 °C in 15 min.

Video S1

A series of optical sections along the z-axis merging three channels (bright field, blue and green emission) obtained by Nikon A1 confocal fluorescence microscopy. The scanning range along the z-axis is 0-8.6 μm.

Reference:

1. Zhai, X.; Zhang, P.; Liu, C.; Bai, T.; Li, W.; Dai, L.; Liu, W., Highly luminescent carbon nanodots by microwave-assisted pyrolysis. *Chem. Commun.* **2012**, 48 (64), 7955-7.
2. Melhuish, W. H., Quantum efficiencies of fluorescence of organic substances: effect of solvent and concentration of the fluorescent solute1. *J. Phys. Chem.* **1961**, 65 (2), 229-235.