Supporting Information

Sonochemical synthesis of highly photoluminescent carbon nanodots

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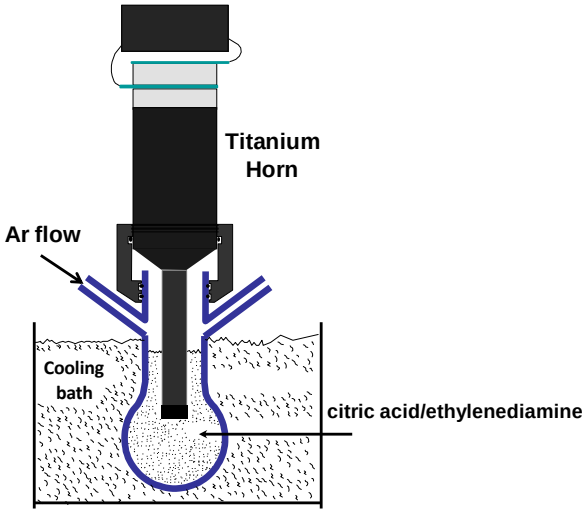
Materials: Citric acid (99%), 1,2-ethylenediamine (98%), and quinine sulfate (99%) were purchased from Sigma-Aldrich. Polyacrylic acid (PAA, M_n=1800) and Fluorescein isothiocyanate (FITC) were purchased from Aladdin Reagents. All materials were used as received without purification. Ultrapure water (18.2 MΩ/cm) from a Milli-Q ultrapure system was used in this study.

Sonochemical synthesis of CDs: In a typical synthesis, 4 g citric acid and 1.8g ethylenediamine were dissolved in 10 ml deionized water, then the mixture was transferred to a sealed vessel and sparged with Ar for 1 h at 0 ^{o}C. Sonication was conducted using an ultrasonic horn (Sonics & Materials, model VCX-750, 1 cm^2 Ti horn at 20 kHz and 40 Wcm^{-2}) for 8 h under continuous Ar flow. The experimental setup is shown below. The obtained products were subjected to centrifuge at 3000 rpm/min for 30 min. Then the supernatant was dialyzed against deionized water through a dialysis membrane (MWCO of 200) for 3 days. In this work, different concentrations of citric acid and ethylenediamine and different sonication times were also used to synthesize CDs.



Schematic illustration of sonochemical setup used in this work.

Characterization: Transmission electron microscopy (TEM) images were obtained from JEOL-2010. Atomic force microscopy (AFM) images were recorded using an Asylum Research MFD-3D. XPS spectra of the samples were measured using a PHI 5000 VersaProbe X-ray Photoelectron Spectrometer. Fourier transform infrared (FTIR) spectra were recorded by a Bruker VECTOR-22 FTIR spectrometer over potassium bromide pellet. NMR spectra of the samples were recorded on a Bruker AVANCE III 400 NMR spectrometer using D_2O as the solvent. Elemental analysis was performed on Elementar Vario EL cube. Powder X-ray diffraction patterns (PXRD) of the product were obtained on a Japan Rigaku DMax-γA rotation anode X-ray diffractometer equipped with graphite monochromatized Cu Kα radiation (λ = 1.54178 Å). UV/Vis absorption spectra were obtained using a Shimadzu 3600 spectrophotometer. Photoluminescence spectra were recorded on a HORIBA FluoroMax-4 fluorometer.

The quantum yields of CDs were calculated by comparing their integrated photoluminescence intensities (the area under the PL curve in the wavelength range from 365nm to 700nm when excited at 350 nm) and absorbance values at 350 nm with those of quinine sulfate according to the following equation :

$$\Phi x = \Phi std(\mathrm{A}std/\mathrm{A}x)(Fx/Fstd)(\boldsymbol{\eta}x/\boldsymbol{\eta}std)^2$$

where std and x denote standard and test, respectively, Φ is the fluorescence quantum yield , A is the absorbance at 350 nm, F is the integrated photoluminescence intensities and $\boldsymbol{\eta}$ is the refractive index of the solvent.

The quantum yields of CDs were calculated by comparing their integrated photoluminescence intensities (excitation at 350 nm) and absorbance values at 350 nm with those of quinine sulfate. Quinine sulfate (literature quantum yield: 0.54) was dissolved in 0.1 M H_2SO_4 and the CDs were dissolved in water. To minimize reabsorption effects, absorbance values of the individual solutions in 10 mm cuvettes were maintained under 0.1 at the excitation wavelength.

MTT assay and confocal microscopy:

HeLa cells were seeded into a 35-mm glass-bottom culture dish (NEST, China) at a density of 5×10^4 cells/well and incubated overnight in 2 ml DMEM (Dulbecco's modified Eagle's medium) containing 10% FBS (Fetal bovine serum) in humidified atmosphere with 5% CO_2 at 37 ^oC.

For testing the cytotoxicity of the CDs, MTT assay was applied. HeLa cells were seeded onto 96-well plates at a density of 1×10^4 cells per well in 200 μL DMEM with 10% FBS at 37 ^oC in a 5% CO_2 humidified atmosphere. After incubation overnight, the original medium was replaced by 2 ml fresh medium containing different concentrations (25~300μg/ml) of CDs. After incubation for 4 h, the medium was removed by fresh medium and MTT solution (20 μl, 5 mg ml^{-1} in PBS buffer) was added to each well, followed by 4 h incubation. The medium in each well was then removed and 200 μl of DMSO was added to dissolve the internalized purple formazan crystals. The plate was gently agitated for 15 min until all the crystals were dissolved. The absorbance at 490 nm was recorded by a microplate reader (Thermo Fisher). Using non-treated cells as control, the relative cell viability was calculated as $Abs_{sample}/ Abs_{control}$.

For confocal microscopy, HeLa cells were seeded in 2-well plate 12 h before use. Then the culture medium was replaced by fresh medium containing CDs and the cells were incubated for 4 h. The cells were then washed with PBS buffer three times. The samples were examined under a Leica confocal laser scanning microscope.

NH_2/COOH Ratio	5/1	1/1	3/4	1/2	1/5
Quantum Yield	9.0%	44.8%	26.6%	33.3%	7.3%

Sonication Time	3 h	7 h	8 h	10 h
Quantum Yield	42.0%	46.0%	44.8%	47.0%

Concentration	0.1M	0.5M	1M	2M
Quantum Yield	57.6%	77.3%	42.0%	38.2%

Table S1 Quantum yields under different synthetic conditions. Upper chart: varying the NH_2/COOH ratios with the same sonication time (8 h) and the same concentration of citric acid (1 M); middle chart: varying the sonication times while using the same NH_2/COOH ratio (1/1) and the same concentration of citric acid (1 M); bottom chart: varying the concentrations of citric acid while keeping the same sonication time (3 h) and the same NH_2/COOH ratio (1/1).

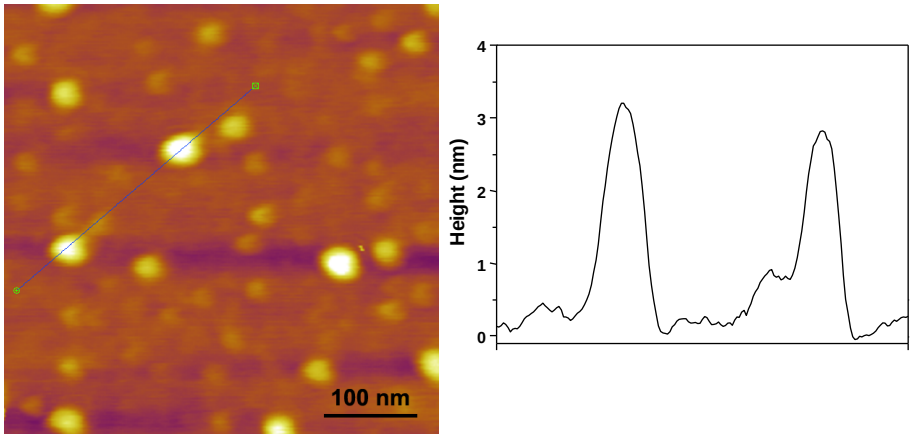


Fig. S1 AFM topography image of CDs on mica substrate and the height profile along the line as shown in the image.

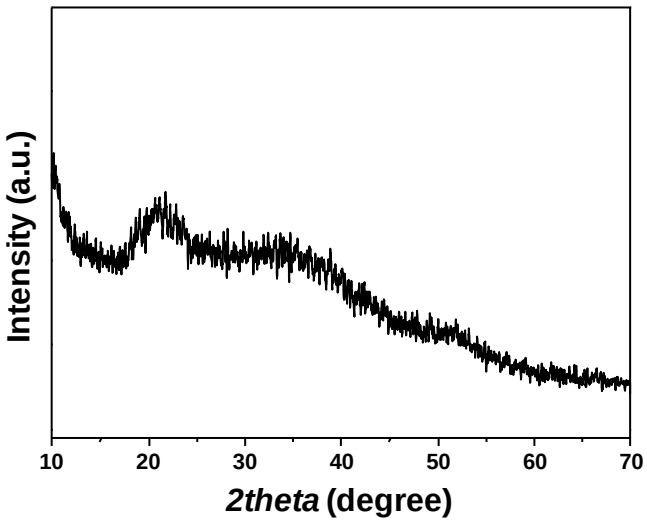


Fig. S2 Powder XRD pattern of sonochemically synthesized CDs.

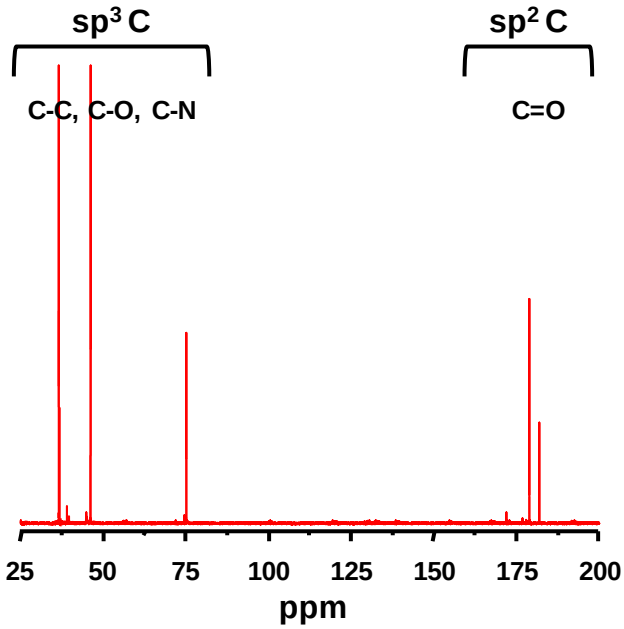


Fig. S3 ^{13}C NMR spectrum of sonochemically synthesized CDs.

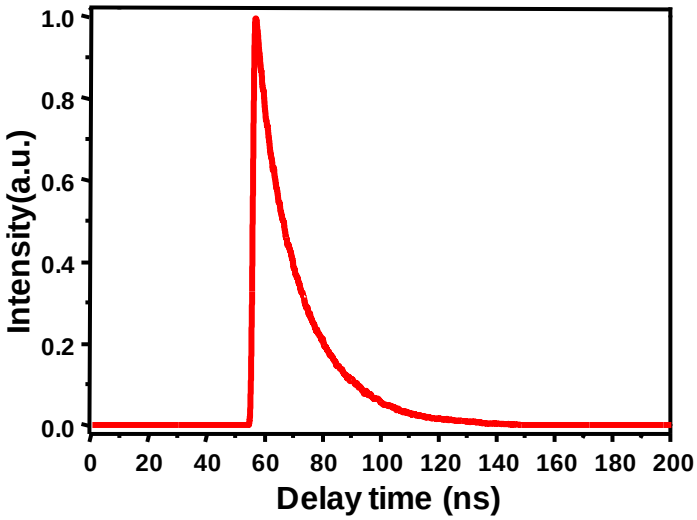


Fig. S4 Time-correlated single-photo counting (TCSPC) of CDs (360 nm excitation and delay time at 450 nm emission), the average lifetime of CDs is *15.52ns* and contains two lifetime components of *3.21 ns* (4%) and *16.07 ns* (96%).

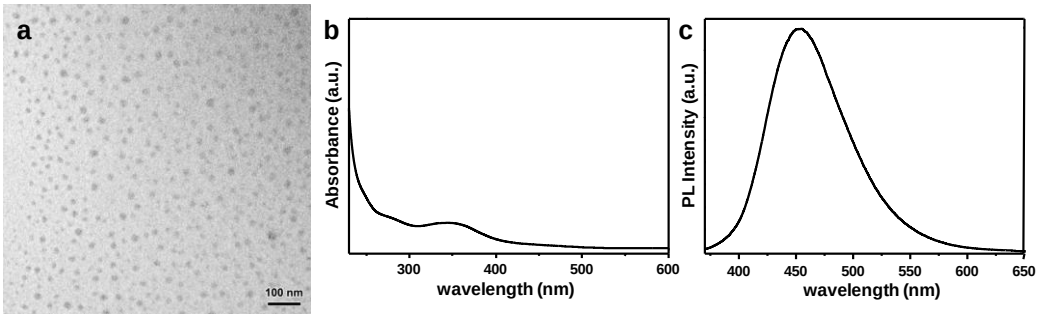


Fig. S5 (a) TEM image, (b) UV/Vis spectrum and (c) emission spectrum (excited at 360 nm) of CDs obtained using 2 M citric acid as carbon precursor.

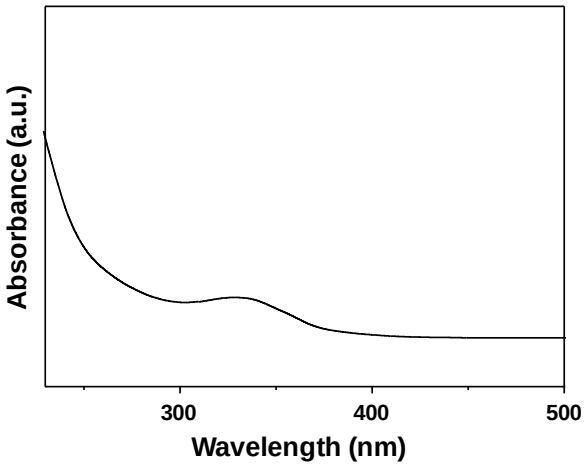


Fig. S6 UV/Vis absorption of CDs synthesized using citric acid without ethylenediamine as

carbon precursor.

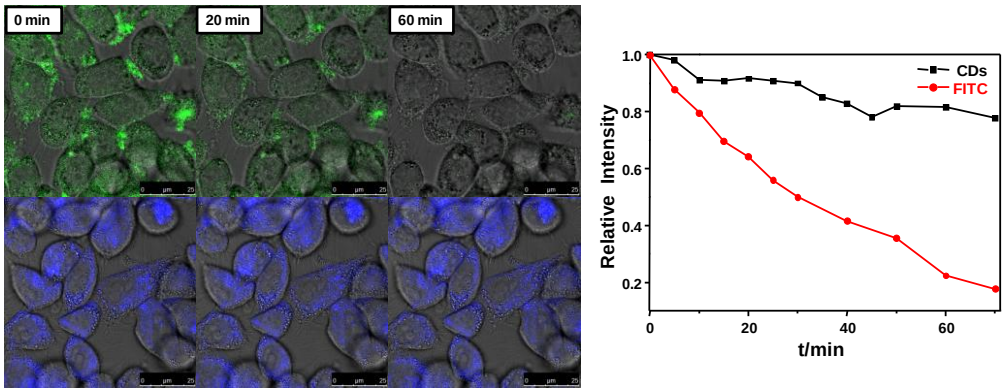


Fig. S7 Photostability comparison of FITC and CDs. Left: Time-dependent stability comparison of fluorescence images of cells labeled by FITC (green) and CDs (blue) under confocal microscopy. Right: Time-dependent photostability comparison of photoluminescence intensity of FITC and CDs under irradiation by a mercury lamp (4.3 mW/cm^2).

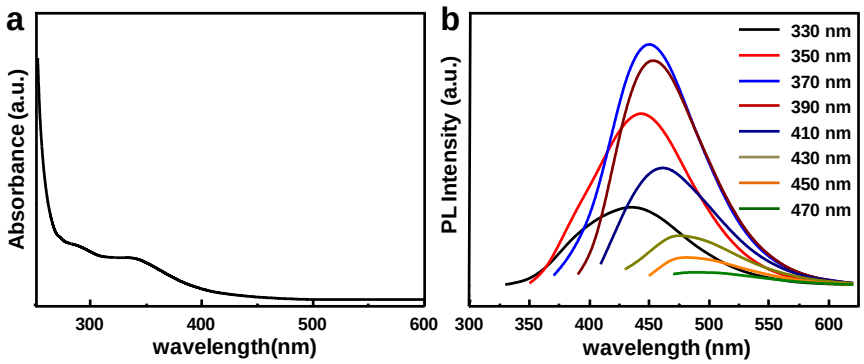


Fig. S8 (a) UV/Vis spectrum and (b) photoluminescence emission spectra of CDs using polyacrylic acid as carbon precursors.