Supplementary information

Nitrogen-doped carbon dots with excitation-independent long-wavelength emission produced by room-temperature reaction

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Experimental Details

1. Materials

The chemicals used in this study were naphthalene (98.0%, Aladdin), sodium (98.0%, Aladdin), ethylene glycol dimethyl ether (99.5%, Aladdin), acetonitrile (99.0%, Aladdin), Tween-20 (TW, 98.0%, J&K Scientific Led), petroleum ether, dichloromethane (≥ 99.5%, Sinopharm), hexamethylene (99.9%, Aladdin), 4',6-diamidino-2-phenylindole(DAPI, Dojindo Laboratories), Cell Counting Kit-8 (CCK-8, Dojindo Laboratories). Unless otherwise stated, all the chemicals and reagents were of analytical grade and used as received without further purification. Deionized (DI) water with a resistivity of 18.2 MΩ·cm was obtained from a Milli-Q Water Purification System and used in all the experiments.

2. Synthesis and purification of the CDs

The CDs were prepared by one-pot method. Typically, naphthalene (1.0 g) and sodium (0.2 g) were added into a flask (100 mL) with ethylene glycol dimethyl ether (20 mL). The mixture was treated using ultrasound for 1 h to form sodium-naphthalene solution. Under vigorous stirring, acetonitrile (0.2 mL) was added. The solution was kept stirring for 1 h and then treated by rotary evaporation. The precipitate was further purified by column chromatography *via* silica gel using petroleum ether first to remove impurities and dichloromethane afterwards to collect CDs, and 0.2g of CDs was obtained.

3. Synthesis of the TW-CDs

The solid CDs (70 mg) were mixed with TW (100 μL) and hexamethylene (20 mL) in a flask (100 mL). The mixture was treated using ultrasound for 20 min. Next, deionized water (30 mL) was added in the flask under vigorous stirring. The solution was then heated to 80 °C for 3 h to evaporate the hexamethylene and obtain the TW-CDs aqueous solution. The products were further purified using an ultrafiltration centrifuge tube by centrifugation for three times.

4. Characterization

The size and composition of the samples were characterized by transmission electron microscopy (TEM, Tecnai G20, FEI Co.), atomic force microscopy (AFM), and X-ray photoelectron spectroscopy (XPS, ESCALAB 250Xi, Thermo Fisher Scientific, using an Al K_α X-ray source). The sample for AFM measurement was prepared by dropping the diluted CDs dispersion (10 μL) on a Si substrate and drying naturally under ambient conditions, and then characterized by MultiMode 8 AFM system (Bruker) with a Si tip. The absorption and infrared spectra were collected by using a ultraviolet-visible (UV-Vis) spectrophotometer (Evolution 300, Thermo Scientific) and a Fourier transform infrared spectrometer (Nicolet 6700, Thermo Scientific). Photoluminescence spectra, steady-state and time-resolved photoluminescence spectra as well as photoluminescence quantum yields of the samples were measured by a fluorescence spectrometer (Fluoromax-4, Horiba Jobin Yvon) equipped with a single photon counting controller FluoroHub and an integrating sphere.

5. *In vitro* cytotoxicity

To examine the cytotoxicity of the TW-CDs, HeLa cells (1×10^4 cells per well) were first seeded on 96-well cell-culture plates and then incubated with the TW-CDs at selected concentrations (0, 10, 20, 50, and 200 μg/mL; dispersed in fresh medium) for 24 h. Afterwards, the cell viability was evaluated using a CCK-8 assay according to the manufacturer suggested procedures. Results were shown as the means of triplicate measurements.

6. *In vivo*/*vitro* bioimaging

HeLa cells were used to study the *in vitro* bioimaging of the TW-CDs. Briefly, HeLa cells were cultured on a 12-well cell-culture plate at a density of 1×10^5 cells per well at 37 °C in a 5% CO_2 atmosphere overnight. Then, the cell culture medium was discarded and the cells were washed three times with phosphate buffer saline (PBS) to remove the dead cells, followed by incubation with the TW-CDs dispersion (50 μg/mL) for 2 or 6 h at 37 °C. Afterwards, the cells were dyed by DAPI, immediately fixed, and then rinsed with PBS and observed under a fluorescence microscope (IX71, Olympus). For *in vitro* imaging, 5-week old mice were subcutaneously injected with the TW-CDs solution (2.5 mg/mL, 100 μL). Under inhalational anesthesia, fluorescence images of the mice were taken by *In-vivo* Imaging System (Carestream FX Pro, Kodak) with the excitation and emission wavelength at 510 and 600 nm, respectively.

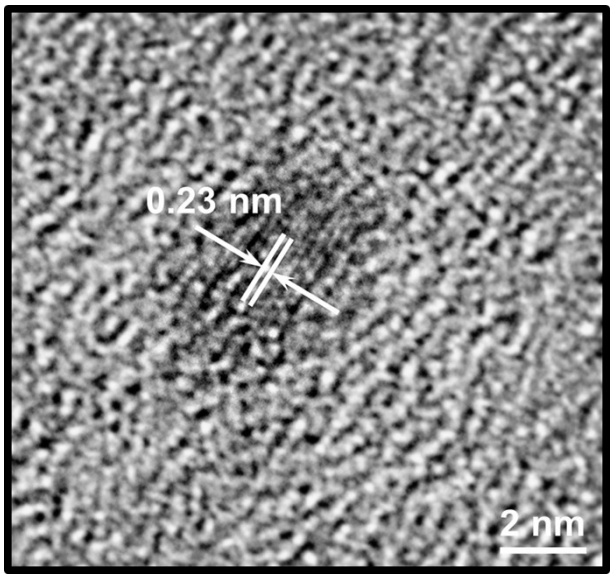

Fig. S1. High-resolution TEM image of a CD.

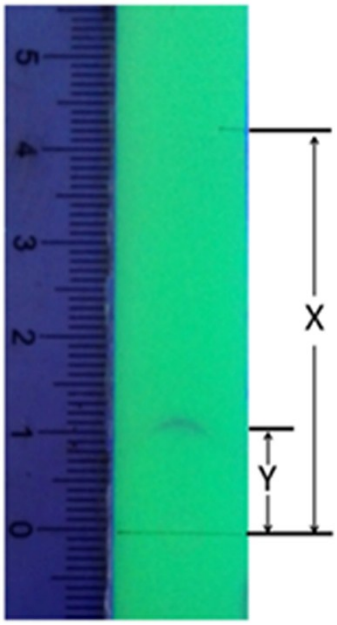

Fig. S2 Thin layer chromatogram of the CDs using petroleum ether and dichloromethane as the developing solvents (9:1). The bottom line is the starting point of spot, the second line is the spot of CDs, and the top line is the solvent front. Rf=Y/X=0.26.

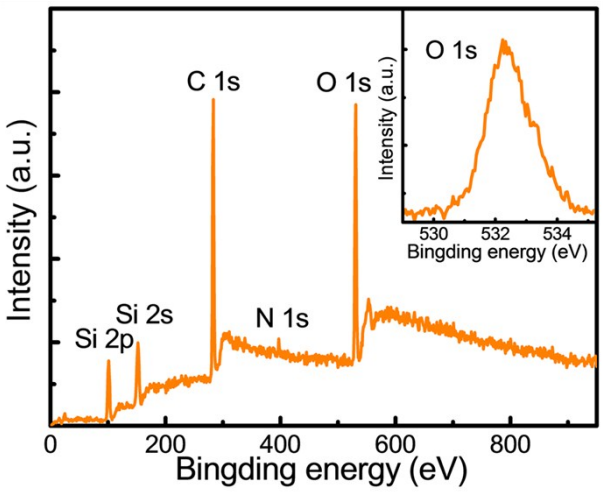

Fig. S3. XPS survey of the CDs. Inset: The high-resolution O 1s XPS spectrum.

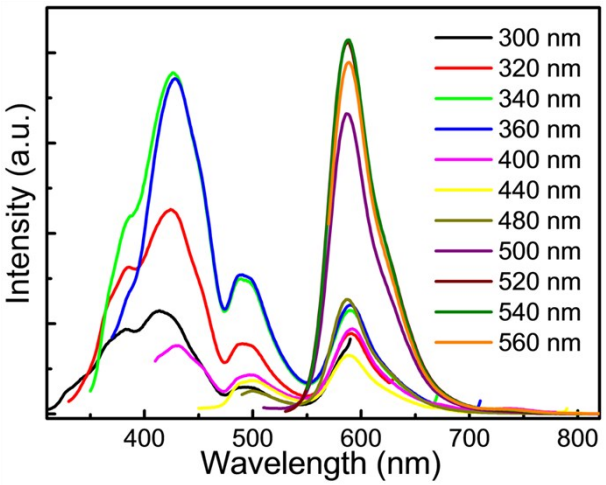

Fig. S4. PL spectra of the CDs under various excitation ranging from 300 to 560 nm.

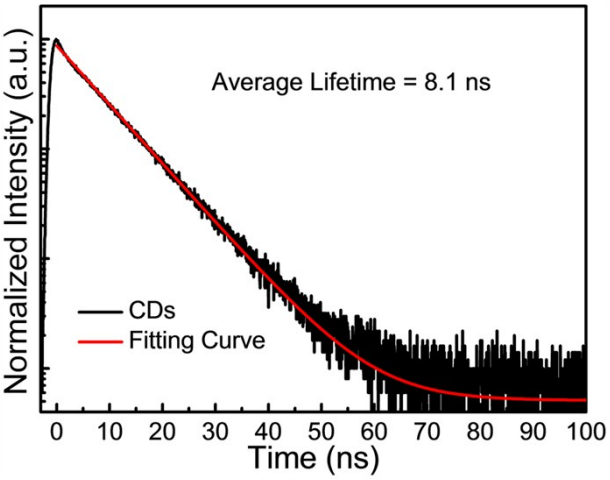

Fig. S5. Time-resolved PL spectrum of the CDs with the excitation and emission wavelength at 455 and 588 nm.

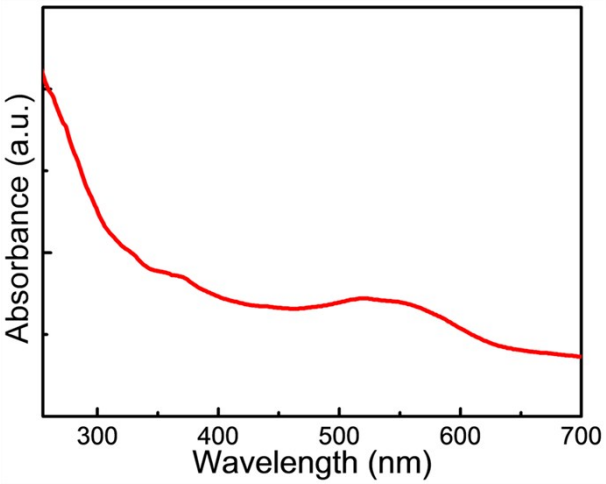

Fig. S6 UV-vis absorption spectrum of the TW-CDs.

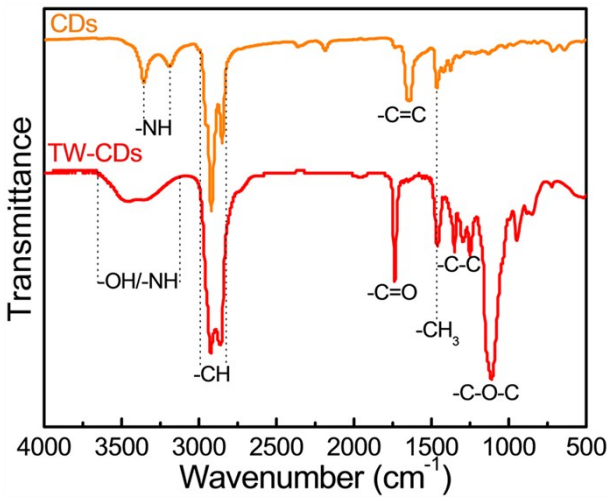

Fig. S7 FT-IR spectra of the CDs and the TW-CDs.

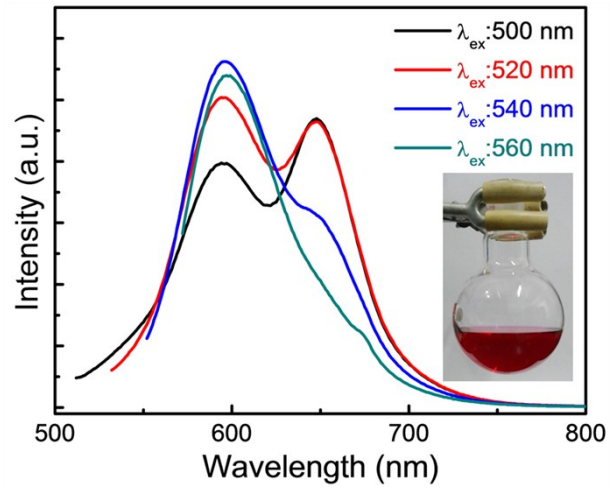

Fig. S8. PL spectra of the TW-CDs under 500-560 nm excitation. Inset: Photograph of the TW-CDs solution.

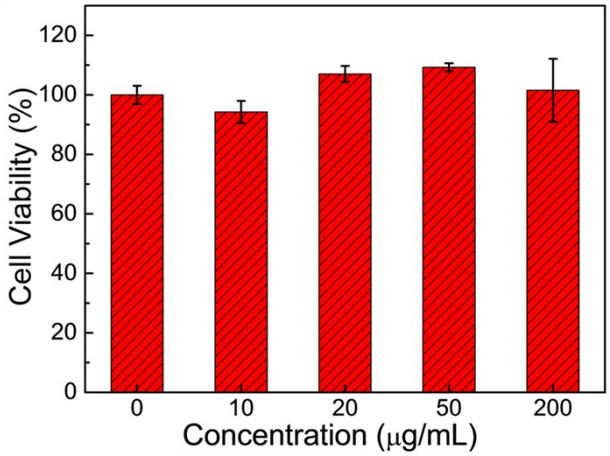

Fig. S9. Hela cells viability after 24-h incubation with the TW-CDs at various concentrations.