Supporting Information

Multifunctional Water-Soluble Luminescent Carbon Dots for Imaging and Hg^{2+} Sensing

*Yanling Zhai, Zhijun Zhu, Chengzhou Zhu, Jiangtao Ren, Erkang Wang and Shaojun Dong**

Experimental sections

Chemicals

Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were obtained from Gibco (USA). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Dingguo Ltd. (Beijing, China). Citric acid and ethylenediamine were purchased from Alfa Aesar. Other reagents were from Beijing Chemical Reagents Company (Beijing, China). All aqueous solutions were prepared with ultrapure water (>18 MΩ cm) from a Milli-Q Plus system (Millipore).

Apparatus A XL30 ESEM scanning electron microscope (SEM) was used to determine the morphology. X-ray diffraction (XRD) spectra were obtained using a D8 ADVANCE (Germany) using Cu K (1.5406 Å) radiation. X-ray photoelectron spectroscopy (XPS) analysis was carried on an ESCALAB MK II X-ray photoelectron spectrometer. Absorption measurements were performed on a Cary 500 UV vis-NIR spectrometer (Varian). Luminescence measurements were carried out on a Fluoromax-4 spectrouorometer (Horiba Jobin Yvon Inc., France) with excitation and emission slit widths of 5 nm. *In vivo* imaging measurements were conducted on In-Vivo Imaging System FX (Kodak). The nanofibers electrospinning precursor was filled into a plastic syringe with 7-gauge needle which was

connected to a High Voltage DC Power Supply (Dongwen, Tianjin), and the flow rate was 1.0 mL/h, which was maintained via a syringe pump (KDS 100, KD–Scientific).

Preparation of CDs

The photoluminescent C-dots was synthesized by a facile green route of microwave assisted pyrolysis method. Briefly, 3.2 g citric acid were dissolved with 80 mL water in a 100 mL beaker, and then 4.2 mL of ethylenediamine were added to the mixture under vigorous stirring to form a homogeneous solution. Then the beaker containing clear transparent solution was placed at the center of the rotation plate of a domestic microwave oven (700 W) and heated for different time intervals. When cooled down to room temperature, 30 mL of water was added, and the color-changed solution was then dialyzed against pure water for 4 days. Finally, a clear, light yellow brown aqueous solution containing CDs was obtained. In this experiment, nine CDs were prepared by varying microwave irradiation time, 4 min, 5 min, 6 min, 7 min, 8 min, 10 min, 12 min and 15 min, respectively.

Printing of photoluminescent CDs ink.

The CDs aqueous solution was first neutralized to pH ≈ 7 by NaOH and then directly injected into a near-vacant cartridge. The cartridge was used for printing until the ink exhausted, and then washed several times to exhaust the residual black ink. It should be noted that filter paper was used as printing paper to avoid the influence of fluorescent additives in office printing papers under the UV lamp. The desired words and images were printed onto filter paper marked letters “CDs” by a common printer.

Preparation of CDs nanofibers (CNFs). 0.6 g polyacrylonitrile (PAN) was first dispersed in 20 mL of DMF stirring for 3 h, then 0.1 g CDs solution was added, and the mixture was magnetically stirred at room temperature overnight. The resulting clear homogenous solution was used for electrospinning precursor. Finally, a plastic syringe with 7-gauge needle, which was connected to a High Voltage DC Power Supply (Dongwen, Tianjin), was filled with the

PAN/CDs aqueous solution. The aluminum foil was used as a collector. The applied voltage was fixed at 15 kV. The distance between the nozzle tip and the collector was fixed at 15 cm, and the flow rate was 1.0 mL/h, which was maintained via a syringe pump (KDS 100, KD–Scientific).

MTT assay and Cell imaging

The vitro cytotoxicity of CDs was assessed through MTT assay. HeLa (human cervical cancer) cells were grown in 5% CO_2 at 37 ˚C in DMEM supplemented with 10% FBS. Cells were firstly seeded at a density of 8000 cells per well on 96-well plates for 24 h, and then incubated for another 24 h with CDs at desired concentrations at 37 ˚C. The medium was removed, and replaced by fresh DMEM/FBS after washed with PBS (100 μL, 3 times). For the MTT assay, 10 μL of MTT (5 mg/mL in PBS) were added and the cells were incubated for an additional 4 h in dark. Finally all medium was removed and 100 μL of dimethylsulfoxide (DMSO) was added, followed by shaking for 15 min. The absorbance was monitored using a microplate reader at 550 nm. For the *in vitro* imaging, HeLa cells were seeded on the glass coverslip in 6-well plate. After incubated with 100 μg/mL of CDs for 2 h, the cells were washed by PBS for 3 times, and the luminescence imaging of the cell pellet was performed under a Leica laser scanning confocal microscope (Mannheim, Germany) equipped with an Ar laser (405/488 nm).

***In vivo* imaging**

For *in vivo* imaging, the Kunming rats were first anesthetized by intraperitoneal injection of chloral hydrate solution (10 wt%), and then 100 μL of CDs dispersion in PBS was subcutaneously injected into the bake of rat. Afterwards, the fluorescence was observed on in-vivo imaging system FX. To determine the fluorescence of the urine of mice, 100 μL of CDs dispersion in PBS was injected into the rat through tail vein. The urine samples were taken at different intervals for fluorescence determination.

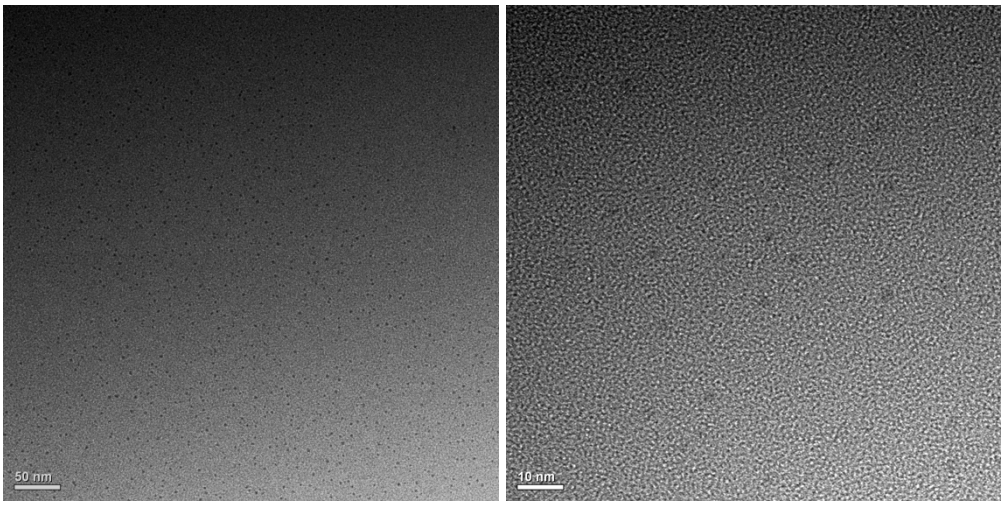

Figure S1. TEM images of the synthesized CDs.

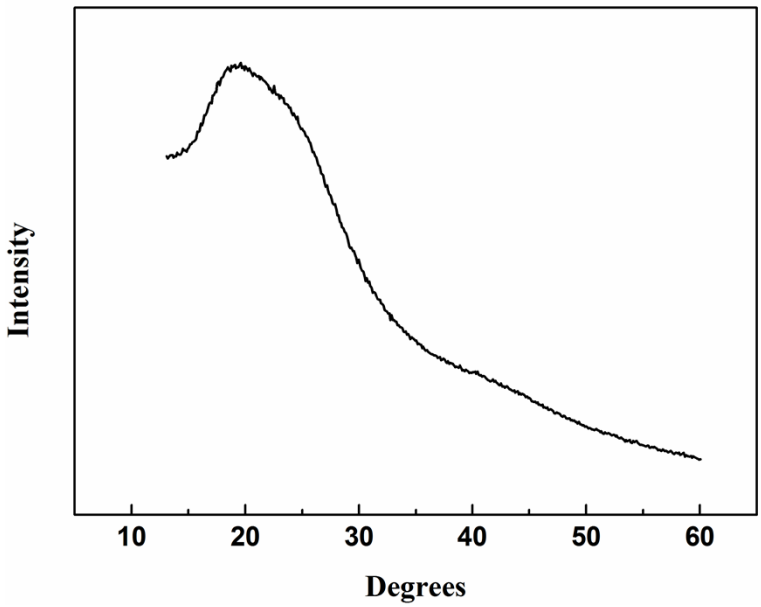

Figure S2. XRD patterns for the CDs.

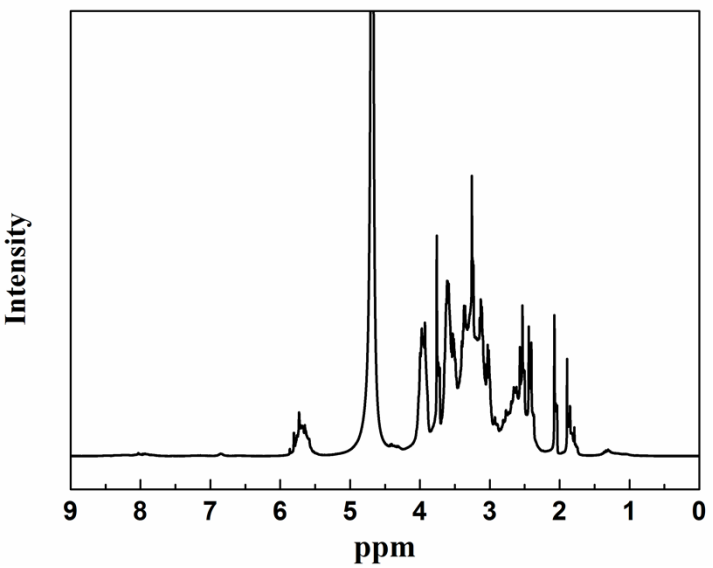

Figure S3. ^{1}H NMR spectrum of the CDs in D_2O.

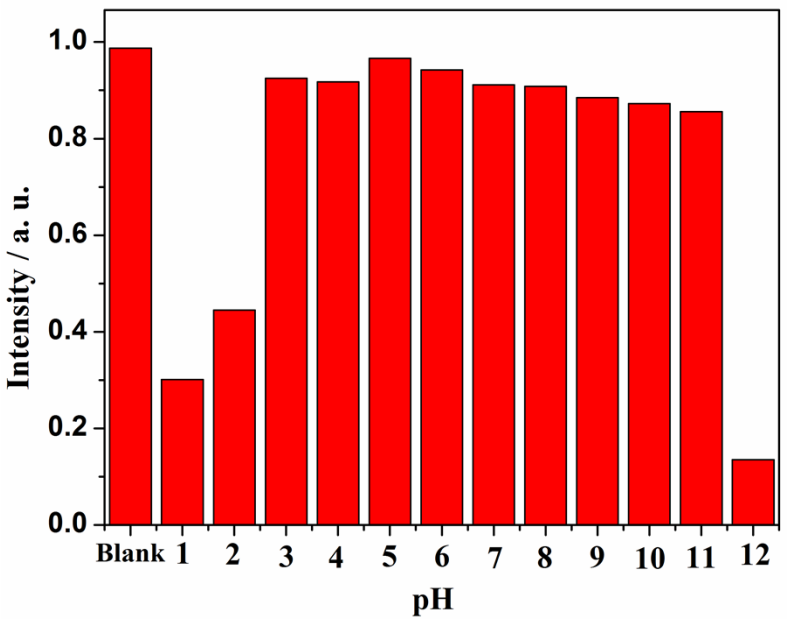

Figure S4. Effect of pH on fluorescence intensity of the CDs dispersion in aqueous solution.

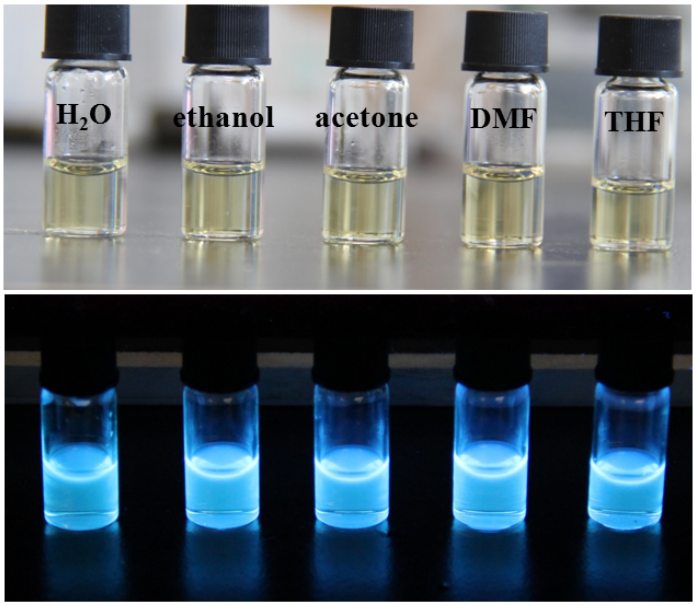

Figure S5. Photographs of the CDs in different solvents after exposure to room light for one month under visible (top) and UV (bottom) light excitation.

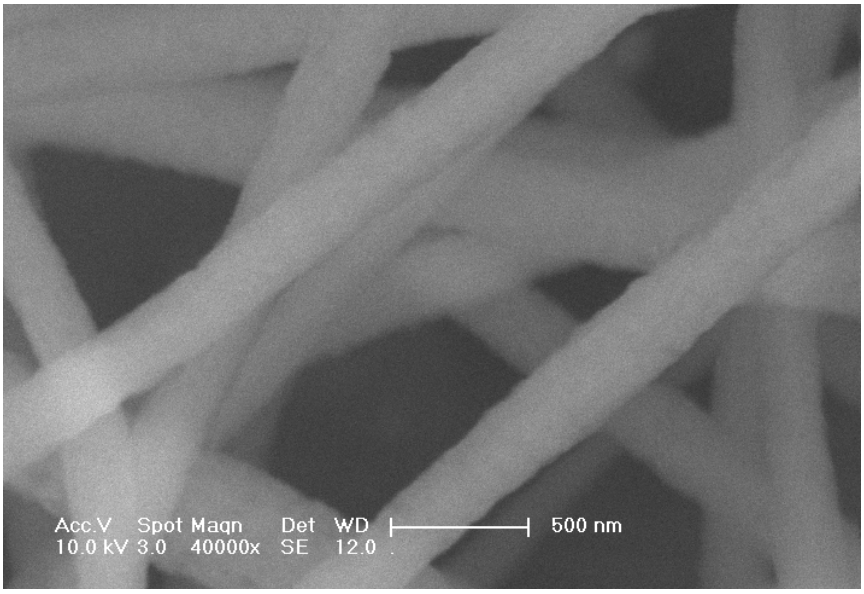

Figure S6. SEM images of the prepared CNFs

Figure S7. Digital photograph of the green diagram printed with the CDs ink under 365 nm UV irradiation after exposure to room light for 2 months.

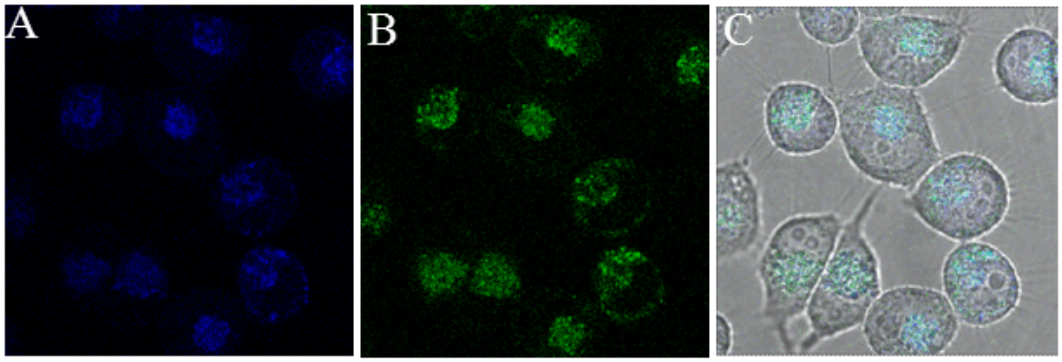

Figure S8. Laser scanning confocal microscopy images of the CDs to HeLa cells. The samples **S8**were observed excited at (A) 405 nm, (B) 488 nm and (C) overlay of A and B.

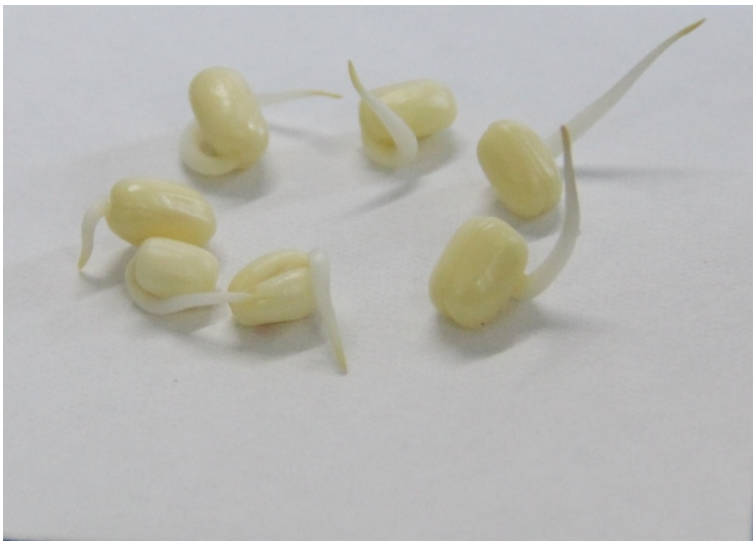

Figure S9. Digital photographs of a bean sprout grown with water under daylight.

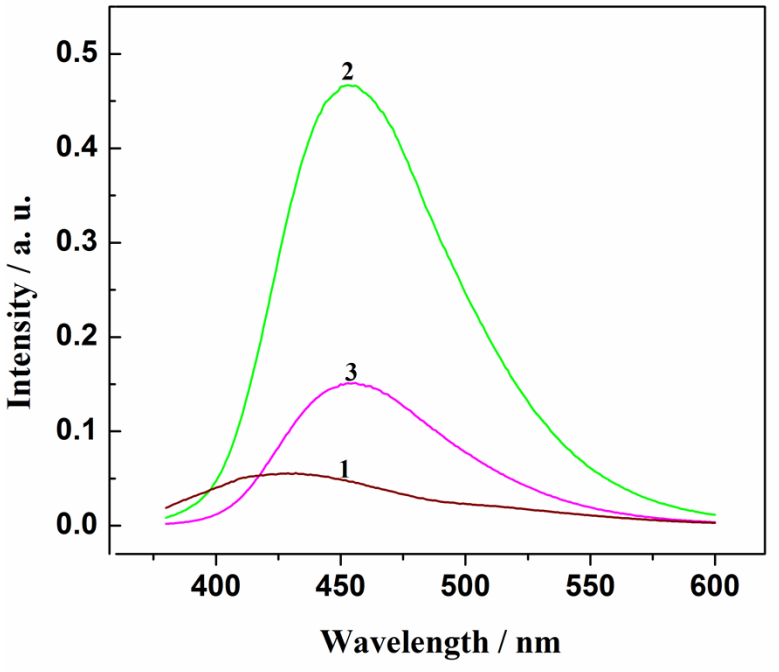

Figure S10. PL spectra of the urine of mice under 365 nm excitation: (1) without injection CDs aqueous after injection of the CDs aqueous solution for (2) 2 h and (3) 4 h.

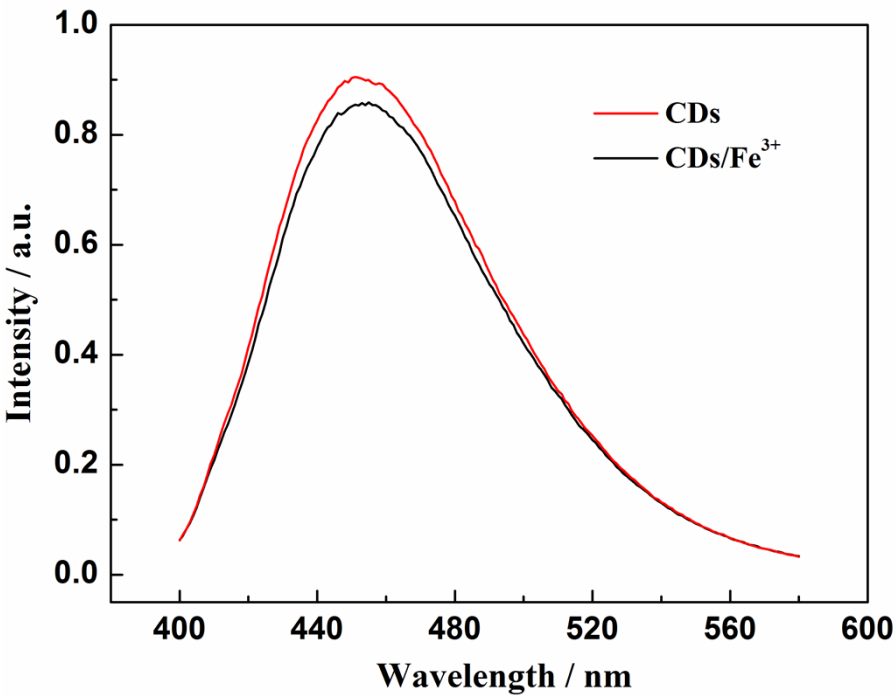

Figure S11. Fluorescence intensities of the CDs before and after addition of Fe^{3+} ions (3 μM).

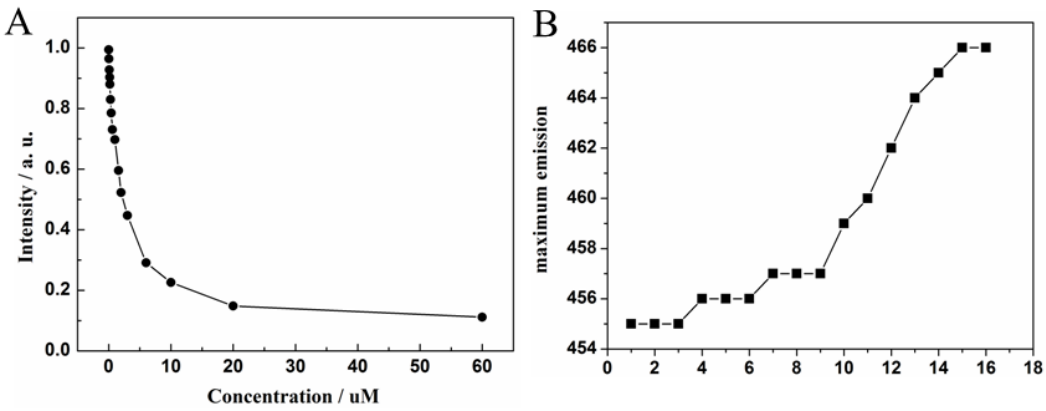

Figure S12. (A) Fluorescence intensity of the CDs in different concentrations of Hg^{2+} ions. (B) The dependence of maximum emission peaks of the CDs on the Hg^{2+} ions concentration in (A).

Table S1. The dependence of reaction time on QY of the CDs.

Reaction time (min)	4	5	6	7	8	10	12	15
QY (%)	35.67	41.39	26.86	25.38	19.73	16.5	14.2	12.7

Table S2. Recovery data for the determination of Hg^{2+} in lake water by nanoprobe with the CDs.

Hg^{2+} added (nM)	Detected (nM)	Found by ICP (nM)	Recovery(%, n=6)
40	39.6±2.1	40.6	102.5
60	62.5±3.8	61.4	98.2
100	101.2±5.4	97.3	96.1
160	158.9±7.3	157.6	99.2