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Supplementary Information

One step synthesis amino-functionalized fluorescent carbon nanoparticles by hydrothermal carbonization chitosan

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Experimental Section

1. Preparation of the fluorescent carbon nanoparticles (CNPs)

In a typical procedure, amino-functionalized fluorescent carbon nanoparticles were synthesized as follows: 2 g chitosan was dissolved in 18 mL 2% acetic acid solutions and then the mixture was sealed into a Teflon equipped stainless steel autoclave, which was then placed in a muffle furnace followed by hydrothermal treatment at 180℃ for 12 h. A programmed temperature profile was adopted to control the heating process. The heating rate was set at 5 ℃•min^{-1}. After the reaction, the autoclave was cooled down naturally. The obtained dark brown solution was centrifuged at a high speed (24 000 *g*) for 15 min to remove the less-fluorescent deposit. The upper brown solution have an average size of 5 nm and exhibits strong blue luminescence under excitation at 365 nm. The deposit nanoparticles have a larger diameters in the range of 30-40 nm and exhibits very weak fluorescence after ultrasonic disperse in water. Pure upper brown luminescent carbon nanoparticles obtained by freeze dried was named as CNPs and had a yield of 7.8%.

2. Characterization methods

High-resolution transmission electron microscopy (HRTEM) observations were performed on a JEOL-2010 electron microscope operating at 200 kV. X-ray diffraction (XRD) patterns were obtained from a MSAL-XD2 X-ray diffractometer with Cu Ka radiation (40 kV, 20 mA, λ = 1.54051 Å). The SEM measurements were taken with a Philips XL-30 scanning electron microscope. The Raman spectrum of as-prepared samples was recorded at ambient temperature on RenishawRM 2000 with an argon-ion laser at an excitation wavelength of 785 nm. Elemental analysis was acquired with a PE CHNS/O EA2400 II. The Fourier transform infrared spectroscopy (FTIR) spectra were measured by an EQUINOX 55 (Bruker) spectrometer with the KBr pellet technique ranging from 500 to 4000 cm^{-1}. Atomic-force microscopy (AFM) image was obtained with a Nanoscope V multimode atomic force microscope (Veeco, USA). X-ray photo-electron spectroscopy (AXIS ULTRA DLD, Kratos) was used to investigate the functional groups present of the surface of the CNPs. Surface charge analysis-zeta potential measurements and size distribution were realized on a Malvern Nano ZS instrument. A Cary 5000 UV-visible-near-infrared (NIR) spectrometer (Varian) was used to follow the absorbance of the CNPs. The fluorescence spectra of the CNPs were measured with a fluorescence spectrometer F-4500 (Hitachi, Japan), with a slit width of 10 nm and 10 nm for excitation and emission, respectively. The excitation wavelength increased by a 20 nm increment starting from 320 nm to 520nm. All measurements used solution were conducted by using DI water as the dispersant with a concertration of 0.1 mg/mL except infinite dilute solution for AFM analysis. The CNPs powder was used to investigate the XPS and XRD measurement.

3. In vitro cytotoxicity against A549 cells

In 96-well plates, 100 μL supspension of A549 cells (5×10^4 cells/mL) in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin (DMEM) were added to per well and incubated in a 5% CO_2 humidified incubator at 37℃ for 24 h. The CNPs were introduced into the wells in a concentration of 25, 50, 100, 200 μg/mL and incubated for another 24h, 48h, 72h, respectively. The medium was removed and cells were washed with phosphate-buffered saline. Then, 20 μL of 5 mg/mL MTT solution was added to each cell well. The 96-well plates were further incubated for 4 h, followed by removing the culture medium with MTT, and then 200 μL of DMSO was added. The resulting mixture was shaken for 10 min at room temperature. The optical density of the mixtures at 490 nm was measured. Cell viability was expressed as percentage of absorbance relative to control, the control was obtained in the absence of CNPs. Experiments were performed in triplicates, with nine replicate wells for each sample and control per assay.

4. Fluorescence Imaging Experiments

A549 cells were seeded in each well of a Confocal Dish (Coverglass-Bottom Dish) and cultured at 37 °C for 24 h. An aqueous solution of the CNPs (0.1 mg/mL) was passed through a 0.2 μm sterile filter membrane. The filtered fluorescent suspension (40-60 μL) was mixed with the culture medium (200 μL) and then added to three wells of the confocal dish (the fourth used as a control) in which the A549 cells were grown. After an incubation of 6 h, the medium was removed and the cells were washed thoroughly three times with PBS (500 μL each time) and kept in PBS for the optical imaging. Cellular uptake of CNPs by A549 cells tracked *via* confocal microscopy and the emission was measured over the range of 450-550 nm, λ_{ex} = 405 nm.

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Table S1 Elemental analysis of the carbon-based materials

Sample	Element Contents			
	%C	%N	%H	%O (Calculated)
Chitosan powder	43.58	8.56	7.35	40.51
CNPs	59.02	7.73	6.51	26.74

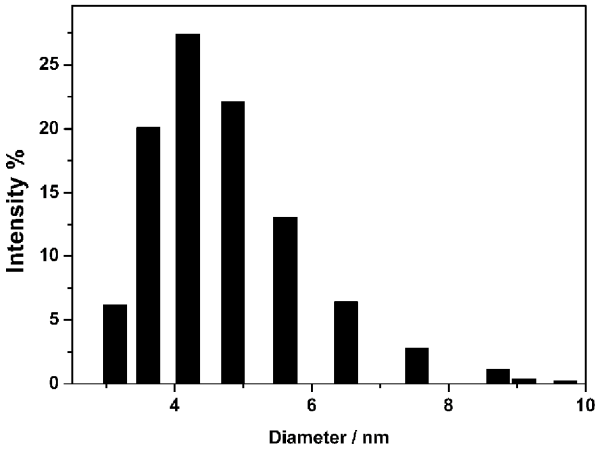

Figure S1. Size dispersion of CNPs obtained by a Malvern NanoZS zetasizer.

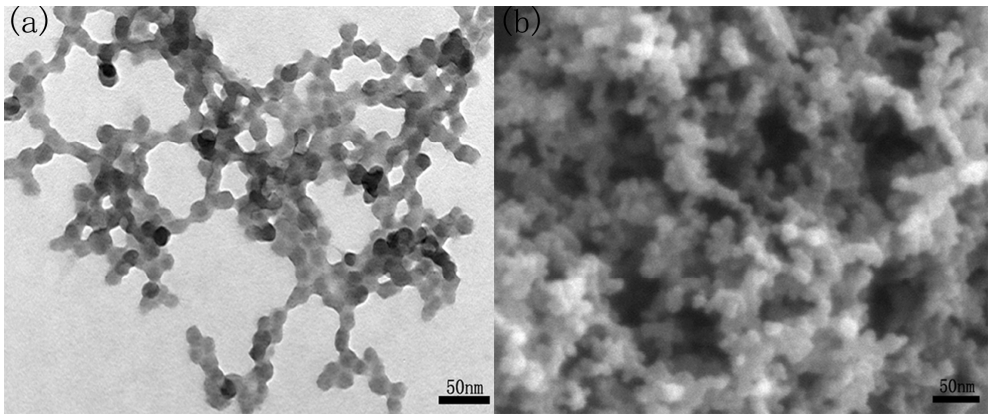

Figure S2. TEM (a) and SEM (b) of the less-fluorescent deposit carbon nanoparticles.

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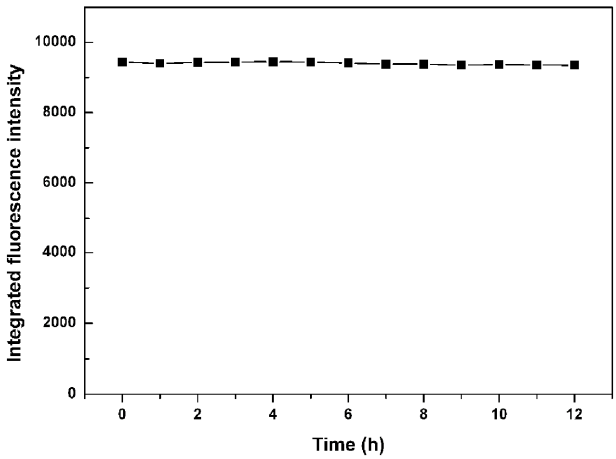

Figure S3. Photostability test of the fluorescent CNPs in a fluorescence spectrophotometer with a 150 W Xe lamp under 360 nm excitation.

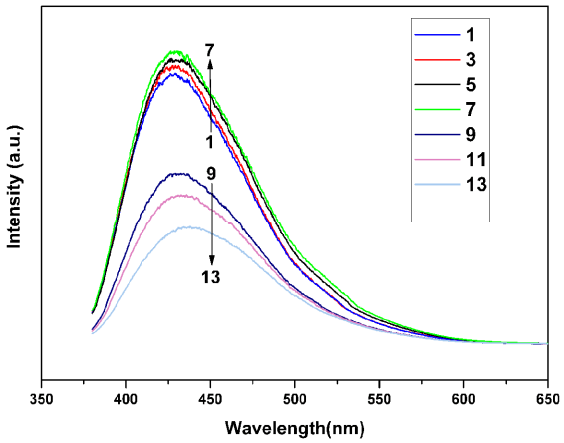

Figure S4. Effect of pH value on the photoluminescence intensity of CNPs at various pHs (λ_{ex} = 340 nm).

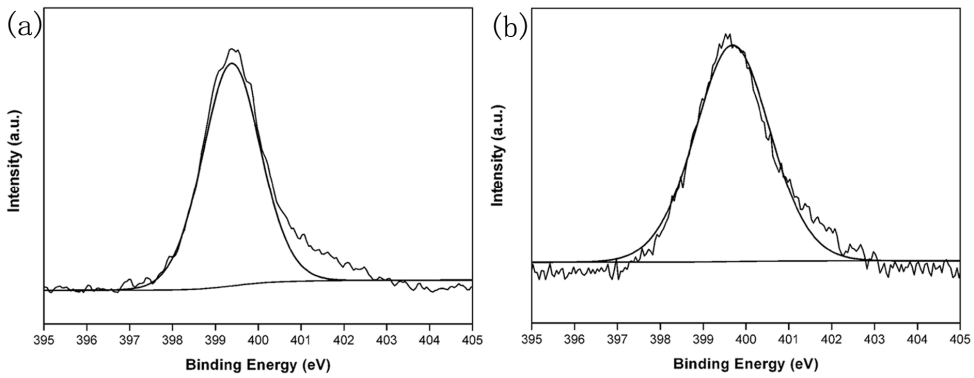

Figure S5. N1s signals of the chitosan (a) and as-prepared CNPs (b).

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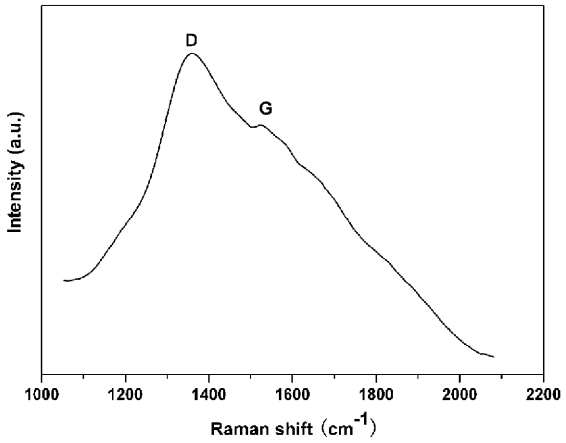

Figure S6. Raman spectra of CNPs measured with 785 nm laser.

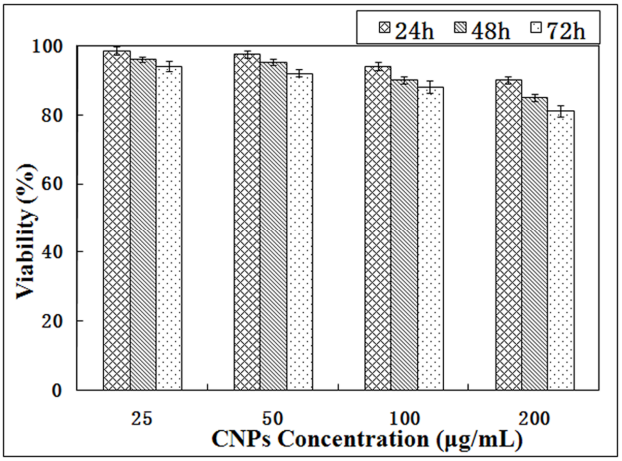

Figure S7. Cytotoxicity evaluations test of A549 cells with different concentrations of CNPs after 24 h, 48 h and 72 h incubation.

5. Quantum Yield Measurements

The quantum yield (Q) of CNPs was calculated with the following equation. Quinine sulfate in 0.1 M H_2SO_4 (literature quantum yield 0.54 at 360 nm) was chose as a standard. Since Q is the quantum yield, I is the measured integrated emission intensity, n is the refractive index, and A is the optical density. The subscript R refers to the reference fluorophore of known quantum yield.

$$Q = Q_R \frac{I}{I_R} \frac{A_R}{A} \frac{n^2}{n_R^2}$$

Table S2 Quantum yield of the fluorescent carbon nanopaiticles

Sample	Intergrated emission intensity (I)	Abs. At 360nm (A)	Refractive index of solvent (n)	Quantum yield at 360nm (Q)
Quinine sulfate	41601	0.07	1.33	0.54 (known)
CNPs sample	101684	0.23	1.46	0.43