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# A facile, green, and solvent-free route to nitrogen-sulfur-codoped fluorescent carbon nanoparticles for cellular imaging

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## Experimental

### Materials

Gentamycin sulfate, 3-(4,5-dimethylthia-zol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and gentamycin were purchased from Sigma Aldrich (USA). Dulbecco's modified Eagle's medium (DMEM) were obtained from Gibco BRL (USA). All the other chemicals were of analytical grade and used as received. All the aqueous solutions were prepared with twice-distilled water in the experiments.

### Instruments

UV-vis absorption spectra were recorded on a Lambda 950 UV-vis spectrophotometer (Perkin-Elmer). Fluorescence spectroscopy experiments were conducted on a LS-45 fluorescence spectrophotometer (Perkin-Elmer). X-ray photoelectron spectroscopy (XPS) was carried out by using a thermoelectron

instrument (Thermo Scientific ESCALAB 250) with Al Kα X-ray radiation as the excitation source (1486.8 eV, 500 μm). Transmission electron microscopy (TEM) analysis was performed on a JEM-2010F transmission electron microscope using an acceleration voltage of 200 KV. X-ray diffraction (XRD) patterns were obtained on a Philips PW3040/60 automatic powder diffractometer using Cu Kα radiation. Fourier transform infrared spectroscopy (FT-IR) spectra were recorded in the form of KBr pellets with a Nicolet 670 FT-IR spectrometer. Zeta potentials were measured on a Malvern Zetasizer Nano ZS dynamic light scattering system. The fluorescence images were acquired using a Leica laser confocal fluorescence microscope (TCS SP5).

**Cell imaging**

Human cervical carcinoma cells (HeLa cells) were cultured in DMEM containing 10% fetal bovine serum and 100 $\mu g \cdot mL^{-1}$ penicillin/streptomycin in a humidified incubator at 37 °C and 5% $CO_2$. The NSCPs suspension was injected into the well of a chamber slide, with the final concentration of 20 $\mu g \cdot mL^{-1}$. After incubation of 24 h, the cells were thoroughly washed with phosphate buffered saline. The fluorescence images were acquired with 488 nm excitation and long pass filter of 530 nm.

**Toxicity assay**

HeLa cells were harvested (the cell density was adjusted to $10^5$ cells$\cdot mL^{-1}$) and seeded in a 96-well plate (90 $\mu L \cdot well^{-1}$) overnight, followed by the addition of the

NSCPs suspension with different concentration (5, 10, 25, 50, 75, 100, 125, and 150 μg·mL$^{-1}$). Five replicate samples were prepared for each concentration. The cells were cultivated for 24 h and then 20 μL of the 1 mg·mL$^{-1}$ MTT solution was put into each cell well. After the cells were incubated for another 4 h, the culture medium was discarded, followed by adding 150 μL of DMSO. The resulting mixture was shaken for 15 min in dark at room temperature. The optical density (OD) of the mixture was measured at 570 nm with a thermo multiskan spectrum microplate spectrophotometer. The cell viability was estimated according to the following equation

$$\text{Cell viability (\%)} = OD_{\text{treated}} / OD_{\text{control}} \times 100\%$$

Where $OD_{\text{control}}$ was obtained without the NSCPs, but $OD_{\text{treated}}$ was obtained in the presence of the NSCPs.

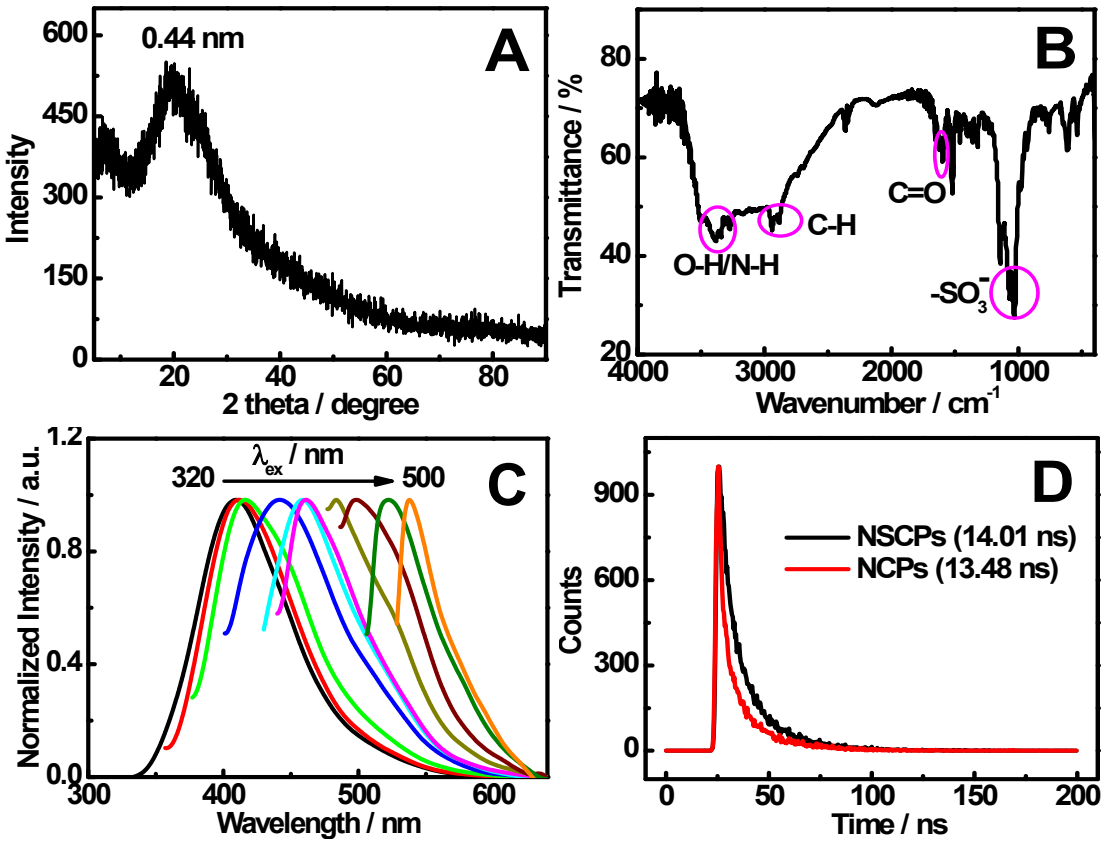


**Fig. S1.** (A) XRD pattern of the NSCPs. (B) FT-IR spectrum of the NSCPs. (C) Normalized fluorescence spectra of the NSCPs. (D) Fluorescence decay trace of the NSCPs and NCPs.

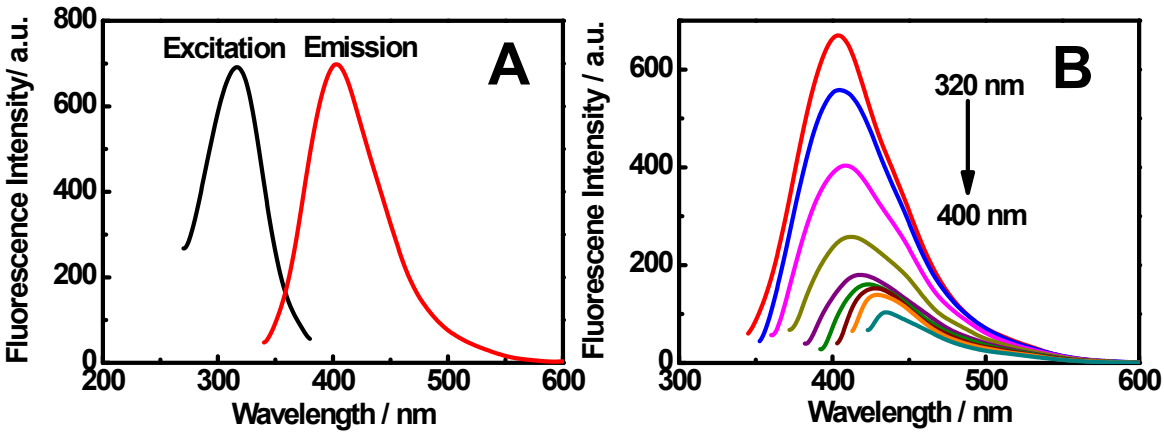


**Fig. S2.** (A) Fluorescence excitation and emission spectra of the NCPs from gentamycin. (B) Emission spectra obtained at different excitation wavelengths with 10 nm increments from 320 to 400 nm.

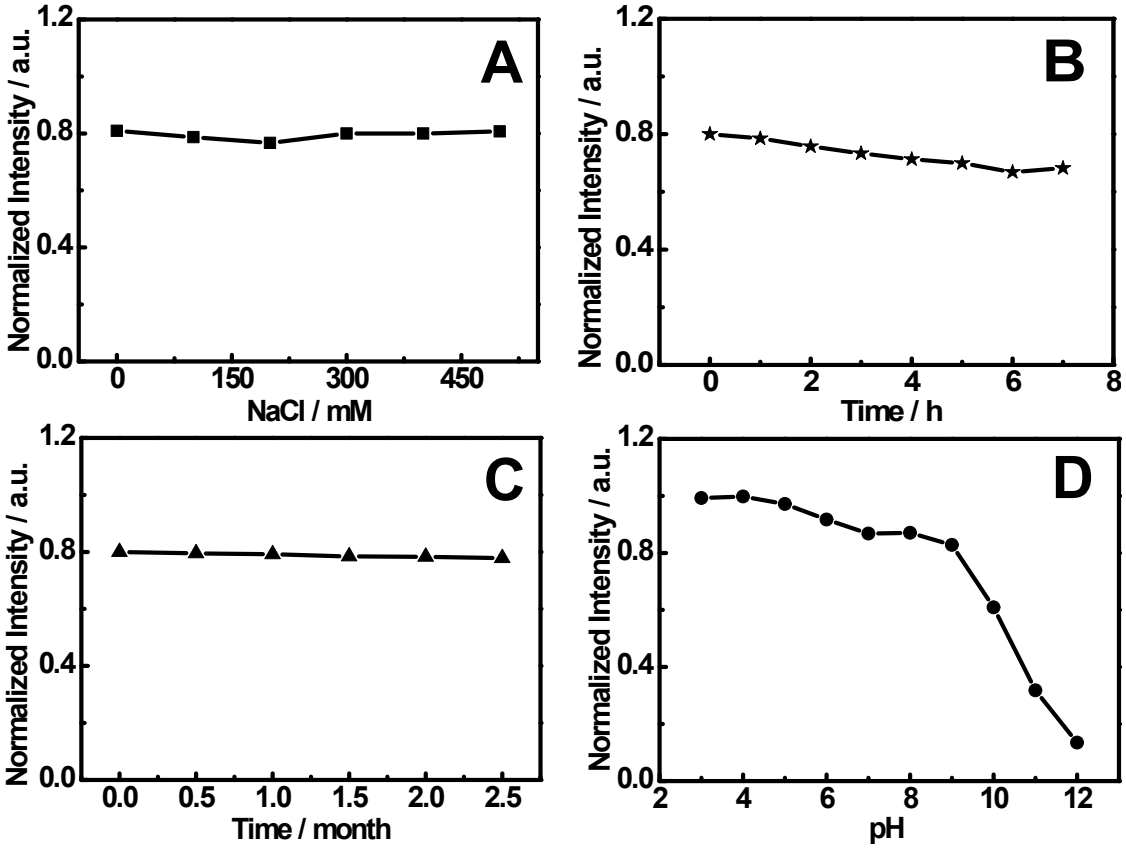


**Fig. S3.** Effects of the ionic strength (A), irradiation time with a 500 W Xe lamp (B), storage time (C), and pH values (D) on the fluorescence intensity of the NSCPs.

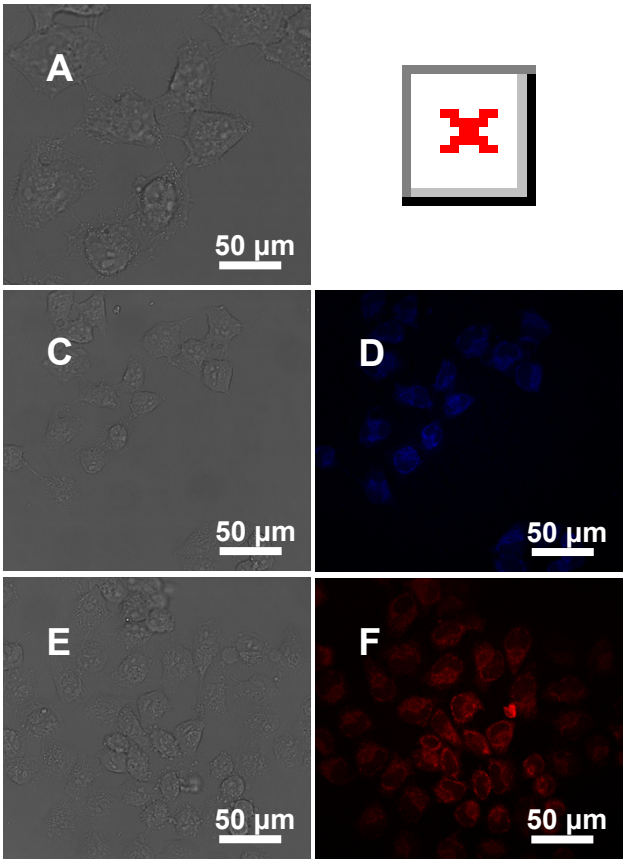


**Fig. S4.** Images of HeLa cells incubated without (A, B) and with (C-F) the NSCPs obtained under bright field (A, C, E) and at the excitation wavelength of 488 nm (B), 405 nm (D), and 543 nm (F).