Supplementary Information

Fluorescent carbon dots with highly negative charge as sensitive probe for real-time monitoring of bacterial viability

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Author Contributions

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1. Experimental Section

1.1 Synthesis of non-doped carbon dots

2 g citric acid was put into a 5 mL beaker and heated to 200 °C for 30 min. The obtained orange liquid was added drop by drop into 100 mL of 10 mg/mL NaOH solution under vigorous stirring. After neutralized to pH 7.0 with NaOH, the aqueous solution of non-doped carbon dots was obtained.

1.2 Synthesis of unary atom-doped carbon dots

The nitrogen-doped carbon dots (NCDs) were synthesized by hydrothermal treatment of CDs (obtained by the developed electrochemical etching method).

1.3 Synthesis of binary atom-doped carbon dots

The nitrogen and sulfur co-doped carbon dots (NSCDs) were synthesized according to a simple hydrothermal method. Briefly, 2 g of citric acid monohydrate and 2 g L-cysteine were mixed within 80 mL deionized water under continuous stirring. The mixture was heated hydrothermally in an autoclave at 80 °C for 72 hours after bubbling N₂ for 30 min. The resulting yellowish-brown solution was cooled to room temperature and filtered with 0.22 mm membranes. To further purify the as-prepared NSCDs, the filtrate was subjected to dialysis against pure water through a dialysis bag (500–1000 MWCO).

1.4 Surface passivation of NPSCDs

Concentrated nitric acid (14.4 mol/L) was added into the solution of NPSCDs (1:10). The mixture was heated at 60 °C for 30 min. The obtained solution was then dialyzed for 1 day (MWCO 1000) to remove unreacted nitric acid.
2. Additional Figures

**Figure S1.** PL spectra of NPSCDs with excitation wavelengths from 280 nm to 500 nm with increment of 20 nm.

**Figure S2.** Fluorescence decay curve of NPSCDs. (300 nm excitation, monitored with 425 nm narrow bandpass filter)

**Figure S3.** The high-resolution XPS spectra of (a) C 1s and (b) O 1s.
Figure S4. Absorbance-based viability assay to HeLa cells incubated with different concentrations of propidium iodide for 2 h.

Figure S5. HeLa cell imaging with different (a) incubation time (200 μg/mL) and (b) concentration of NPSCDs (3 h).
Figure S6. PL spectra of NPSCDs during (a) heating and (b) cooling processes from 30 °C to 90 °C with increment of 10 °C.

Figure S7. CLSM images of *E. coli* (60 °C for 15 min) incubated with (a) CDs, (b) NCDs and (c) NSCDs. (d) $\zeta$ potential of the four types of CDs (Blue) and calculated *E. coli* alive rate (red).

References


S2. J. Liu, S. Y. Zhao, C. X. Li, M. M. Yang, Y. M. Yang, Y. Liu, Y. Lifshitz, S. T.
