## **Electronic Supplementary Information**

## Near-infrared emissive carbon dots for two-photon fluorescence bioimaging

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## **Supporting Figures**



**Fig. S1** Fluorescence decay (red lines) and fitting (blue lines) curves of NIR-CDs160 recorded at emission wavelengths of 685 nm with excitation at 420 nm in deionized water.



**Fig. S2** Two-photon emission of NIR-CDs160 (aqueous solution) under different excitation wavelengths of femtosecond pulse laser.



Fig. S3 FT-IR spectra of NIR-CDs160 in acidic, neutral and alkaline conditions.



**Fig. S4** Fluorescence emission spectra of NIR-CDs140 (a), and NIR-CDs180 (b) under different excitation wavelengths.



Fig. S5 Comparison of the emission intensities of NIR-CDs140, NIR-CDs160 and NIR-CDs180 at the same mass concentration ( $\lambda_{ex}$ =420 nm).



Fig. S6 TEM image of NIR-CDs180 (insert: HRTEM).



**Fig. S7** Photostability of NIR-CDs140, NIR-CDs160 and NIR-CDs180 (the same mass concentration) under contineous irridiation of a xenon lamp (150 W) for 60 min .



Fig. S8 Stabilities of the NIR-CDs160 in different media after standing for 2 days. a-b) 10  $\mu$ g/mL, and c-d) 50  $\mu$ g/mL (in a and c: 1, water; 2, PBS (10 mM pH 7.4); 3, cell culture medium).



Fig. S9 Cell viabilities of MCF-7 after incubation with NIR-CDs160 for 24 h by MTT assay.



**Fig. S10** One-photon fluorescence images of NIR-CDs160 against MCF-7 living cells. The images (a) and (b) are collected in the ranges of 550–750 nm (collection for NIR-CDs160) and 493–540 nm (collection for fluorescein), respectively, under excitation of 488 nm; the image (c) is bright field, and the image (d) is the overlay of (a), (b) and (c).



Fig. S11 FT-IR spectra of the NIR-CDs160 (black line), FITC (blue line) and NIR-CDs160-FITC (red line).



**Fig. S12** a) Plot of PL intensity of FITC with different concentrations (i.e. calibration line); b) PL emission spectrum of the NIR-CDs160-FITC (5  $\mu$ g/mL). The amount of FITC linked to the NIR-CDs160 can be easily calculated based on this calibration line (i.e. 8.9 mg per gram of the NIR-CDs160).



**Fig. S13** One-photo fluorescence images of MCF-7 cells after being incubated with FITC (a-c:  $0.8 \ \mu\text{g/mL}$ ; d-f:  $5.0 \ \mu\text{g/mL}$ ). The images of (a) and (d) are collected in the ranges of 493–540 nm under excitation of 488 nm; images (b) and (e) are bright fields; and (c) and (f) are the overlays of (a) and (b), and (d) and (e), respectively.

## **Supporting Tables**

| sample           | solvent          | $\lambda_{ex}/nm$ | $\Phi_1$ | $\Phi_2$ | $\Phi_3$ | $\Phi_4$ | $\Phi_5$ | $\Phi_{\mathrm{ave}}$ | $\Phi_{\rm corr.}$ |
|------------------|------------------|-------------------|----------|----------|----------|----------|----------|-----------------------|--------------------|
| Rhodamine        | EtOH             | 488               | 88.0%    | 87.4%    | 87.8%    | 86.9%    | 87.1%    | 87.4%                 | 95%                |
| oG<br>NIR-CDs140 | H <sub>2</sub> O | 420               | 22.5%    | 21.3%    | 21.2%    | 21.3%    | 22.0%    | 21.7%                 | 23.6%              |
| NIR-CDs160       | H <sub>2</sub> O | 420               | 14.6%    | 15.1%    | 16.1%    | 15.7%    | 15.9%    | 15.5%                 | 16.8%              |
| NIR-CDs180       | H <sub>2</sub> O | 420               | 11.5%    | 11.9%    | 11.4%    | 12.5%    | 12.1%    | 11.9%                 | 12.9%              |

 Table S1. QYs of Rhodamine 6G and NIR-CDs under excitation at 420 nm.

Table S2. FL lifetimes of CNP160 under excitation at 420 nm.

| $\lambda_{ex}/nm$ | $\lambda_{em}/nm$ | B[%]  | $\tau_1/ns$ | B[%]  | $\tau_2/ns$ | τ(avg)/ns | $\chi^2$ |
|-------------------|-------------------|-------|-------------|-------|-------------|-----------|----------|
| 588               | 685               | 23.24 | 3.07        | 76.76 | 5.27        | 4.52      | 1.10     |