Supporting Information

A Ratiometric fluorescent nanoprobe based on naphthalimide derivative-functionalized carbon dots for imaging lysosomal formaldehyde in HeLa cells

Shuai Chen,[a] Ye Jia,[b] Guang-Yue Zou,[b] Yong-Liang Yu,[b,*] and Jian-Hua Wang[b]

[a] *College of Life and Health Sciences, Northeastern University, Shenyang 110169, China.*

[b] *Research Center for Analytical Sciences, Department of Chemistry, College of Sciences, Northeastern University, Shenyang 110819, China*

Corresponding Author

*E-mail: yuyl@mail.neu.edu.cn (Y.-L. Yu)

Tel: +86 24 83688944; Fax: +86 24 83676698

Table of contents

1. Chemicals

EDC.HCl (1-(3-(dimethylamino)propyl)-3-ethlcarbodiimidhydrochloride), DMAP (dimethylaminopyridine), citric acid, cellular uptake inhibitors including chlorpromazine, genistein, methyl betacyclodextrin, and amiloride were purchased from Aladdin Chemistry Co., Ltd. (Shanghai, China). 6-aminocaproic acid, formaldehyde, and dimethyl sulfoxide (DMSO) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). L-methionine was purchased from Biotopped. 1,8-naphthalic anhydride was purchased from Sigma Aldrich. Fetal bovine serum (FBS) and DMEM were supplied by Hyclone. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was from Dalian Meilun Biotechnology. All of chemicals were analytical grade and used without further purification. Milli-Q water was used in all synthetic and analytical experiments.

2. Apparatus

UV-vis absorption spectra were recorded by using a U-3900 UV-vis spectrophotometer (Hitachi High-Technologies, Japan) with a 1.0-cm optical path. Fluorescence spectra were conducted on an F-7000 FL spectrophotometer (Hitachi High-Technologies, Japan) with a 0.5-cm quartz cuvette. The excitation and emission slits were both set at 10.0 nm. Fourier transform infrared (FTIR) spectra were performed on a Nicolet-6700 spectrophotometer (Thermo Instruments Inc., USA) from 400 to 4000 cm^{-1}. X-ray photoelectron spectroscopy (XPS) scanning curves were obtained on an ESCALAB 250 surface analysis system (Thermo Electron, England) with an Al Kα 280.00 eV excitation source. Transmission electron microscopy (TEM) images were recorded on a JEM-2100 high resolution transmission electron microscope (JEOL, Japan). MTT assay was conducted by using a Synergy H1 ELISA plate reader at 490 nm (BioTek, USA).

3. Cytotoxicity assay

A standard MTT assay was carried out to evaluate the cytotoxicity of CDs and CDs-ND with HeLa cells. Briefly, HeLa cells were first seeded in a 96-well plate at a density of ca. 200 cells per well for 12 h. Once attached to each well, the cells were treated with 100 μL of CDs or CDs-ND solutions (0, 0.1, 0.2, 0.5, 1, 2, 5, 10, 20 mg mL^{-1}) for the ensuing incubation. After another 24 h, 20 mL of 5 mg mL^{-1} MTT solution was added into each well, and the cells were further incubated for 4 h to form violet-colored formazan. Finally, the supernatant MTT medium was removed thoroughly, and 150 μL of DMSO was added into each well to dissolve formazan. After shaking for 10 min, absorbance (A) was measured by using an ELISA plate reader at 490 nm. The cell viability was estimated based on the following equation:

$$\text{Cell viability } (\%) = \frac{A_{\text{sample}} - A_{\text{blank}}}{A_{\text{control}} - A_{\text{blank}}} \times 100\% \quad (1)$$

4. Cellular uptake pathways

In order to investigate the cellular uptake pathways of the CDs-ND (CDs or ND), HeLa cells were pre-incubated in DMEM for 1 h at 37°C with different pathway inhibitors such as chlorpromazine (10 μM, clathrin-mediated endocytosis inhibitor), genistein (185 μM , caveolae-mediated endocytosis inhibitor), methyl beta-cyclodextrin (5 mM, lipid raft mediated endocytosis inhibitor) and amiloride (100 μM, macropinocytosis inhibitor). Moreover, HeLa cells were pre-cultured at 4°C for 1 h to investigate the influence of energy on the cell internalization process. Then, a final concentration of 0.1 mg mL^{-1} CDs-ND or 0.1 mg mL^{-1} CDs or 5 μM ND were added into the culture medium, and cultured with cells for 4 h. After that, the cells were washed three times with 10 mM PBS physiological buffer at pH 7.4. Finally, fluorescence imaging was performed on a confocal fluorescence microscope with a 40× objective lens (λ_{ex} = 405 nm, λ_{em} = 420-460 nm and 520-560 nm). The optical density was evaluated using software Image J.

5. Synthesis of CDs-ND

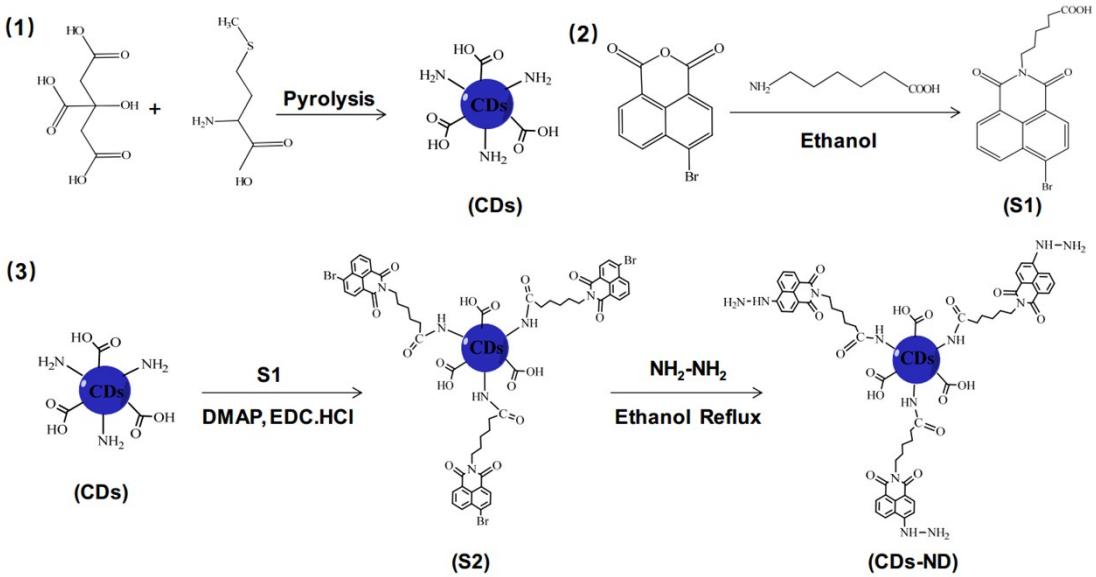

Fig. S1 Schematic illustration for the preparation of CDs-ND.

6. FTIR spectra of CDs, ND, and CDs-ND

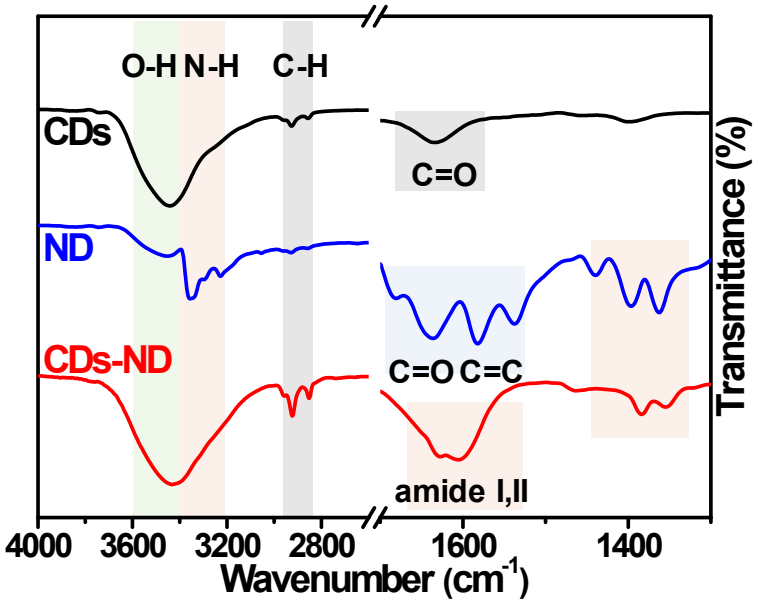

Fig. S2 FTIR spectra of CDs, ND, and CDs-ND.

7. 3D fluorescence mapping of CDs

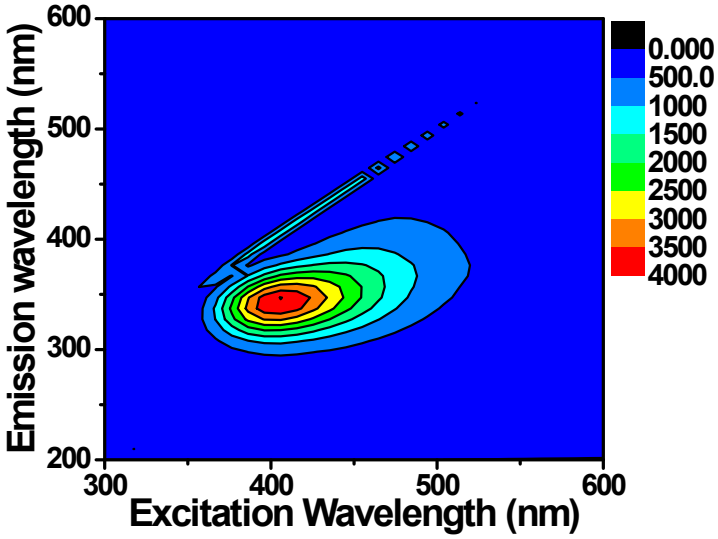

Fig. S3 Three-dimensional fluorescence mapping of CDs.

8. HR-MS spectra

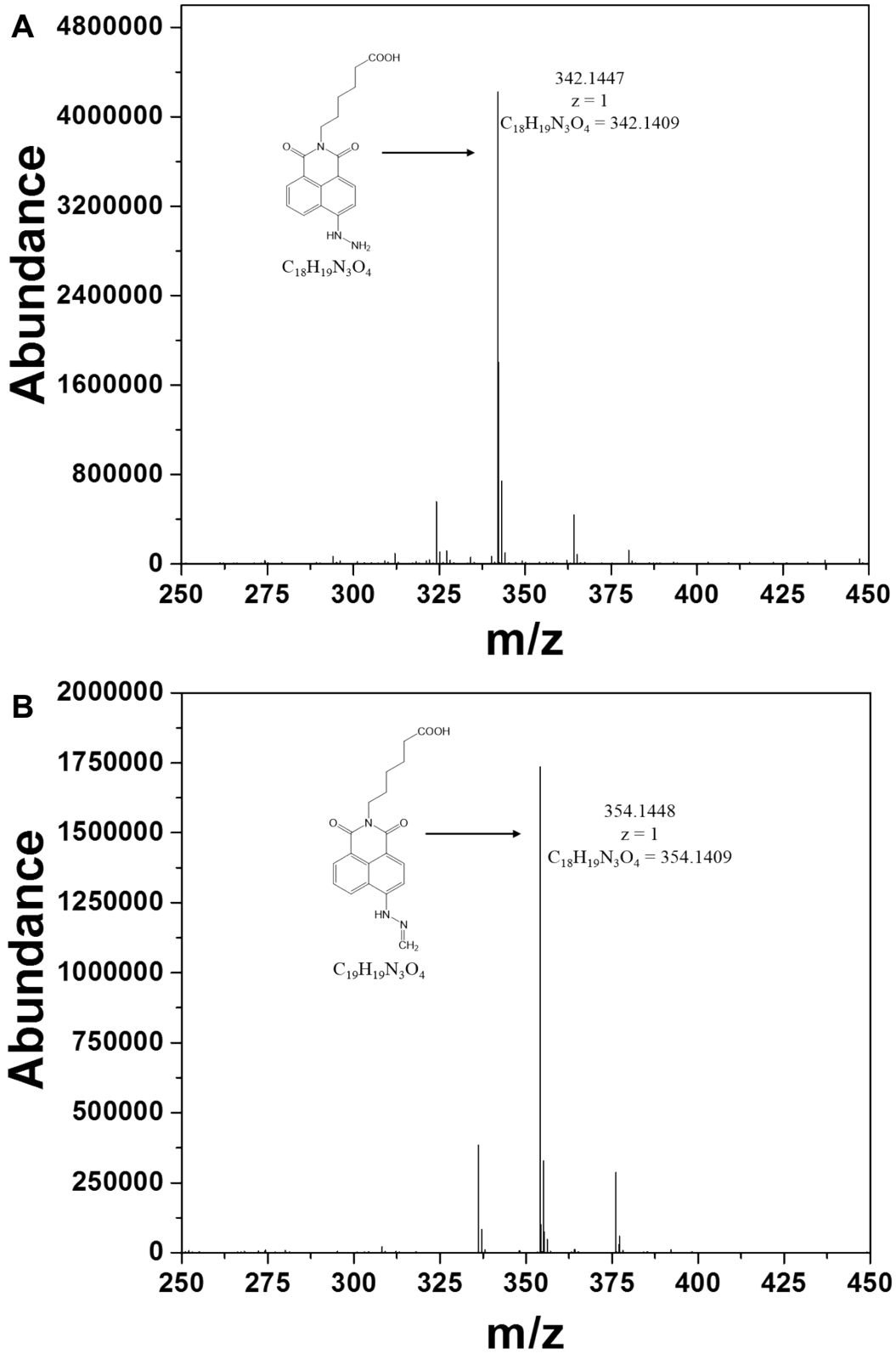

Fig. S4 HR-MS spectra of ND unit before (A) and after (B) treated with FA.

9. Kinetic studies

The rate constant was determined from the fluorescence titration data based on a reported method. The reaction of the probe CDs-ND (0.1 mg mL^{-1}) with FA in PBS (10 mM, pH 7.4) was monitored using the fluorescence intensity ratio of F_{535}/F_{414}. The reaction was carried out at room temperature. The pseudo-first-order rate constant for the reaction was determined by fitting the fluorescence intensity ratio of the sample to the pseudo-first-order equation:

$$Ln[(F_{max} - F_t) / F_{max}] = - k't \qquad (2)$$

Where F_t and F_{max} are the fluorescence intensity ratios of F_{535}/F_{414} at time t and the maximum value obtained after complete reaction. k' is the pseudo-first order rate constant. The pseudo-first-order plot for the reaction of CDs-ND with 20 μM of FA is shown in Fig. S4, The negative slope is pseudo-first-order rate constant for FA: k = 0.28 min^{-1}.

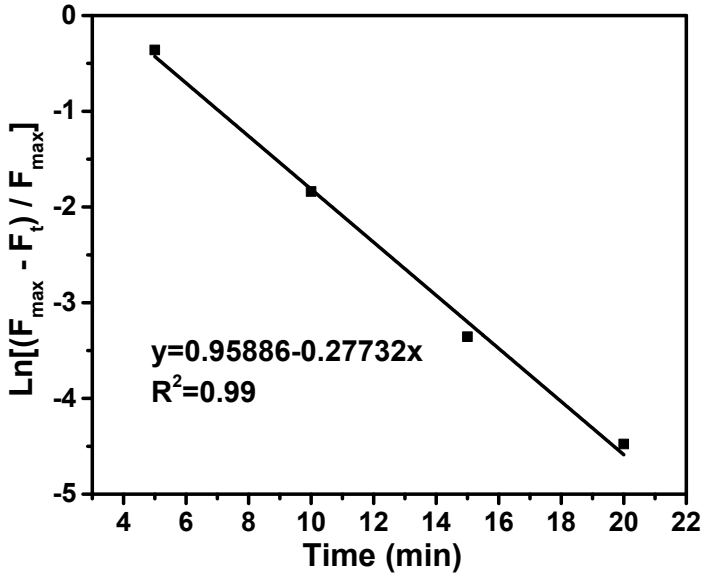

Fig. S5 Pseudo-first-order kinetic plot of the reaction of CDs-ND with FA, Slope = 0.28 min^{-1}.

10.Stability of probe

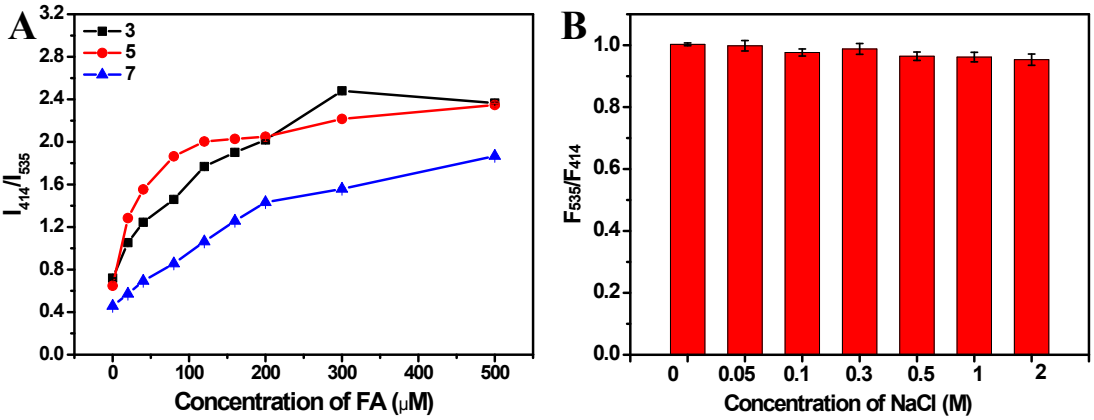

Fig. S6 (A) Fluorescence intensity response of probe at different pH values. (B) Fluorescence intensity ratio of probe at various concentrations of salt.

11. Cell viability

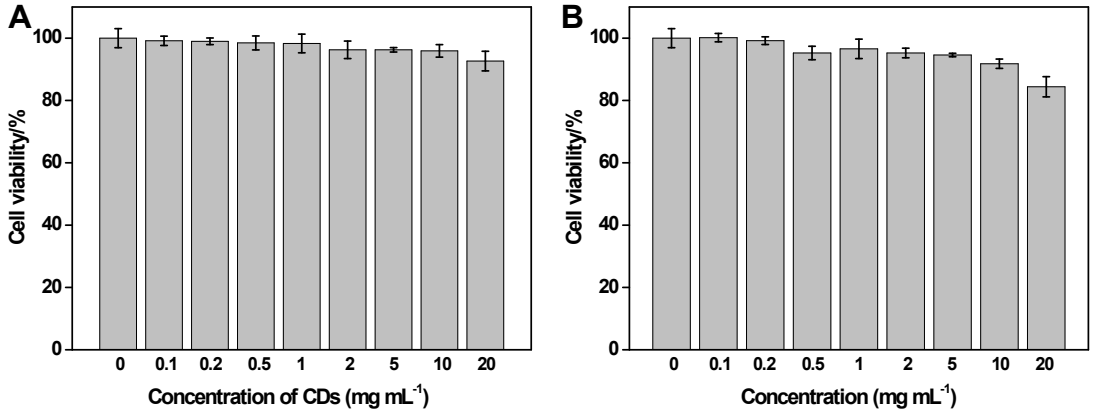

Fig. S7 Cell viability of HeLa cells after 24 h treatment with (A) CDs and (B) CDs-ND calculated from the MTT assay.

12. Quantification of FA in HeLa cells

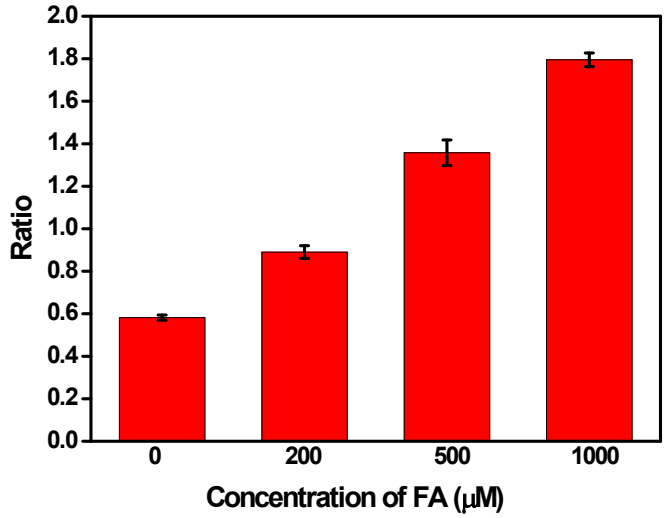

Fig. S8 The average I_{green}/I_{blue} fluorescence intensity ratios for the images in Fig. 3.

13. FA inhibitor test

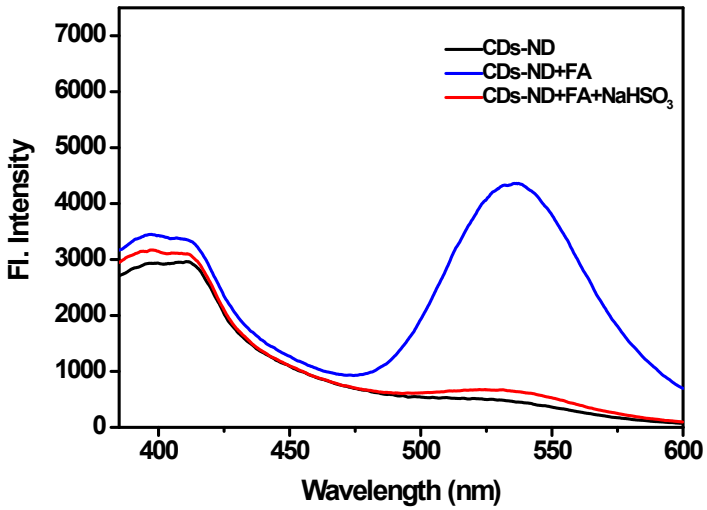

Fig. S9 Fluorescence spectra of the nanoprobe CDs-ND (0.1 mg mL^{-1}), CDs-ND (0.1 mg mL^{-1}) treated with FA (30 μM), and CDs-ND (0.1 mg mL^{-1}) treated with $NaHSO_3$ (200 μM). Incubation time: 30 min.

14. Colocalization Experiment

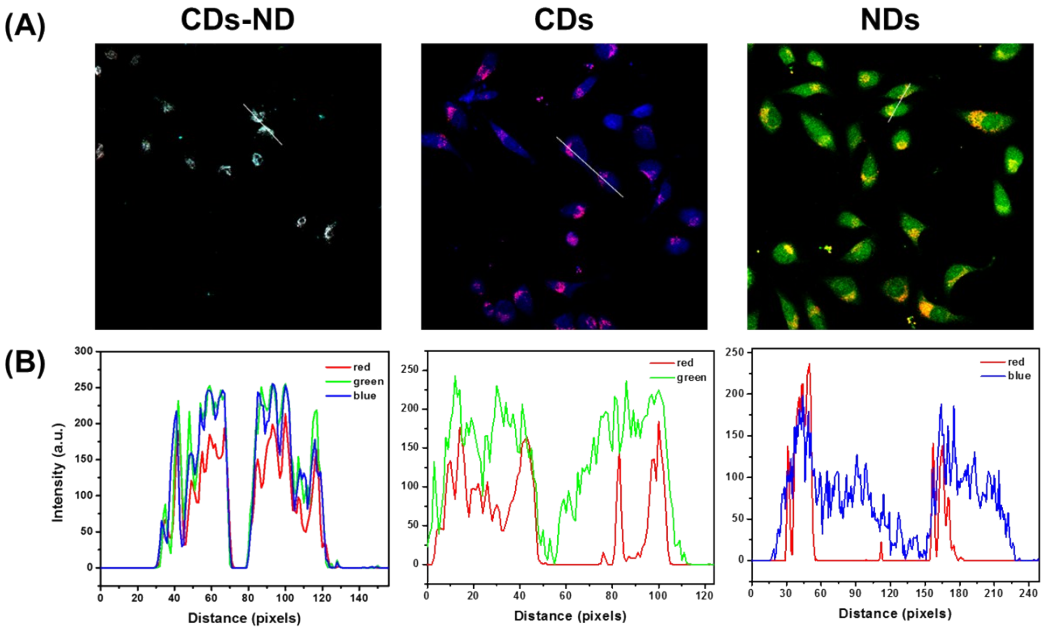

Fig. S10 (A) Fluorescence images of colocalization experiment in overlap; (B) Intensity profiles of regions of interest (ROI) across HeLa cells. The intensity profiles of the linear regions of interest (ROI) across cells stained with CDs-ND and LysoTracker Red vary in close synchrony.

15. XPS spectra

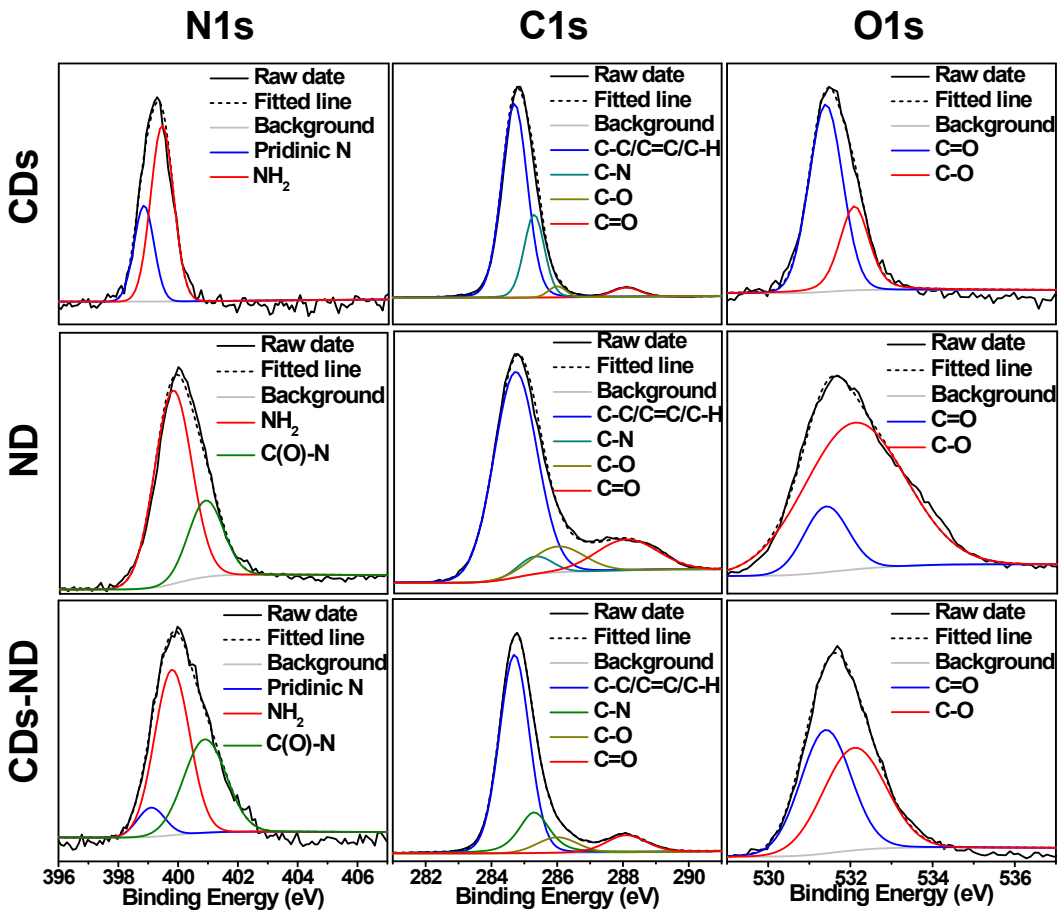

Fig. S11 High-resolution C1s/O1s/N1s spectra of CDs, ND and CDs-ND.

16. Ninhydrin test

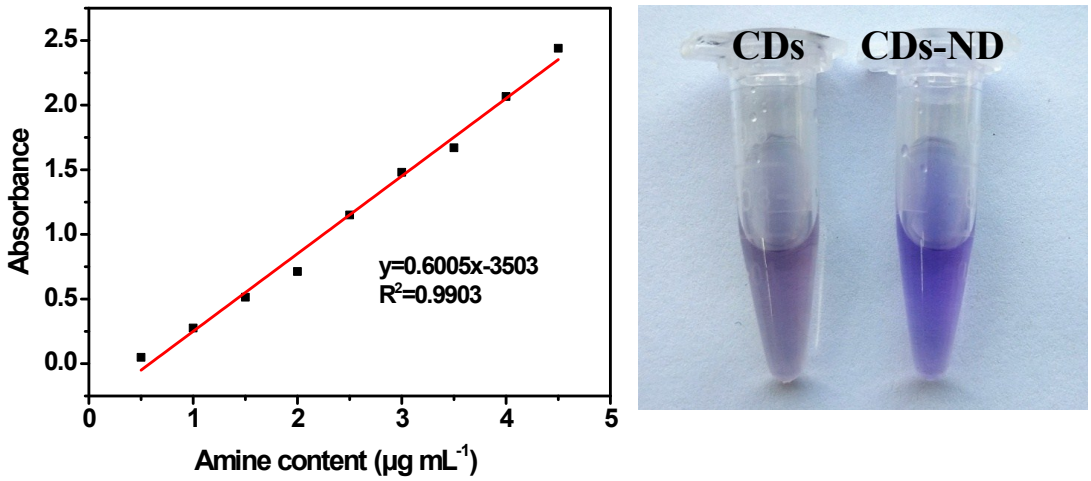

Fig. S12 Calibration curve for determination of amine groups in CDs and CDs-ND by Ninhydrin test with Leucine as the standard.

17. Distribution of CDs-ND at different pH values

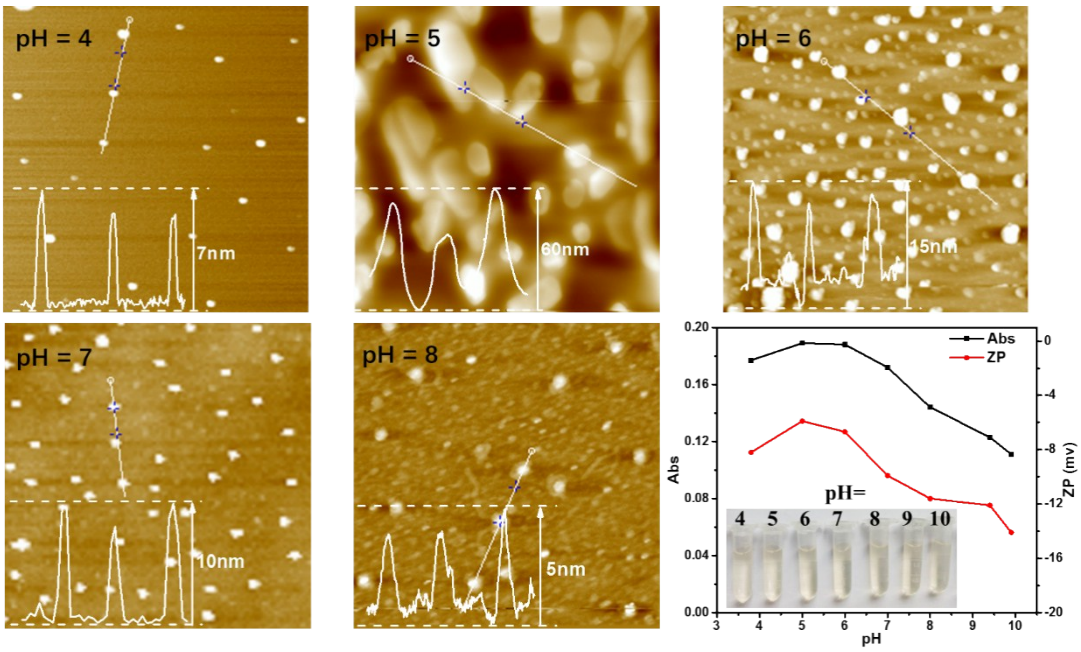

Fig. S13 AFM images of CDs-ND at different pH values. The absorption intensity at 416 nm and Zeta potential variation within pH 4-10, insert is the photo of CDs-ND solutions at different pH values.

18. ^{1}H NMR spectra of ND, CDs and ND-CDs

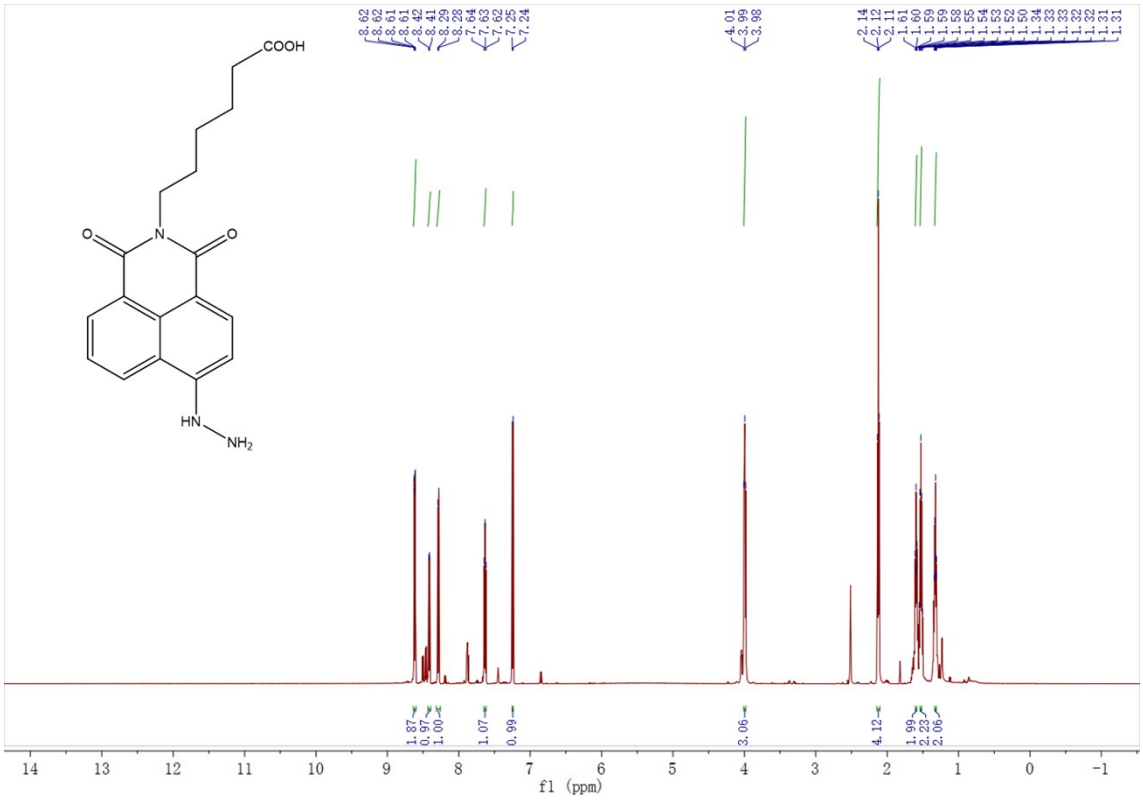

Fig. S14 ^{1}H NMR spectrum of ND.

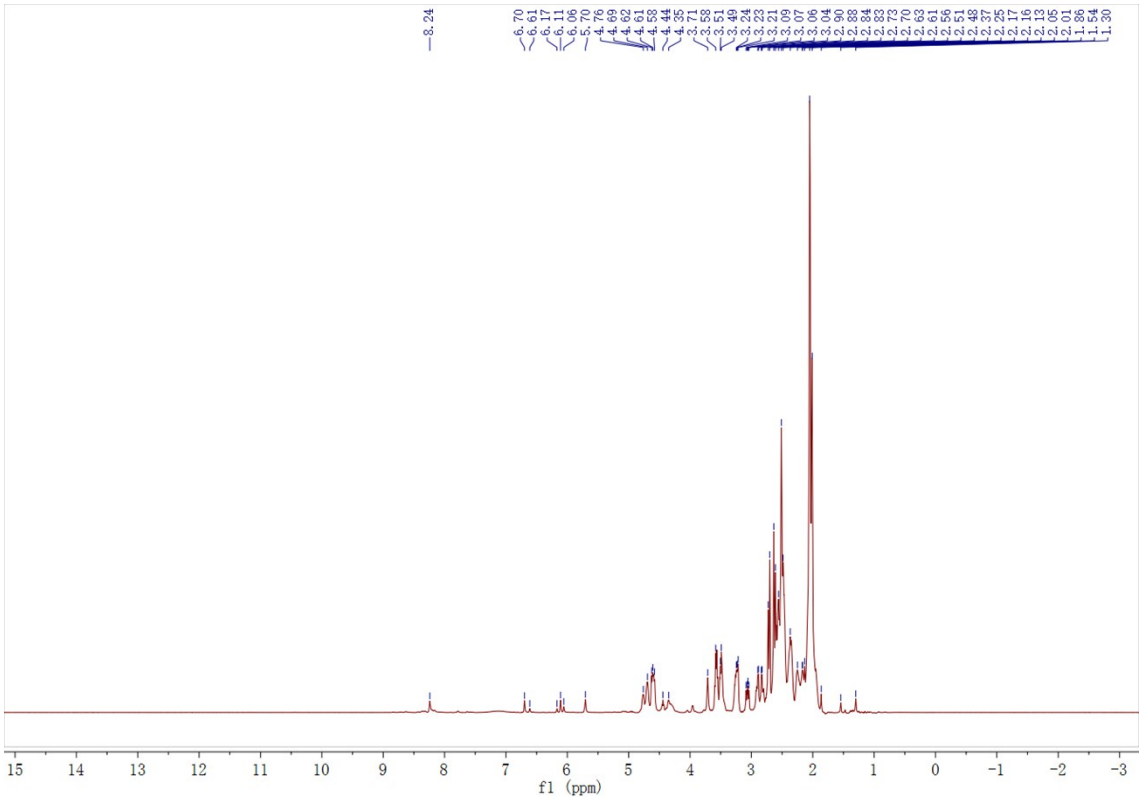

Fig. S14 ^{1}H NMR spectrum of CDs.

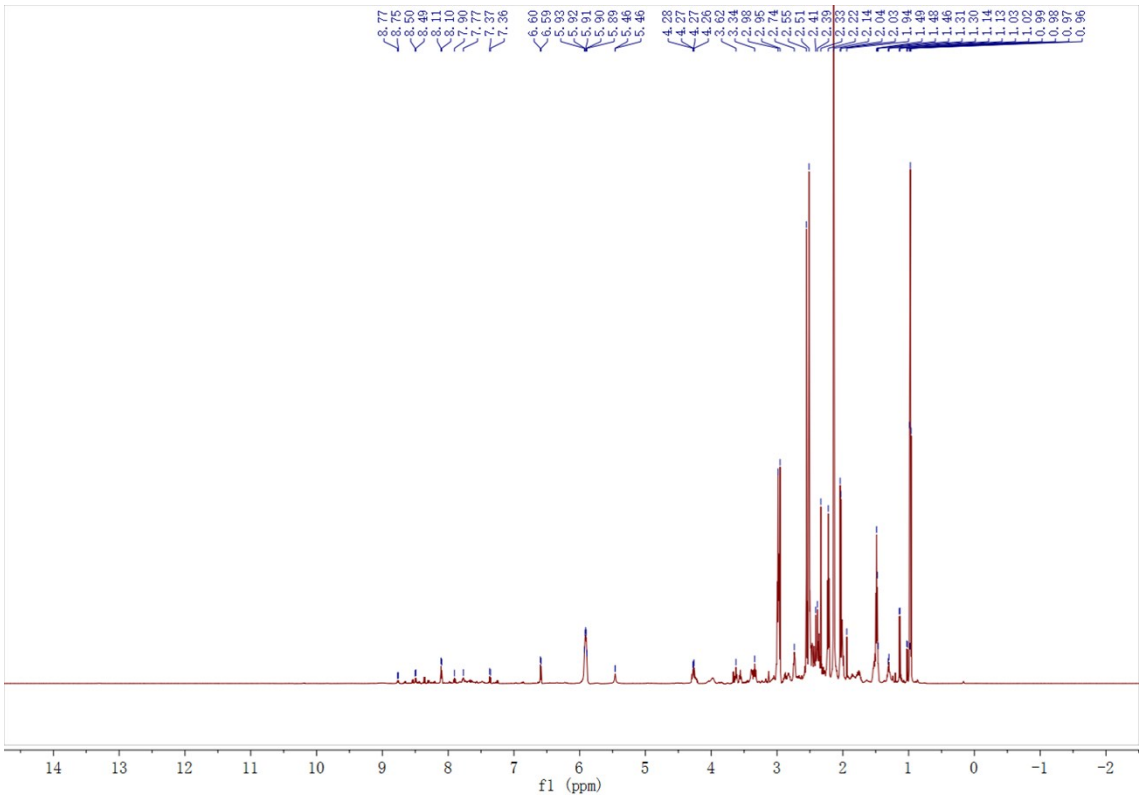

Fig. S15 ^{1}H NMR spectrum of ND-CDs.