Electronic Supplementary Material (ESI) for Nanoscale.

Supplementary Information

Carbon dots for Lysosome Targeting and Imaging of Lysosomal pH and Cys/Hcy in Living Cells

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1. Determination of pK_a

pK_a can be calculated by using the Henderson-Hasselbalch equation:

$$pH = pK_a + \log ([\text{conjugate base}]/[\text{weak acid}])$$

$$pH = pK_a + \log ([A^-]/[HA]$$

At half the equivalence point,

$$pH = pK_a \quad (1)$$

2. Determination of fluorescence quantum yield

Fluorescence quantum yield was measured by a standard method in air-equilibrated sample at room temperature. The fluorescence quantum yield was determined by using Rhodamine B (Φ=0.31 in water) as reference. [1, 2]

$$\Phi_{sam} = \Phi_{ref} \frac{I_{sam}}{I_{ref}} \cdot \frac{A_{ref}}{A_{sam}} \cdot \left(\frac{n_{sam}}{n_{ref}}\right)^2 \quad (2)$$

Where Φ is the fluorescence quantum yield, I is the integrated emission intensity, A is the absorbance, and n is the refractive index. The subscripts $_{sam}$ and $_{ref}$ stand for sample and reference, respectively. Herein, the SCy-CDs and Rhodamine B were dissolved in ultrapure water (n =1.33) and excited under 420 nm and kept the absorbance below 0.05.

3. Supplementary figures

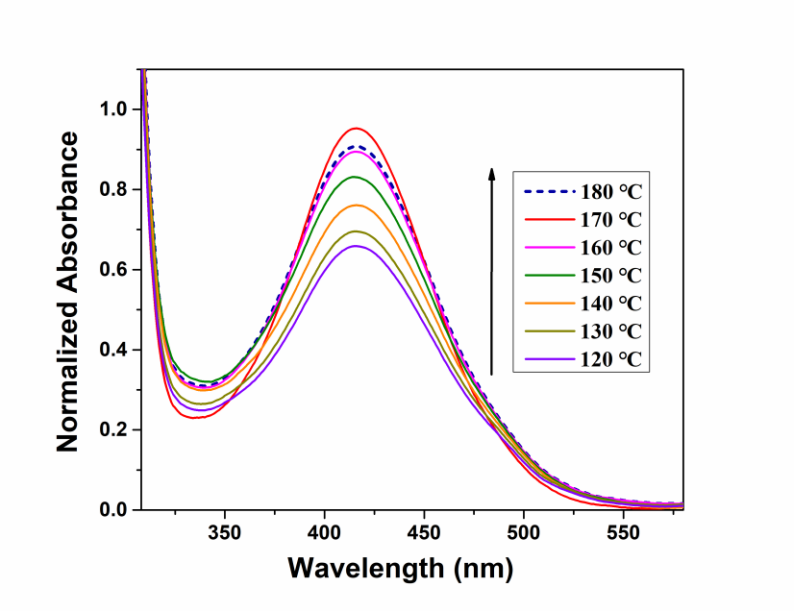


Fig.S1. The absorption spectra of the o-CDs at the different reaction temperatures.

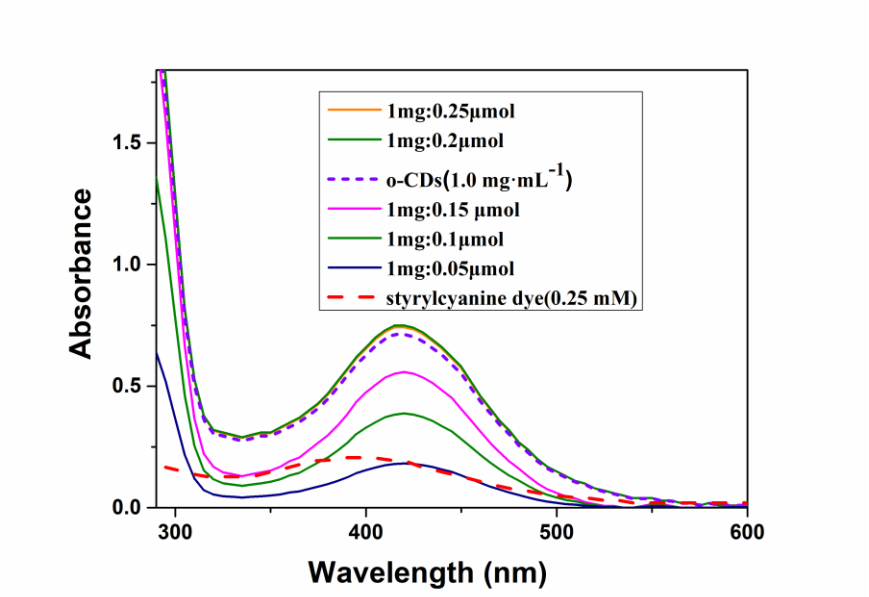


Fig.S2 The absorption spectra of o-CDs and styrylcyanine at different ratio by ethanol reflux.

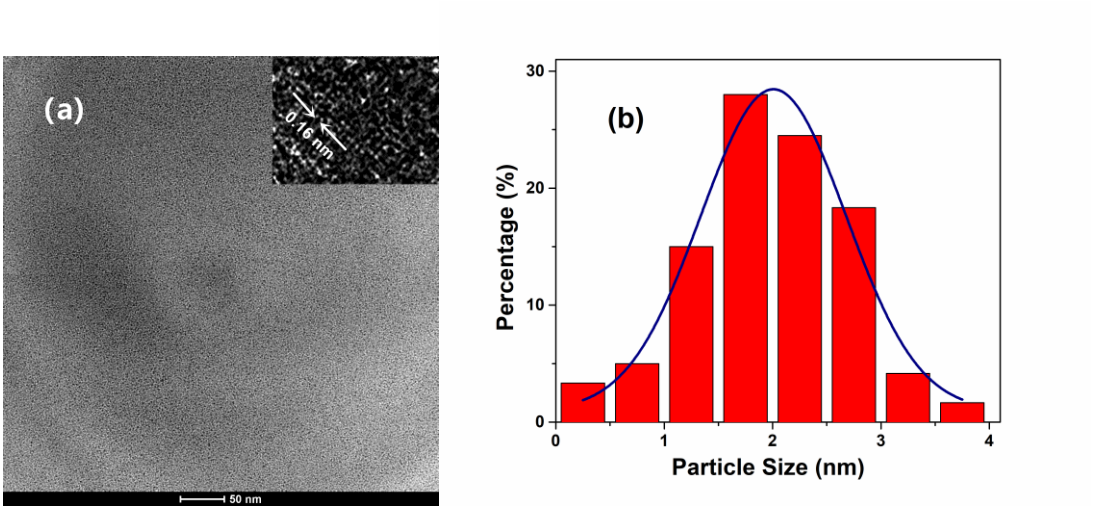


Fig.S3 TEM image of the o-CDs (inset: corresponding HRTEM image). (b) The size distribution of the o-CDs.

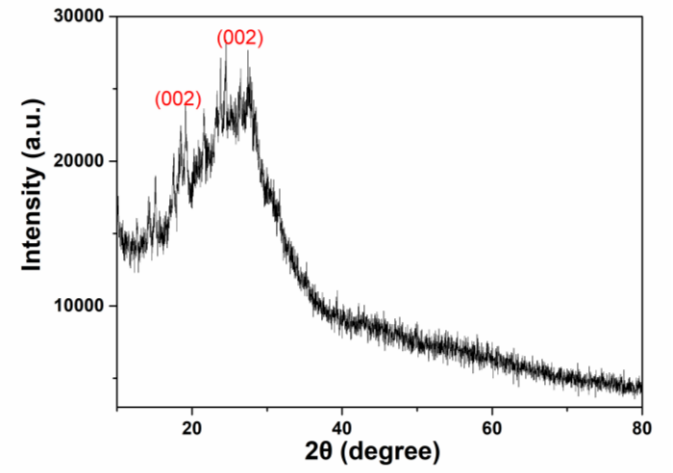


Fig. S4 XRD diffraction pattern of the Scy-CDs.

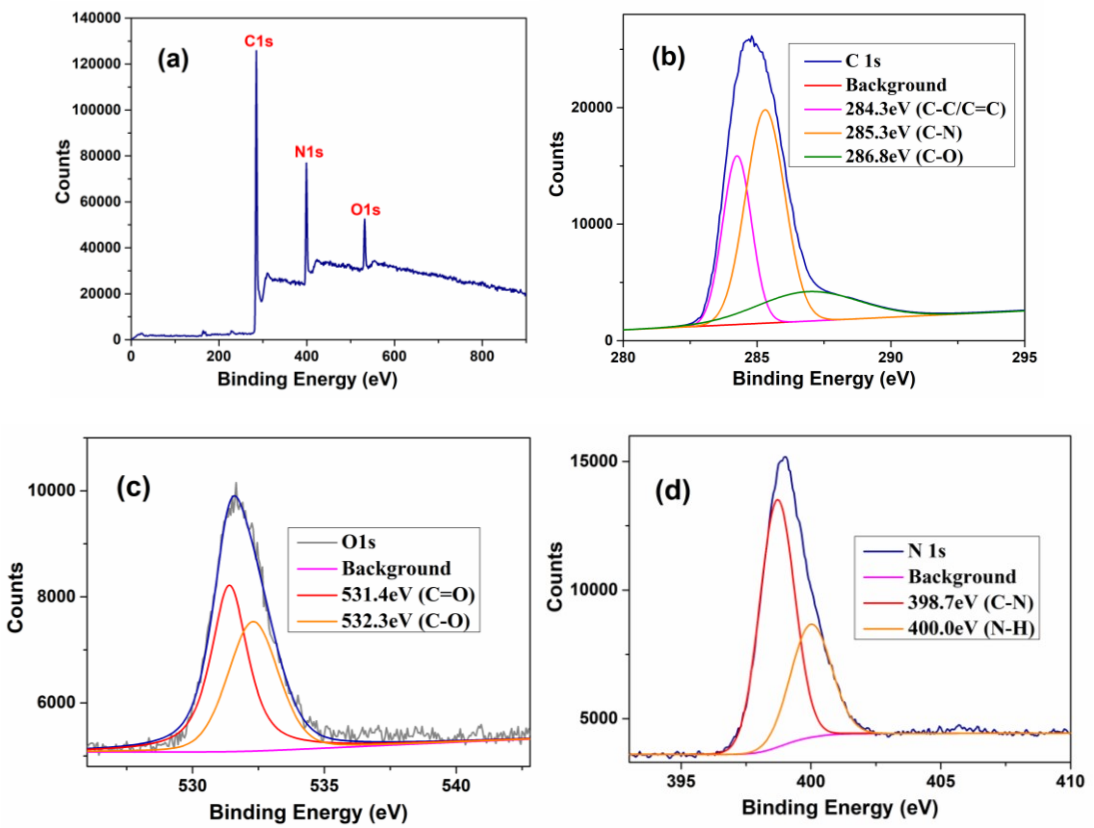


Fig.S5 (a) The full scan XPS of o-CDs. High resolution XPS of C 1s (b), O 1s (c) and N 1s (d).

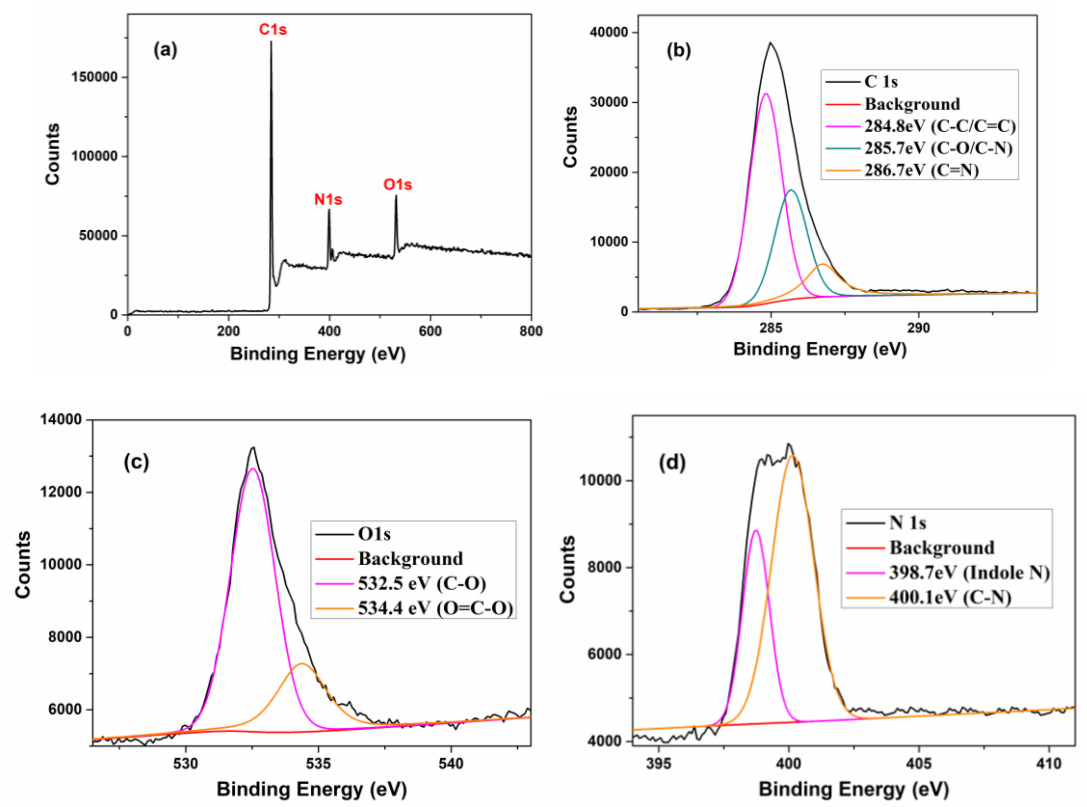


Fig.S6 (a) The full scan XPS of Scy-CDs. High resolution XPS of C 1s (b), O 1s (c) and N 1s (d).

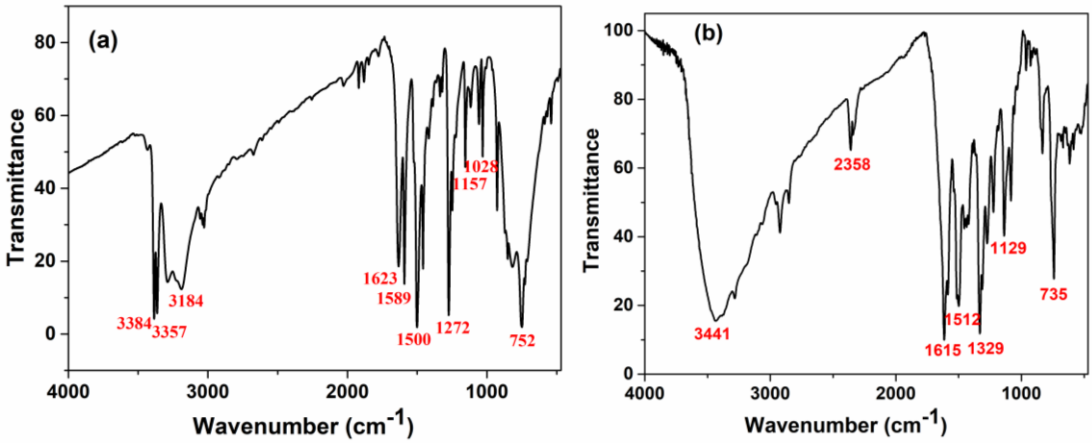


Fig.S7 Fourier transformed infrared spectra of o- CDs (a) and Scy-CDs (b).

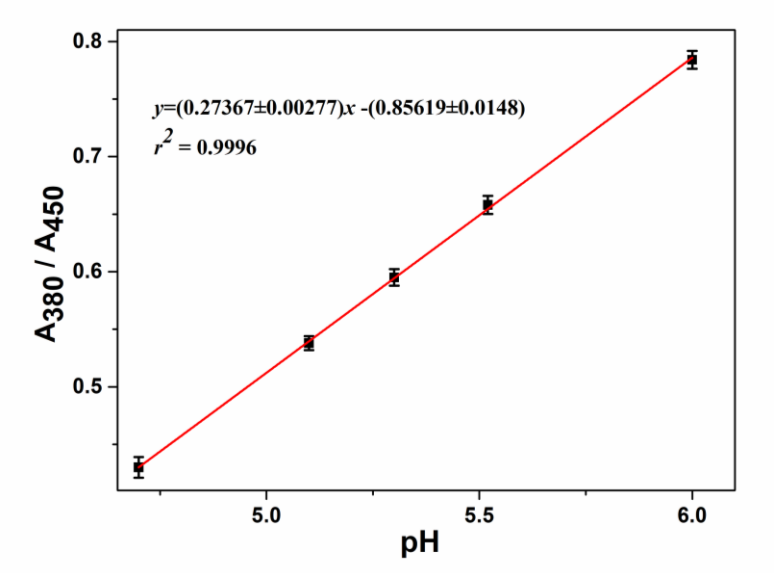


Fig. S8 The linear region between the ratiometric absorbance (A_{380} /A_{450}) and pH in the range of 4.7-6.0.

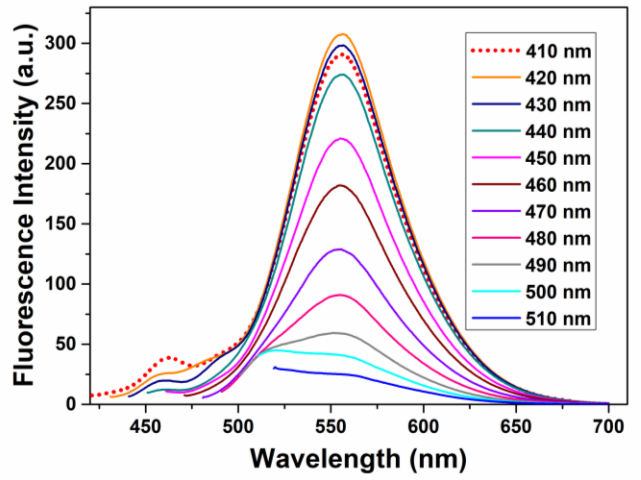


Fig. S9 The fluorescence emission spectra of the Scy-CDs with different excitation wavelengths.

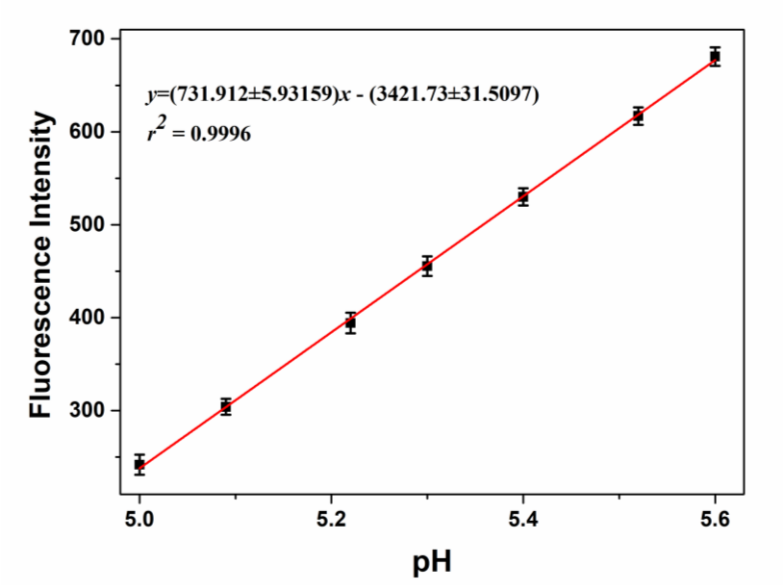


Fig.S10 The linear region between the fluorescence intensity of Scy-CDs at 556 nm and pH in the range of 5.0-5.6.

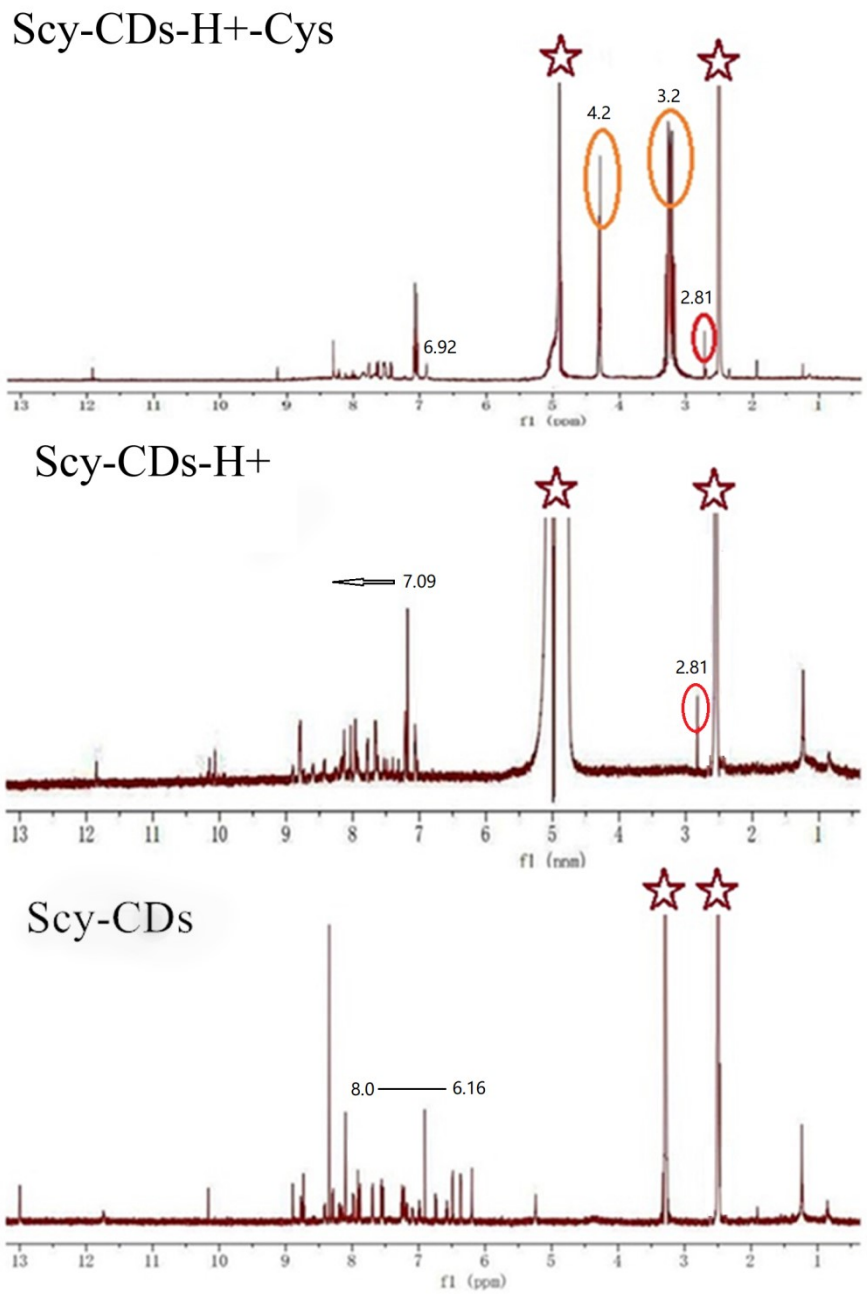


Fig. S11 ^{1}H NMR spectra of Scy-CDs, Scy-CDs-H^{+} and Scy-CDs-H^{+}-Cys complex in DMSO-d_6. The water and solvent peaks are marked with asterisks.

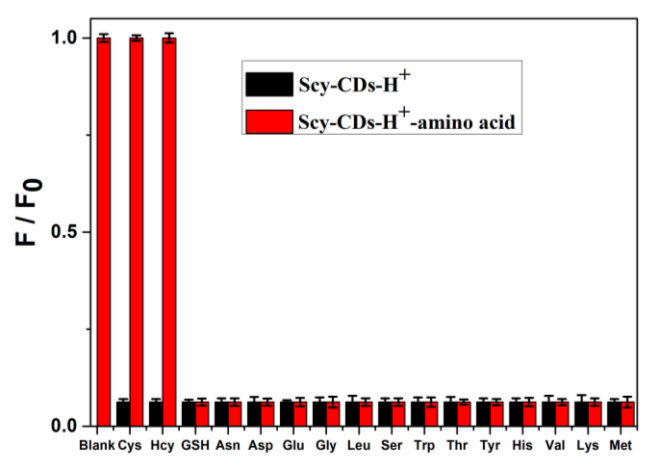


Fig.S12 Relative fluorescence intensity of the Scy-CDs (0.04 mg/mL) in the presence of 120 μM H^+, and then addition of different amino acids (120μM) in aqueous solution, where F_0 and F are the fluorescence intensities of the Scy-CDs in the absence and the presence of H^+ or (H^+ and amino acids), respectively. The excitation wavelength is 450 nm.

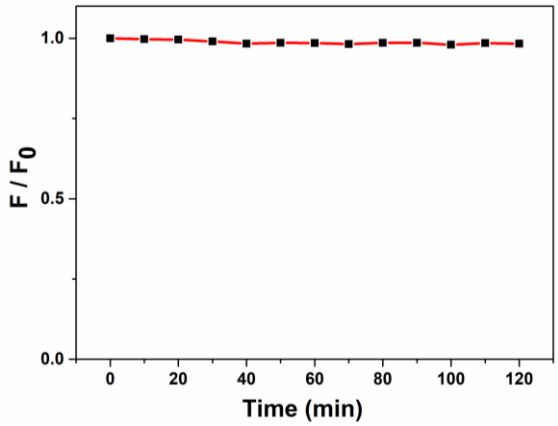


Fig. S13 Photostability of the Scy-CDs as a function of the continuous ultraviolet light at 365 nm for 120 min.

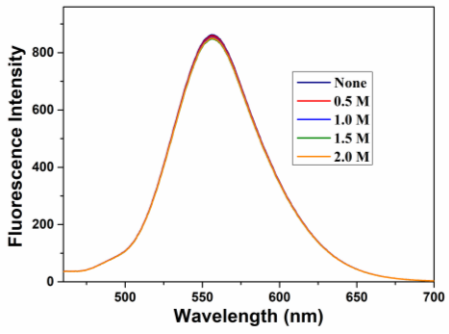


Fig. S14 The effects of the different ionic strengths (0.0-2.0 M KCl) on the fluorescence emission spectra of the Scy-CDs (0.04 mg/mL) at 420 nm excitation wavelength.

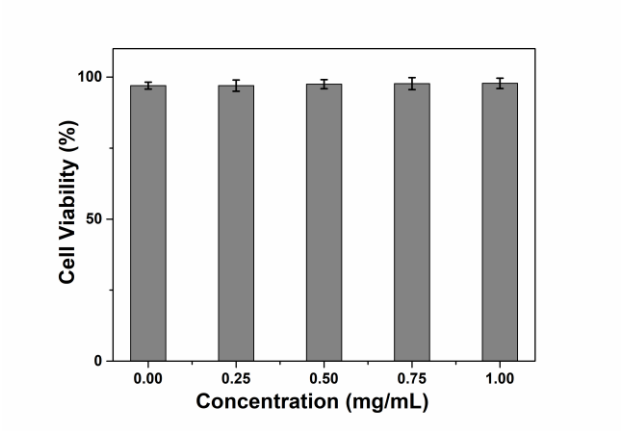


Fig. S15 Cytotoxic assay of the different concentrations of Scy-CDs on Hep-2 Cells.

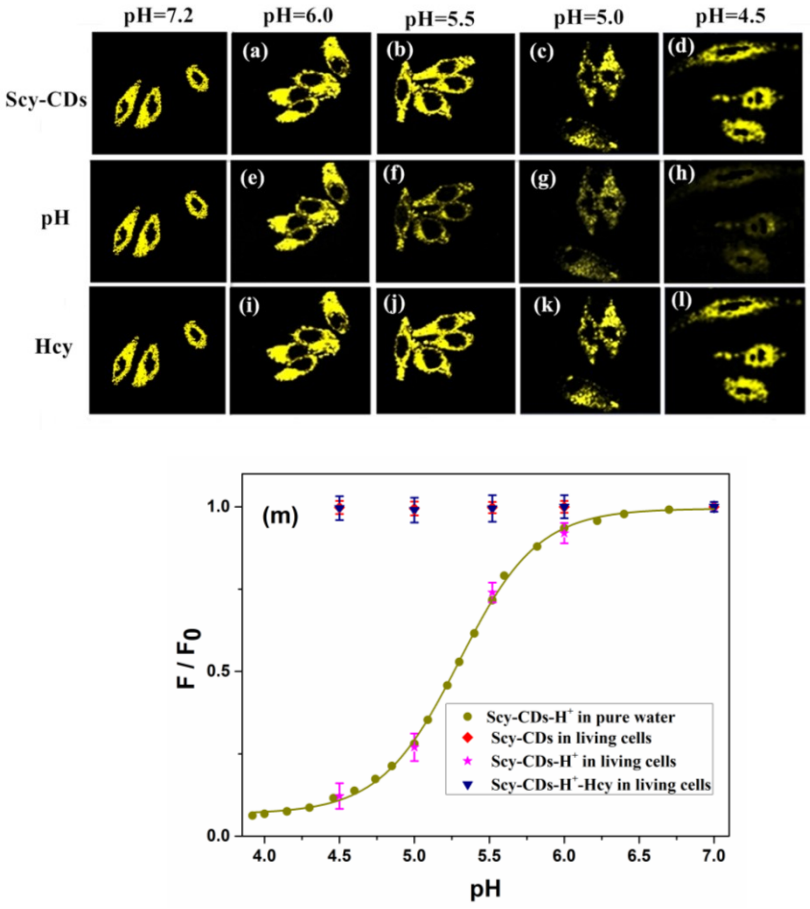
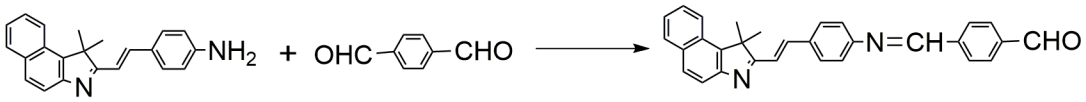


Fig. S16 HEp-2 cells at pH 7.2 as control group. (a)-(d) Confocal fluorescence images of 0.5 mg/mL Scy-CDs in HEp-2 cells at pH 7.2.(e)-(h) Followed by incubation with decreased pH (6.0, 5.5, 5.0 and 4.5, respectively) in high-K^+ buffered solutions for 30 min at 37 °C. (i)-(l) Further subsequent incubation with Hcy (50, 80, 105, 120 μM) for another hour at 37 °C. (m) Relatively mean fluorescence intensities of Scy-CDs in pure water and HEp-2 cells at different pH with absence or presence of Hcy (Data are presented as mean ± SD with replicates (n=3)).

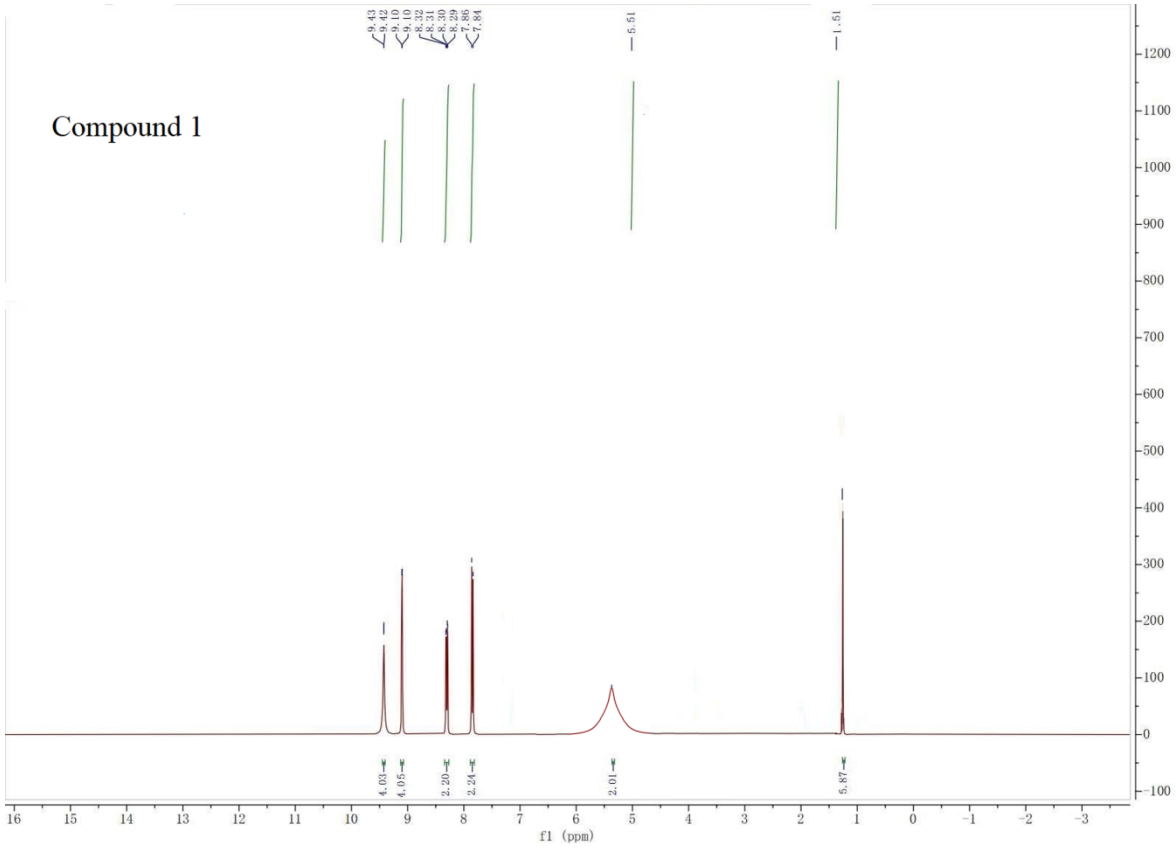
4. Synthesis of the styrylcyanine dye

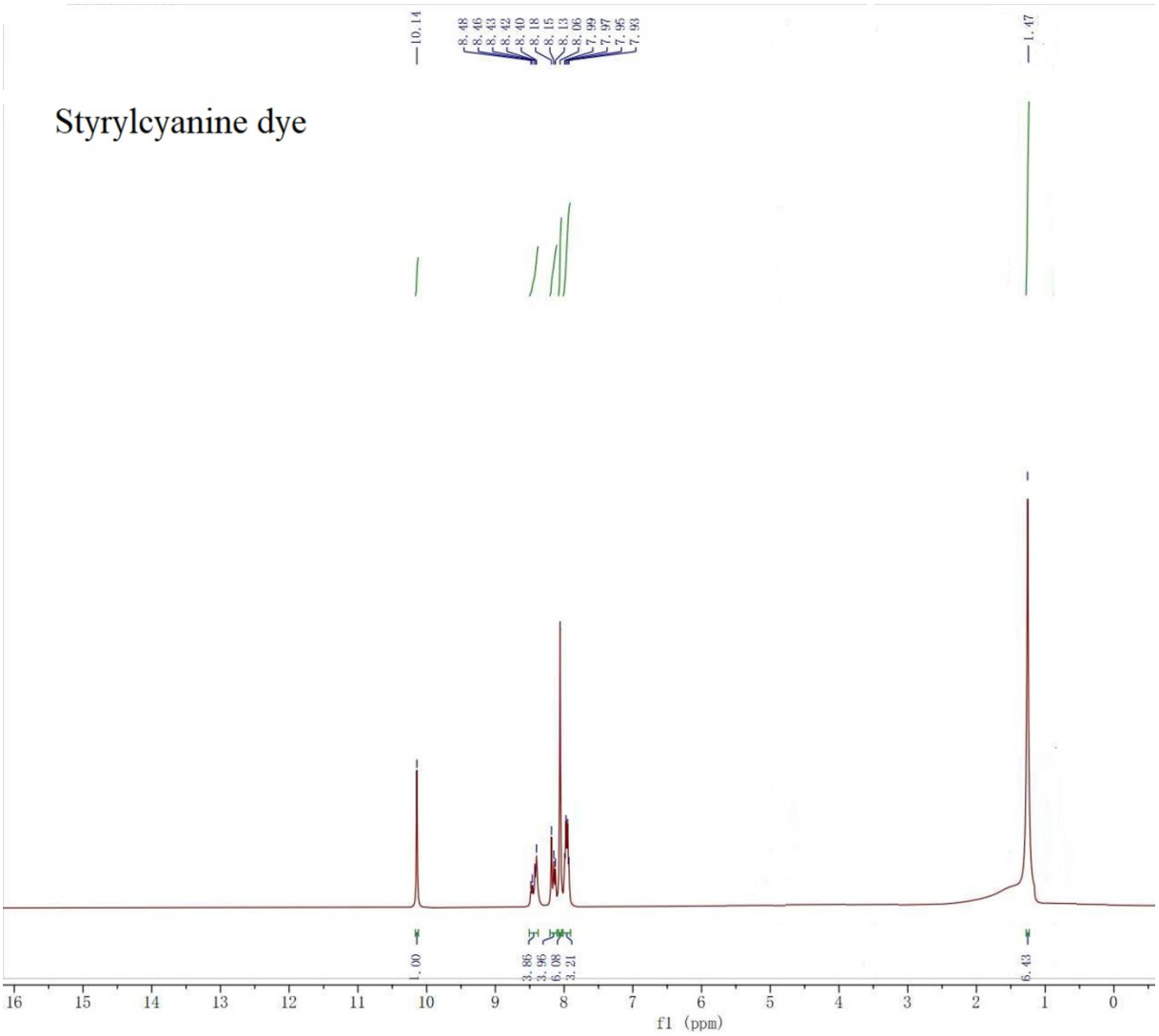


compound 1 styrylcyanine dye

Compound 1(laboratory-made, 1.24g, 3.97 mmol) and p-diformylbenzene (0.64g, 4.77mmol) were dissolved in 50 mL dry dichloromethane, The mixture solution was stirred and heated to reflux for 5 h. Finally, dichloromethane was removed on a rotary evaporator and the precipitate was purified by column chromatography using dichloromethane /ethyl acetate (3:1) as the eluent to obtain the orange solid styrylcyanine dye (1.37 g, 80.6 % yield). The ^{1}H NMR (600 MHz, $CDCl_3$) data are δ 10.1 (s, -CHO, H), 8.48 (s, -CH, H), 8.46 (d, Ar-H, H), 8.43(d, Ar-H, 3H), 8.42 (d, Ar-H, H), 8.40 (d, Ar-H, 3H), 8.18 (d, Ar-H, 2H), 8.15 (d, -CH, H), 8.06 (t, Ar-H, H), 7.99 (t, Ar-H, H), 7.95 (d, Ar-H, 2H), 7.93 (d, -CH, H), and 1.47 (s, $-CH_3$, 6H).

5. ^{1}H NMR spectra





6. References

1. Zhang, M.; Gao,Y.; Li, M.; Yu, M.; Li, F.; Li, L.; Zhu, M.; Zhang, J.; Yi, T.; Huang, C. *Tetrahedron Lett.* **2007**, *48*, 3709-3712.

2. Qu, Z.; Zhou, X.; Gu, L.; Lan, R.; Sun, D.; Yu, D.; Shi, G. *Chem. Commun.*, **2013,** *49*, 9830-9832.