Supporting Information

Near-infrared fluorescent probe based on chloroacetate modified naphthofluorescein for selectively detecting cysteine/homocysteine and its application in living cells

Jianxi Wang[a], Cheng Zhou[b], Wei Liu[a], Jianjian Zhang[a], Xinyue Zhu[a], Xiaoyan Liu[a], Qin Wang[b], Haixia Zhang[a,*]

[a]Key Laboratory of Nonferrous Metals Chemistry and Resources Utilization of Gansu Province and State Key Laboratory of Applied Organic Chemistry, College of Chemistry and Chemical Engineering, Lanzhou University, Lanzhou 730000, P. R. China.

[b]Department of Cell Biology, School of Life Sciences, Lanzhou University, Lanzhou 730000, P. R. China.

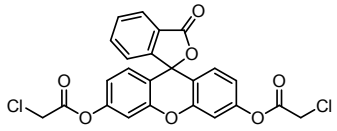
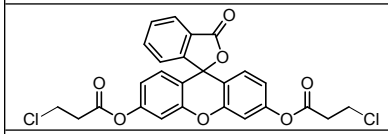
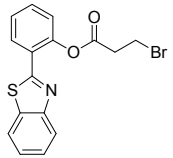
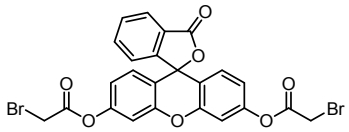
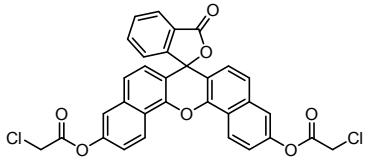
[*]Corresponding author

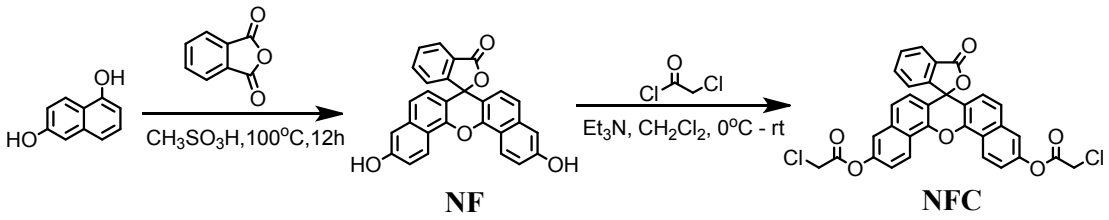
Tel.: +86 931 8912058;

Fax: +86 931 8912582.

E-mail: zhanghx@lzu.edu.cn

Table S1 Comparison of fluorescent probes based on conjugate nucleophilic substitution/cyclization sequences for Cys and Hcy

Probe	NIR or not	E_x/E_m (nm)	Detection System	Range of linear correlation (μM)	LOD (μM)	Time (min)	Application	Ref.
	NO	450/513	EtOH/ H_2O 1:20	Cys: 20-280 Hcy: 40-280	Cys: 4 Hcy: 7	30	Determination of Cys/Hcy in EtOH/H_2O and imaging in living cells	24
	NO	490/518	DMSO/ PBS 1:4	Cys: 16.6-166.5	Cys: 12.8	10	Determination of Cys in PBS buffer, human plasma and imaging in living cells	25
	NO	330/467	DMSO/ HEPES 4:1	Cys: 10-50	Cys: 2.8	15	Determination of Cys in HEPES buffer and imaging in living cells	26
	NO	500/525	DMSO/ HEPES 1:1	Cys: 0-16	Cys: 35	10	Determination of Cys in HEPES buffer and imaging in living cells	27
	YES	608/660	DMF/ PBS 3:7	Cys: 1-40 Hcy: 1-80	Cys: 0.30 Hcy: 0.42	10	Determination of Cys/Hcy in PBS buffer, human plasma and imaging in living cells	**This work**



Scheme S1 Synthesis of **NFC**.

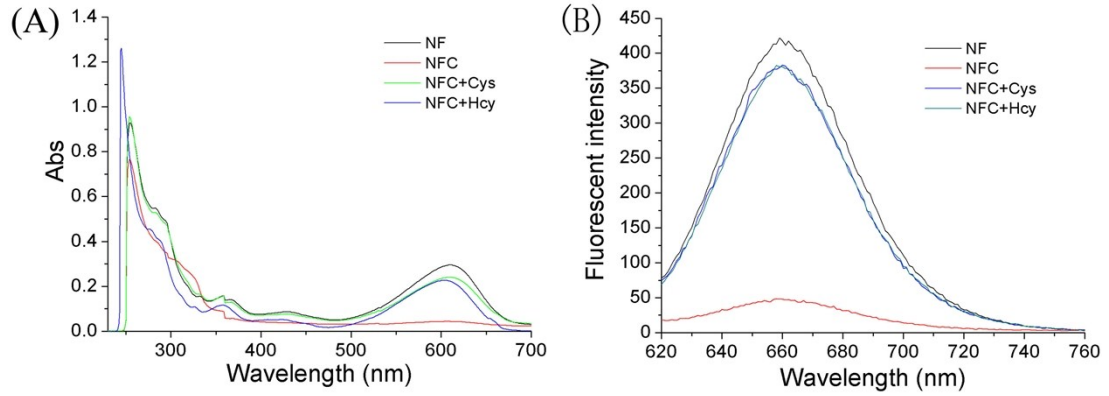


Fig. S1 (A) Absorption and (B) fluorescence emission spectra of **NF** (20 μM, black line) and **NFC** (20 μM), before (red line) and after reacting with Cys (100 μM, green line) and Hcy (100 μM, blue line) in PBS (10 mM, pH 7.4) containing 30% DMF, λ_{ex} = 608 nm.

Determination of the fluorescence quantum yield. The fluorescence quantum yields of **NFC** (Φ = 0.008) and **NF** (Φ = 0.081) were determined in PBS (10 mM, pH 7.4) containing 30% DMF (v/v), using Rhodamine B (Φ_s = 0.89, EtOH) as standard. The quantum yield was calculated using the following equation:

$$\Phi_x = \Phi_s (A_s F_x / A_x F_s) (n_x^2 / n_s^2)$$

where A_x and A_s are the absorbance of the sample and the reference, respectively, at the excitation wavelength, F_x and F_s are the corresponding relative integrated fluorescent intensities, and n_x and n_s is the corresponding refractive index of the solvent.

Kinetic studies. The reaction of **NFC** (20 μM) with Cys and Hcy was monitored by measuring the fluorescence intensity F at 660 nm. The apparent rate constant for the reaction was determined by fitting the fluorescence intensity of the samples to the pseudo first-order equation:

$$\ln ((F_{max} - F_t) / F_{max}) = -k' t$$

where F_t and F_{max} are the fluorescence intensities at 660 nm at times t and the maximum value obtained after the reaction was completed. K' is the apparent rate constant. The pseudo-first-order rate constant k (M^{-1} s^{-1}) was obtained from equation k' = k [M], where [M] is the concentration of Cys and Hcy.

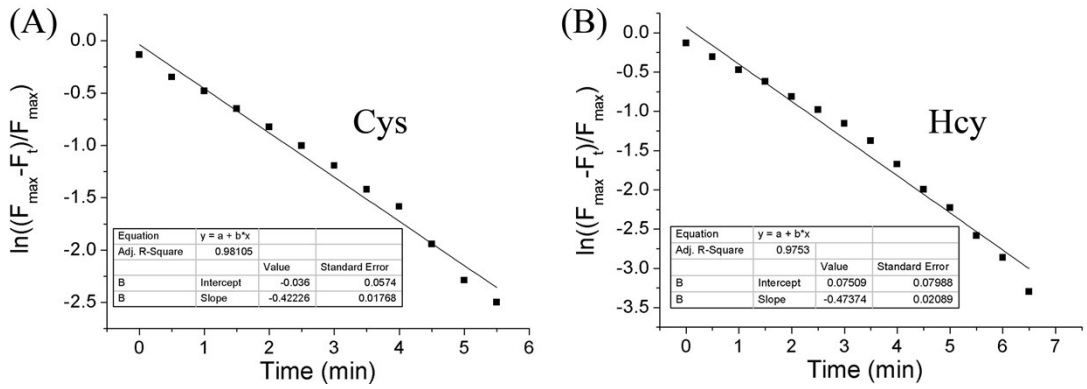


Fig. S2 (A) Pseudo first-order kinetic plot of reaction of **NFC** (20 μM) with Cys (100 μM), slope = -0.422 so k' = 0.422 min^{-1} and k = 70.38 M^{-1} s^{-1}. (B) Pseudo first-order kinetic plot of reaction of **NFC** (20 μM) with Hcy (100 μM), slope = -0.474 so k' = 0.474 min^{-1} and k = 78.96 M^{-1} s^{-1}.

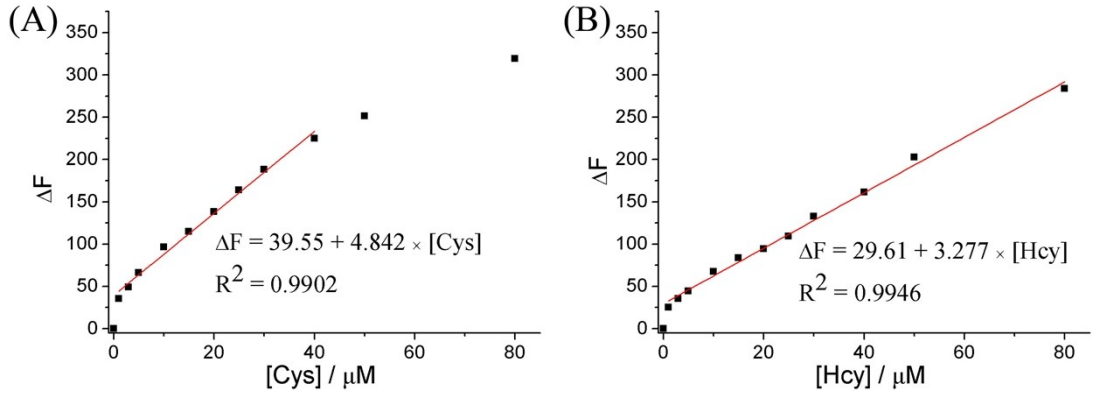


Fig. S3 Calibration curves of **NFC** (20 μM) for Cys (A) and Hcy (B) at 660 nm, respectively. $\Delta F = F - F_0$. Where, F is the fluorescent intensity of **NFC** (20 μM) upon addition of various

concentration of Cys or Hcy. F_0 is the fluorescent intensity of **NFC** (20 μM).

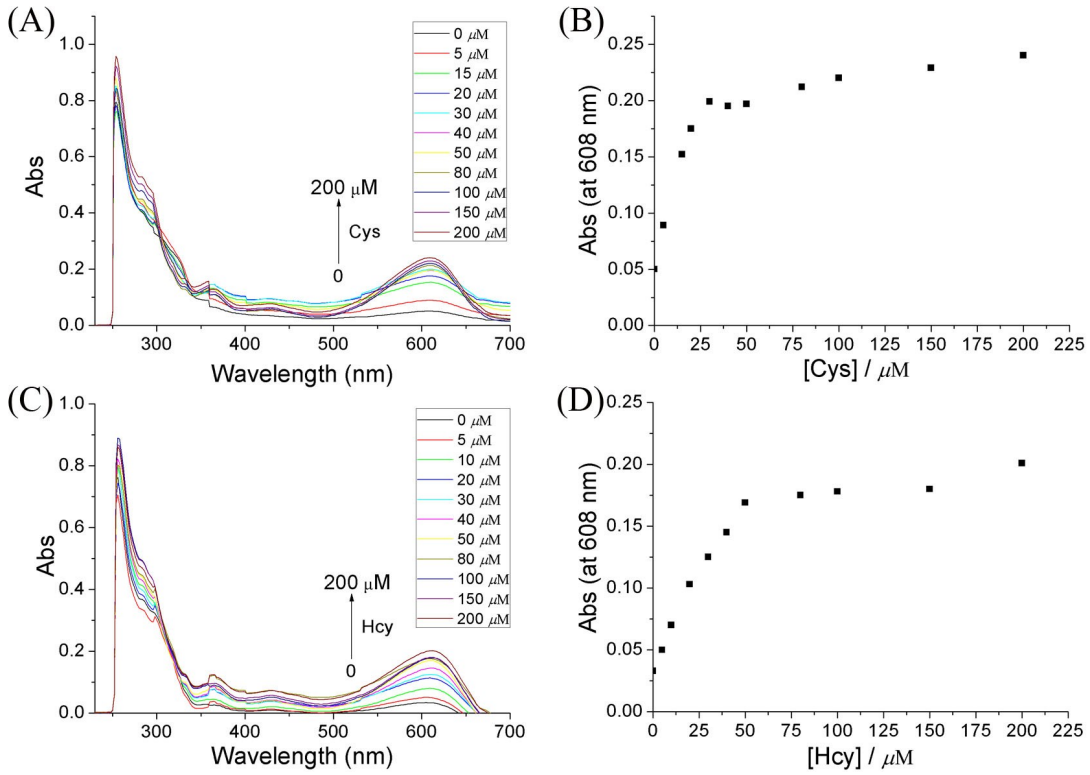


Fig. S4 (A) and (C): UV-Vis spectra changes of **NFC** (20 μM) upon addition of different concentrations of Cys and Hcy. (B) and (D): Absorbance changes of **NFC** at 608 nm against concentrations of Cys and Hcy.

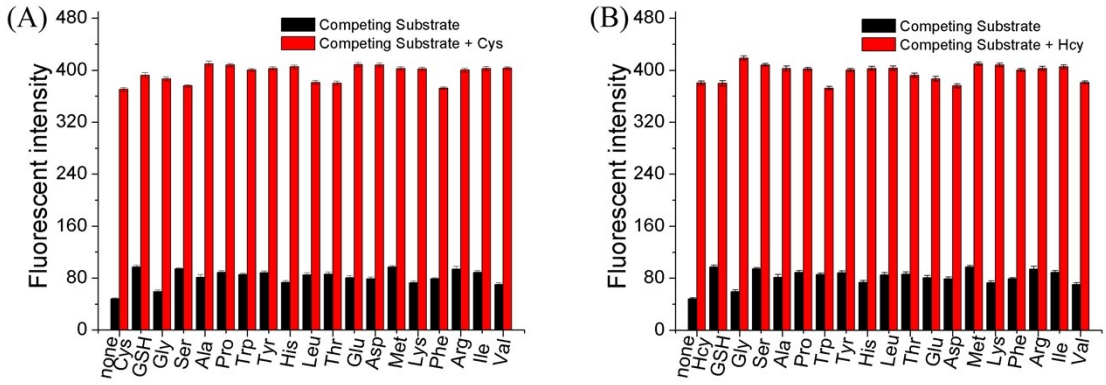


Fig. S5 The fluorescence responses of **NFC** (20 μM) toward Cys (100 μM) (A) and Hcy (100 μM) (B) in presence of various amino acids (100 μM) and GSH (1 mM). Black bars represent the addition of a single analyte. Red bars represent the subsequent addition of Cys (100 μM) or Hcy (100 μM) to the analytes. All data were obtained 10 min after addition of each analyte in PBS (10 mM, pH 7.4) containing 30% DMF at 25 °C and reported as the mean ± standard deviation of triplicate experiments, λ_{ex} = 608 nm.

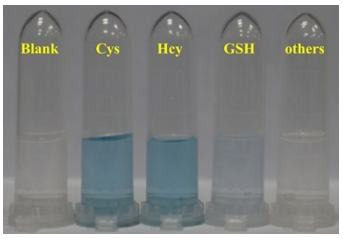


Fig. S6 The color change of **NFC** (20 μM) with various analytes (100 μM). Others are Gly, Ser, Ala, Pro, Trp, Tyr, His, Leu, Thr, Glu, Asp, Met, Lys, Phe, Arg, Ile, Val. The picture was obtained 10 min after addition of each analyte in PBS (10mM, pH 7.4) containing 30% DMF.

Probe **NFC** (15.0 mg, 0.026 mmol) was dissolved in DMF (1 mL), and Cys (20 mg, 0.13 mmol) was then added into the solution. After stirring 2 hours at room temperature, the mixture was diluted with water, and extracted by ethyl acetate. The organic layer was evaporated under reduced pressure, and the crude product was purified by flash column chromatography to afford the product.

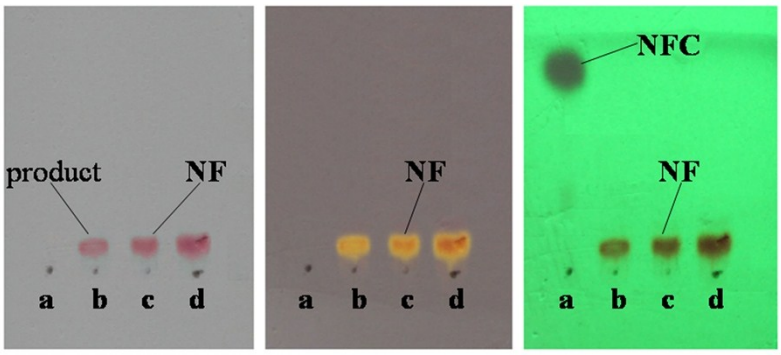


Fig. S7 The photos of the TLC plate under different light used to compare **NFC**, the reference sample of **NF** and the isolated reaction product of **NFC** with Cys. (A) under room light, (B) under light of 365 nm, (C) under light of 254 nm. Spots on the TLC plate are: a. **NFC**, b. the isolated reaction product of **NFC** and Cys, c. **NF**, d. mixture of the isolated reaction product and **NF**. The eluent for TLC: petroleum ether: ethyl acetate 1:1 (v/v).

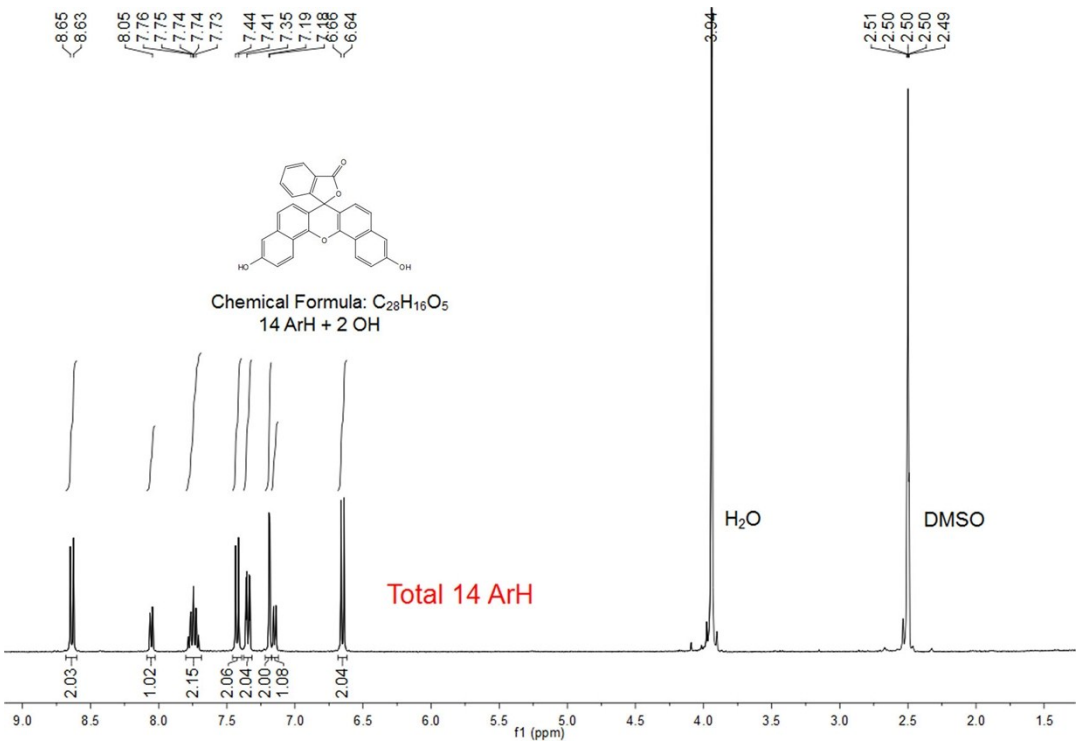


Fig. S8 ^{1}H NMR spectrum of the isolated fluorescent reaction product of **NFC** and Cys.

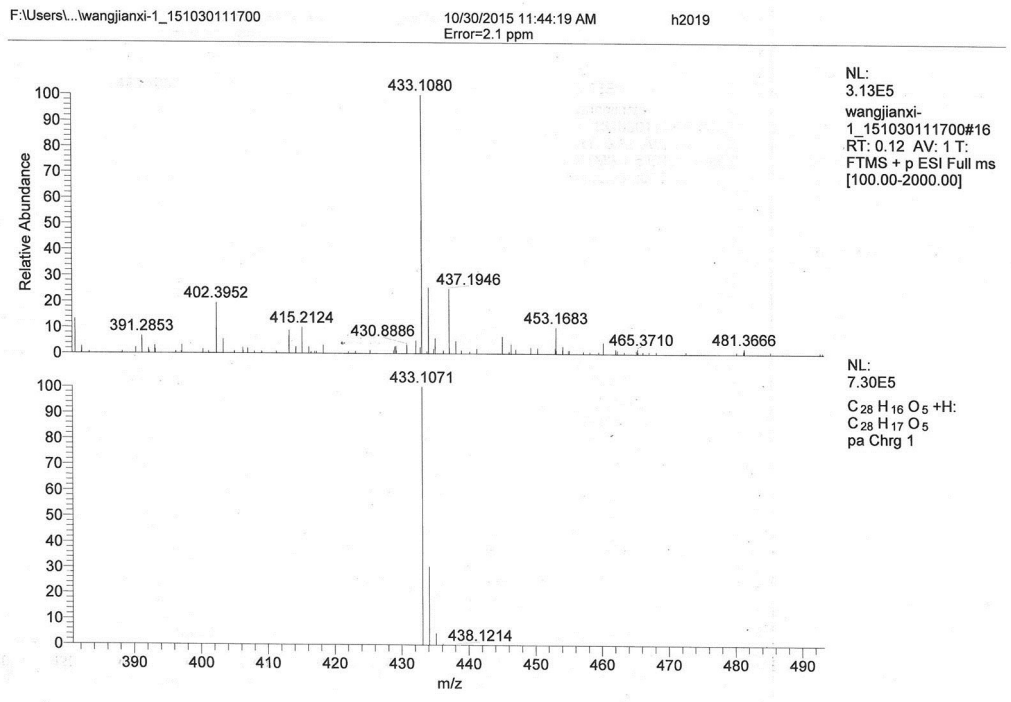


Fig. S9 HRMS spectrum of the isolated fluorescent reaction product.

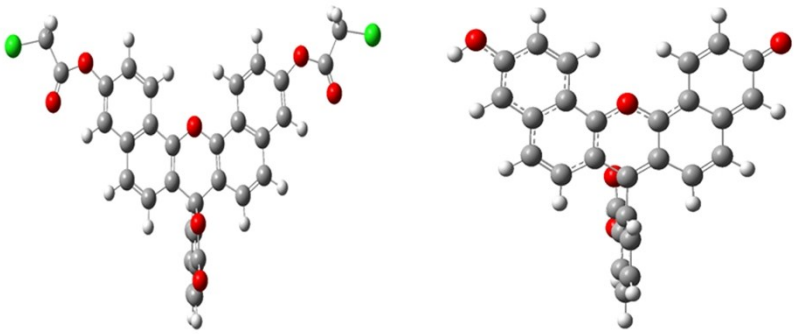


Fig. S10 The optimized conformation of **NFC** and **NF**. In the ball-and-stick model, carbon, oxygen, chlorine atoms are colored in gray, red and green, respectively.

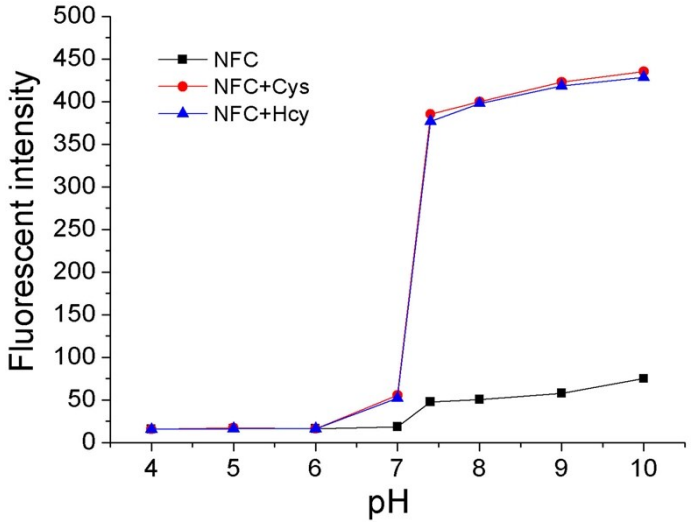


Fig. S11 Fluorescence intensity of **NFC** (20 μM) and **NFC** (20 μM) with Cys (100 μM) and Hcy (100 μM) in 10 min at various pH.

Human plasma (1 mL) was deproteinized using acetonitrile (3 mL) and centrifuging at 8,000 rpm for 30 min. The supernatant was diluted in PBS (pH 7.4, 10 mM). The total content of Cys and Hcy in the plasma sample was determined using the same procedure above and the standard calibration curve in Fig. S3A. Condition: PBS (10mM, pH 7.4) containing 30% DMF at 25 °C for 10 min, λ_{ex} = 608 nm.

Table S2 Determination of the total content of Cys and Hcy in human plasma

Analyte in plasmas	Added Cys (μM)	Found[a] (μM)	Recovery[a] (%)
Cys and Hcy	0	180.8 ± 4.5	–
	100	278.7 ± 6.7	98 ± 6.7
	200	376.3 ± 7.6	97 ± 3.8
	400	588.6 ± 9.4	102 ± 2.3

[a]Mean of three determinations ± standard deviation.

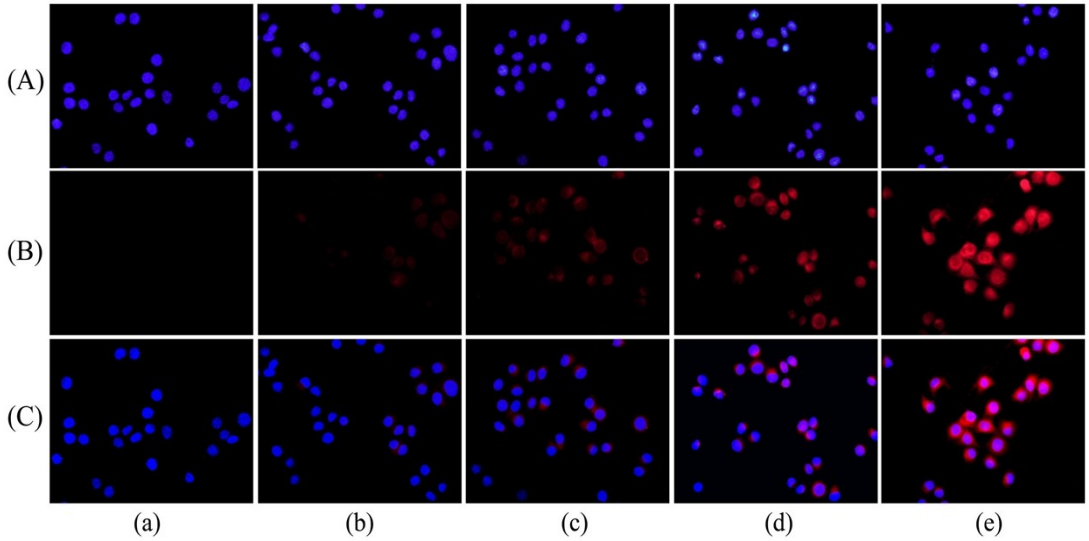


Fig. S12 Fluorescent images of HeLa cells with different concentration of **NFC** (a-e: 0, 5.0, 10.0, 20.0, 40.0 μM) at 40 min. (A) Fluorescence images for cells co-stained with 4', 6-diamidino-2-phenylindole (DAPI) to identify cell nuclei (blue dots); (B) Fluorescence images of cells treated with different concentration of **NFC**; (C) Merge.

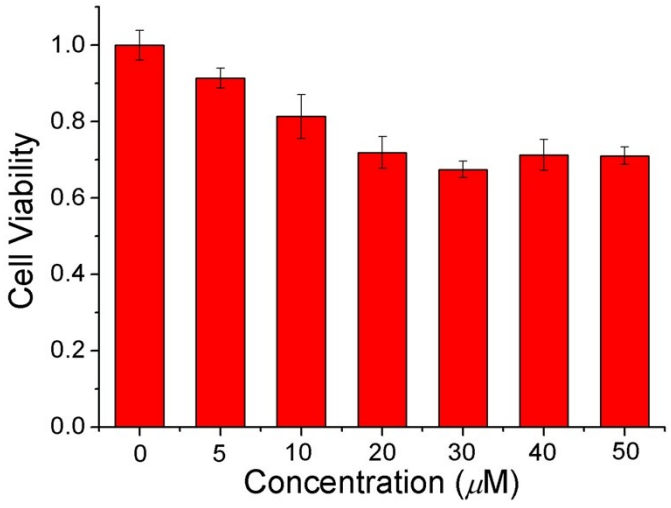


Fig. S13 Viable HeLa cells after treatment with various concentrations of **NFC** after 24 hours. The

cell viability was observed via MTT assay.

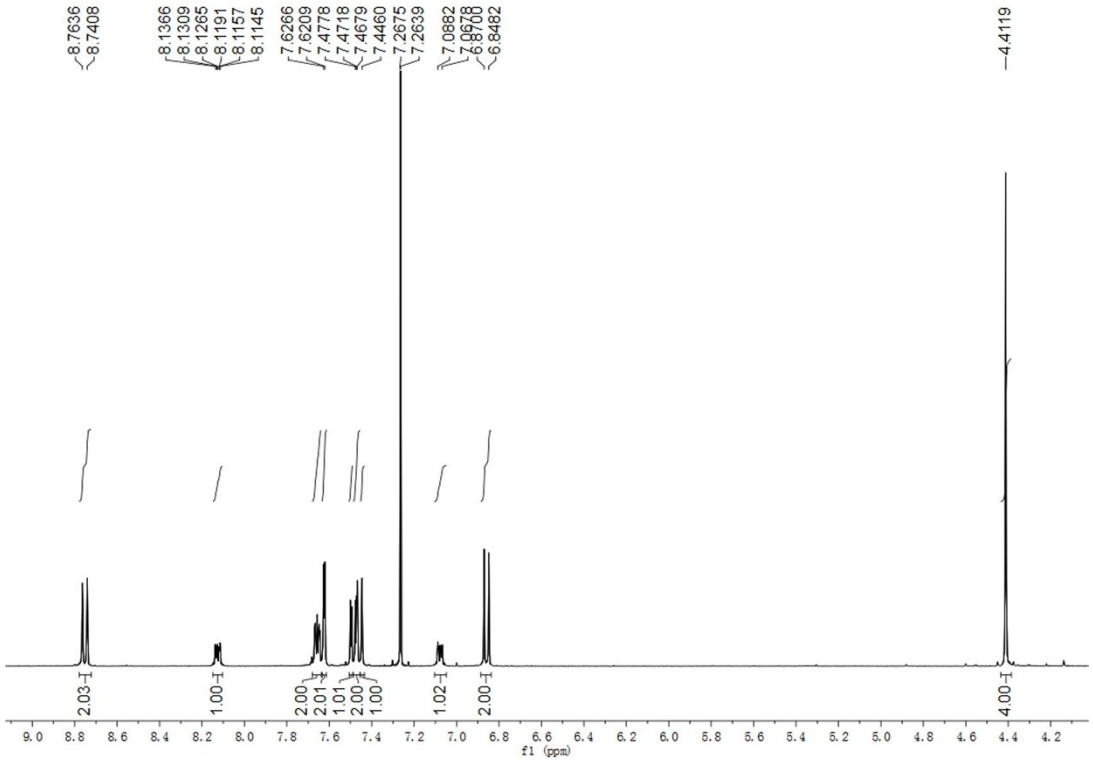


Fig. S14 ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **NFC**.

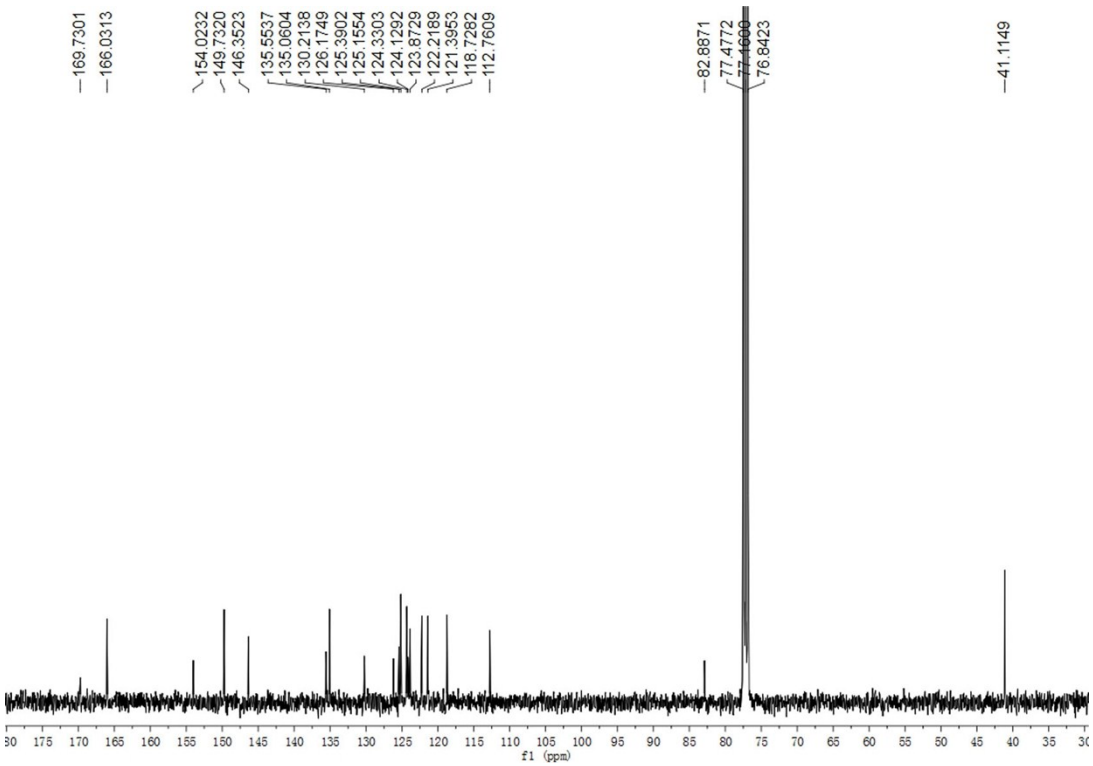


Fig. S15 ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of **NFC**.

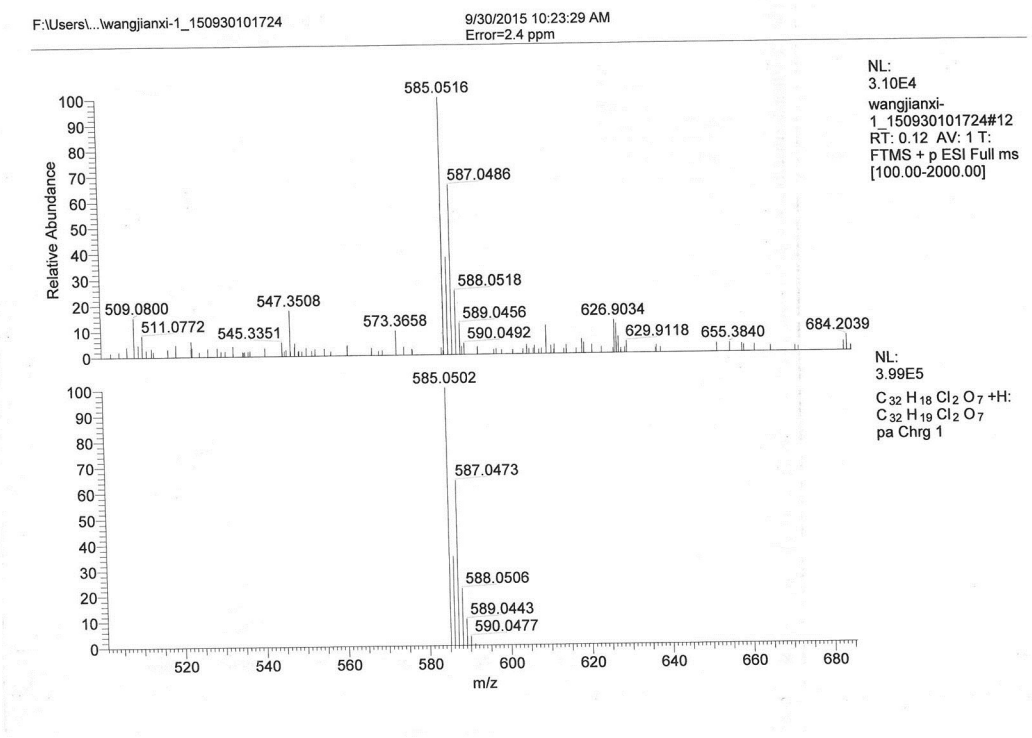


Fig. S16 HRMS spectrum of **NFC**.