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Supporting information for

Constructing a far-red to near-infrared fluorescent probe for highly specific detection of cysteine and its bioimaging application in living cells and zebrafish

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Materials and instruments. Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. Solvents used were purified by standard methods prior to use. Twice-distilled water was used throughout all experiments. Mass spectra were performed using an LCQ Advantage ion trap mass spectrometer from Thermo Finnigan or Agilent 1100 HPLC/MSD spectrometer. NMR spectra were recorded on an INOVA-400 spectrometer, using TMS as an internal standard. Electronic absorption spectra were obtained on a Labtech UV Power PC spectrometer. Photoluminescent spectra were recorded at room temperature with a HITACHI F4600 fluorescence spectrophotometer with the excitation and emission slit widths at 5.0 and 5.0 nm respectively. The fluorescence imaging of cells was performed with Zeiss LSM 780 confocal microscopy; The pH measurements were carried out on a Mettler-Toledo Delta 320 pH meter. TLC analysis was performed on silica gel plates and column chromatography was conducted over silica gel (mesh 200-300), both of which were obtained from the Qingdao Ocean Chemicals.

Determination of the fluorescence quantum yield:[1-3] Fluorescence quantum yields for **FR-NIR-Cys** were determined by using rhodamine 6G ($\Phi_{F(S)}$ = 0.9 in ethanol) as a fluorescence standard. The quantum yield was calculated using the following equation:

$$\Phi_{F(X)} = \Phi_{F(S)} (A_S F_X / A_X F_S) (n_X / n_S)^2$$

Where Φ_F is the fluorescence quantum yield, A is the absorbance at the excitation wavelength, F is the area under the corrected emission curve, and n is the refractive index of the solvents used. Subscripts S and X refer to the standard and to the unknown, respectively.

References

1. Valeur, B. Molecular Fluorescence: Principles and Applications, Wiley-VCH, 2001.

2. Magde, D.; Rojas, G. E.; Seybold, P. Photochem. Photobiol. 1999, 70, 737-744.

3. Oushiki, D.; Kojima, H.; Terai, T.; Arita, M.; Hanaoka, K.; Urano, Y.; Nagano, T. J. Am. Chem. Soc. 2010, 132, 2795-2801.

Kinetic Studies:

The reaction of the probe **FR-NIR-Cys** (5 μM) with Cys (100 μM) in pH 7.4, 10 mM PBS was monitored using the fluorescence intensity at 626 nm. The reaction was carried out at 25 ˚C. The *pseudo*-first-order rate constant for the reaction was determined by fitting the fluorescence intensities of the samples to the *pseudo* first-order equation:

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

Where F_t and F_{max} are the fluorescence intensities at 626 nm at time t and the maximum value obtained after the reaction was complete. k' is the *pseudo*-first-order rate constant.

A549 cell Culture and Imaging Using FR-NIR-Cys. A549 cells were seeded in Dulbecco's modified Eagle'smedium (DMEM) supplemented with 10% fetal bovine serum for 24 h. The cells were cultured in confocal glass dishes and then incubated at 37°C for 36 hours. The cells were divided into three groups, and then incubated with N-ethylmaleimide (as a thiol blocking agent), Cys, PBS, respectively, in the culture medium for 30 min at 37 °C. After washing with PBS three times to remove the remaining N-ethylmaleimide or Cys in culture medium, the cells were further incubated with **FR-NIR-Cys** (5 μM) for 30 min at 37 °C. After washing the cells with PBS three times, the cells were imaged using Carl Zeiss confocal microscope (LSM 710, Jena, Germany) with an excitation filter of 543 nm and 633 nm.

Cytotoxicity assays. The toxicity of **FR-NIR-Cys** towards living A549 cells was determined by MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide) assays. A549 cells were grown in the modified Eagle's medium (MEM) supplemented with 10% FBS (fetal bovine serum) in an atmosphere of 5% CO_2 and 95% air at 37 ºC. Immediately before the experiments, the cells were placed in a 96-well plate, followed by addition of increasing concentrations of probe **FR-NIR-Cys** (99% MEM and 1% DMSO). The final concentrations of **FR-NIR-Cys** was 5, 10, 15,

30 μM (n = 5), respectively. The cells were then incubated at 37 ºC in an atmosphere of 5% CO_2 and 95% air at 37 ºC for 24 h, followed by MTT assays. Untreated assay with MEM (n = 5) was also conducted under the same conditions.

Fluorescence imaging in living zebrafish.

Wild type zebrafish were purchased from the Nanjing EzeRinka Biotechnology Co. Ltd. Animal handing procedures were approved by the Animal Ethics Committee of Guangxi Normal University (No. 20150325-XC). The zebrafish were kept at 28 °C and optimal breeding conditions. For confocal microscope imaging experiments, 3-day-old zebrafish were divided into three groups, and then incubated with N-ethylmaleimide (as a thiol blocking agent), Cys, PBS, respectively, in the culture medium for 30 min, followed by washing away gently. After that, the zebrafish were transferred into another glass bottom dishes for confocal microscope imaging. The imaging experiments were recorded with a 10x objective lens using Zeiss LSM 780 confocal microscopy. The fluorescence emission was collected at green channel (575-675 nm) and red channel (675nm-775nm) when excitation at 543 nm or 633 nm, respectively.

Preparation of the test solution.

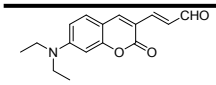
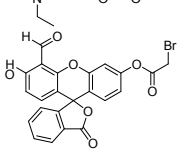
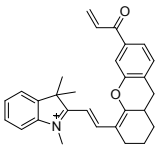
The stock solution of the **FR-NIR-Cys** probe was prepared at 0.5 mM in ethanol. The solutions of various testing species were prepared from $CaCl_2$, NaCl, S_8, $Na_2S_2O_3$, Na_2SO_3, Na_2SO_4, NaSH, Ala, Gly, Arg, Glu, Lys, Leu, Ser, Val, GSH, Hcy, Cys, twice-distilled water. The test solution of the **FR-NIR-Cys** probe (5 μM) in 3 mL aqueous buffer (pH 7.4, 10 mM PBS buffer with 1% ethanol) was prepared by placing 0.03 mL of the **FR-NIR-Cys** probe stock solution in 2.97 mL of 10 mM PBS buffer (pH=7.4). The test solution was shaken well at room

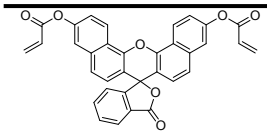
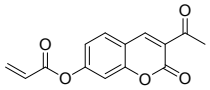
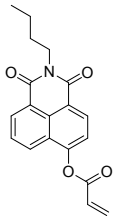
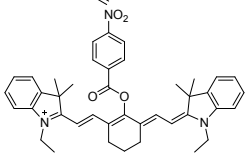
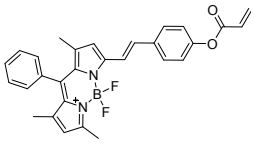
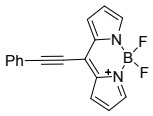
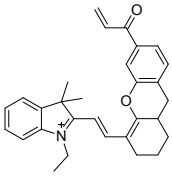
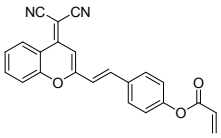
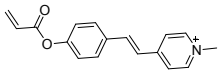
temperature before recording the spectra. Unless otherwise noted, for all measurements, the excitation wavelength was 550 nm and 630 nm, the excitation slit widths were 5 nm, and emission slit widths were 5 nm.

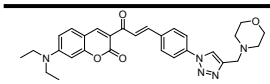
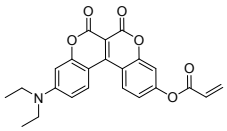
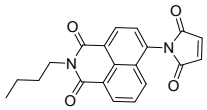
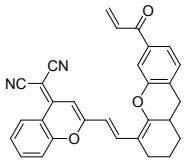
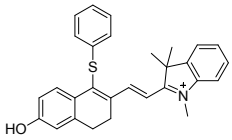
Synthesis of FR-NIR-Cys probe.

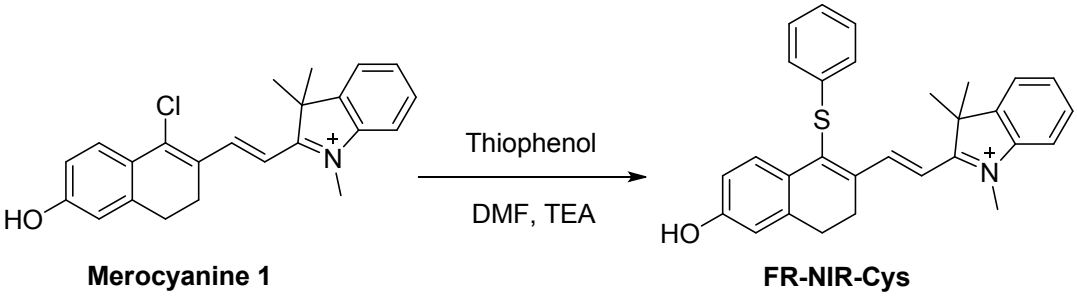
Compound merocyanine **1** (49.1 mg, 0.1 mmol) and thiophenol (33 mg, 0.3 mmol) were placed in a flask containing dry DMF (4.0 ml), and then two drops of TEA was added. After heating overnight at 90℃ under nitrogen, the solution was concentrated under reduced pressure. The resulting crude product was purified by silica gel flash chromatography using ethyl CH_2Cl_2/MeOH (35: 1) as eluent to give the probe **FR-NIR-Cys** as a red solid (29 mg, yield 51.0%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.96 (d, *J* = 15.5 Hz, 1H), 7.51 (d, *J* = 8.7 Hz, 1H), 7.43-7.39 (m, 3H), 7.20 (s, 1H), 7.15-7.00 (m, 6H), 6.88 (s, 1H), 6.65 (d, *J* = 6.9 Hz, 1H), 4.17 (s, 3H), 2.64-2.68 (m, 4H), 1.64 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.4, 161.2, 151.2, 148.9, 141.9, 141.4, 140.9, 138.0, 134.9, 131.5, 128.6, 128.0, 127.4, 125.8, 124.0, 121.6, 114.8, 114.5, 113.1, 110.2, 50.8, 34.3, 28.9, 26.9, 26.4, 24.8. MS (ESI) m/z =438.1 $[M]^+$; HRMS (ESI) Calcd for $C_{29}H_{28}NOS^+$ ($[M]^+$): 438.1886, Found, 438.1880.

Table S1. Comparison of some fluorescent probes for selective detection of cysteine.

probes	Detection limit	Living cell Imaging	Living Animals Imaging	Selectivity	Reference
	0.75μM	exogenous	no	specific	Chem.Commun. 2011, 47, 6275-6277
	0.51μM	endogenous	no	selective	Sensors and Actuators B, 2015,209, 652-657
	0.16μM	exogenous	no	Highly Selective	Anal.Chem. 2015, 87, 9, 4856-63

	0.18μM	exogenous	no	selective	Biosensors and Bioelectronics, 2015, 68,316-321
	0.66μM	exogenous	no	selective	Biosensors and Bioelectronics,2014, 59, 35-39
	0.08μM	endogenous	no	selective	Biosensors and Bioelectronics, 2014, 55, 72-75
	0.20μM	endogenous	yes	selective	Biosensors and Bioelectronics, 2015,74,156-164
	15nM	endogenous	no	specific	Chem. Commun., 2015, 51, 16932-16935
	0.38nM	endogenous	no	Highly selective	Anal.Chem. 2015, 87, 11475-11483
	14.5nM	endogenous	yes	selective	ACS Appl. Mater. Interfaces, 2015, 7, 27968-27975
	0.2 μM	endogenous	no	selective	ACS Sens., 2016, 1, 882-887
	No data	endogenous	no	Highly Selective	Anal.Chem., 2016, 88 , 1908-1914

	1.2 μM	endogenous	yes	Specific	Anal.Chem., 2018, 90 , 7018-7024
	0.4 μM	endogenous	no	Specific	Anal.Chem., 2018, 90, 6138-6143
	2.0 nM	endogenous	no	Specific	Chem. Eur. J. 2016, 22, 9247-9256
	48. nM	endogenous	yes	selective	Anal. Chem., 2018, 90,1014-1020
	0.15μM	endogenous	yes	Highly Specific	This work



Scheme S1. Synthesis of the probe **FR-NIR-Cys**.

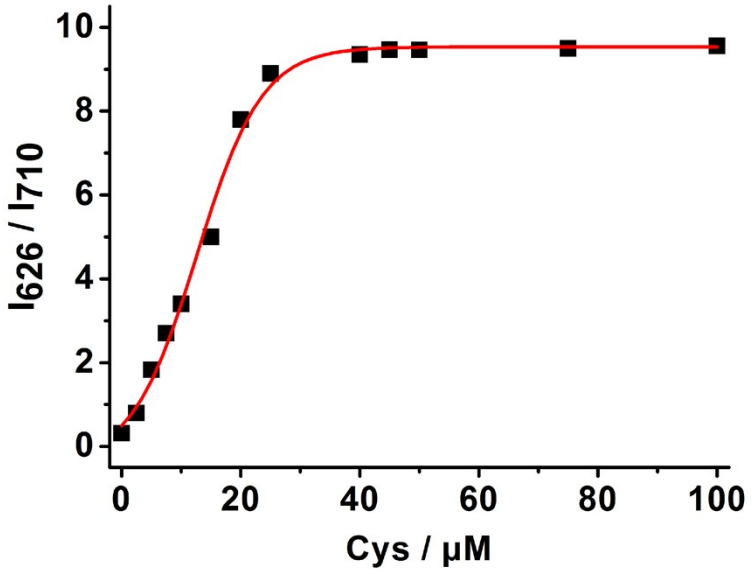


Figure S1. Fluorescence intensity ratio $F_{626\ nm}/F_{710\ nm}$ changes of the probe (5 μM) with the amount of Cys.

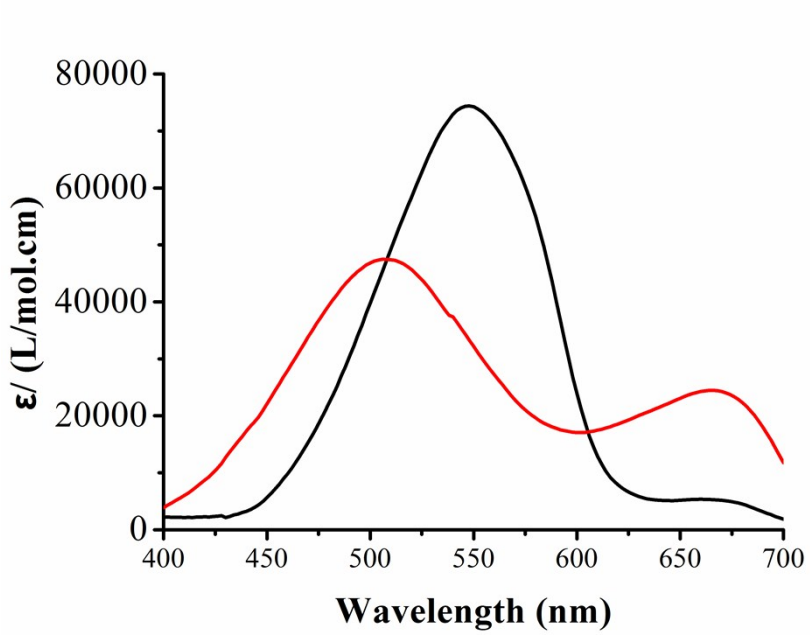


Figure S2. Molar extinction coefficient spectra of the far-red to near-infrared probe **FR-NIR-Cys** (5 μM) in the absence (red line) and presence (black line) of Cys (25 μM) in PBS buffer (pH 7.4, 10 mM, containing 1% EtOH as a cosolvent).

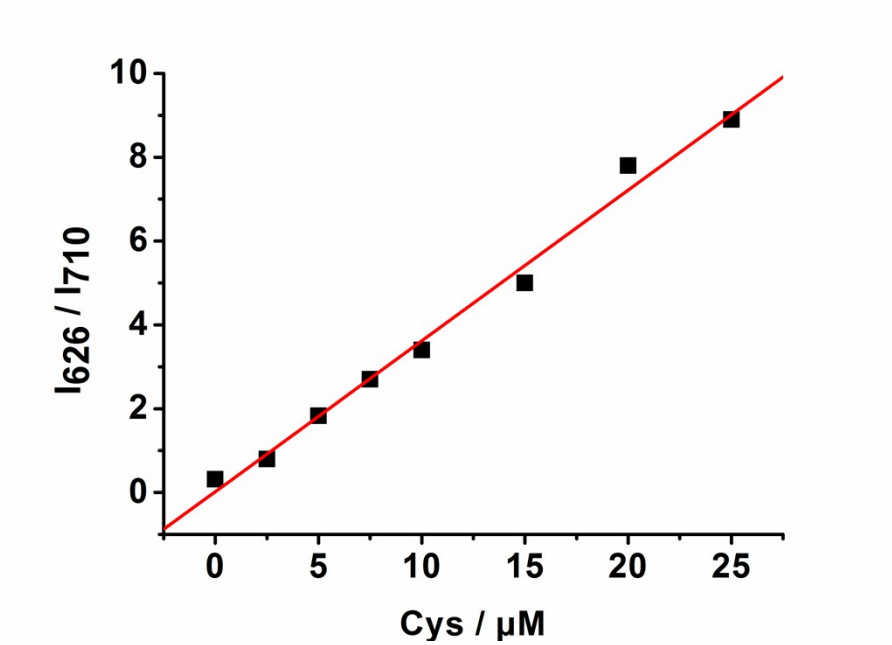


Figure S3. Linear fitting between I_{626}/I_{710} (**FR-NIR-Cys** 5μM, excitation at 550 nm) and the concentration of Cys in PBS buffer (pH 7.4, 10 mM, containing 1% EtOH as a cosolvent).

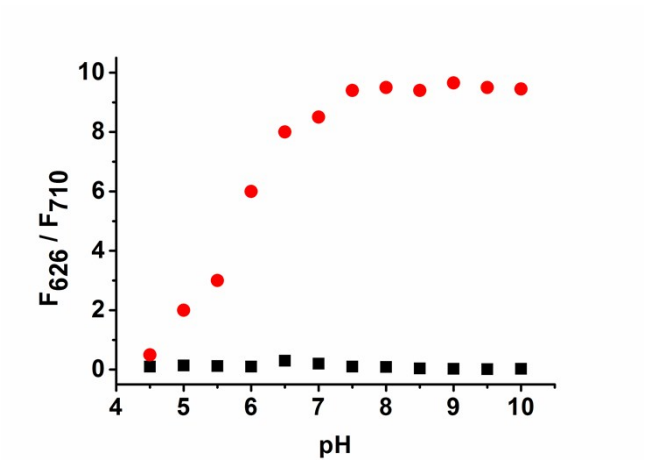


Figure S4. The pH effects of **FR-NIR-Cys** in the absence (■) or presence (●) of Cys.

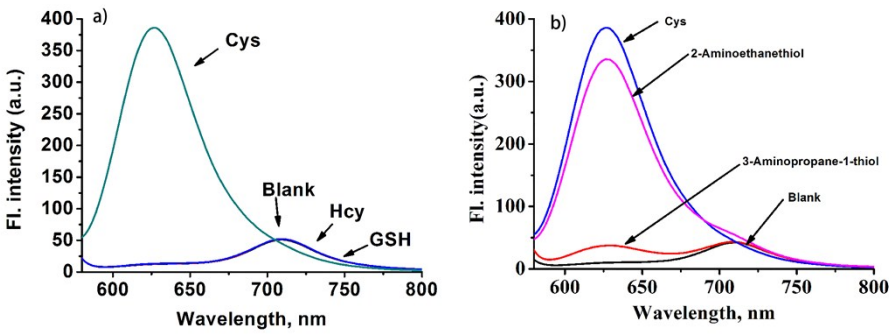
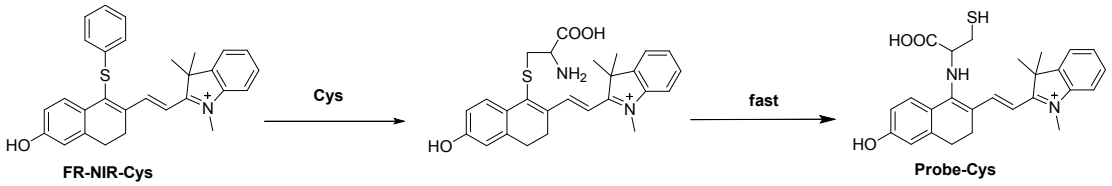


Figure S5. The comparison fluorescence response of probe **FR-NIR-Cys** with Cys, Hcy, GSH, 2-Aminoethanethiol,3-Aminopropane-1-thiol.



Scheme S2. The proposed sensing mechanism of the probe **FR-NIR-Cys** for Cys.

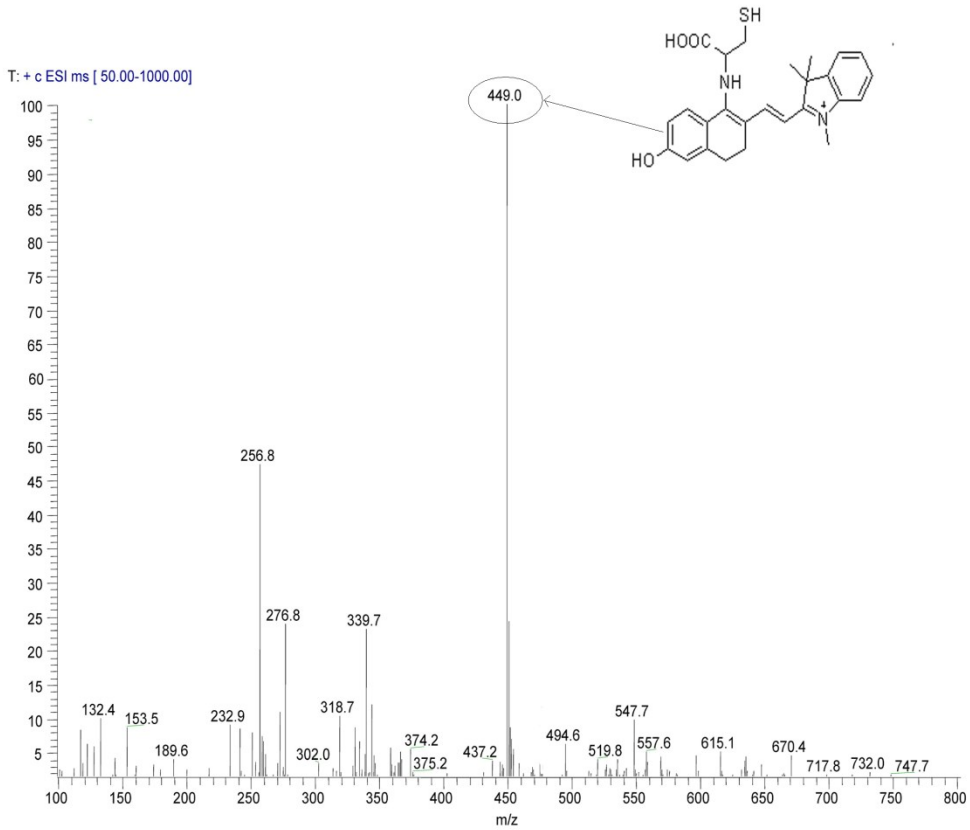


Figure S6. Mass spectrum (ESI) of the reaction mixture of the probe **FR-NIR-Cys** reacted with Cys.

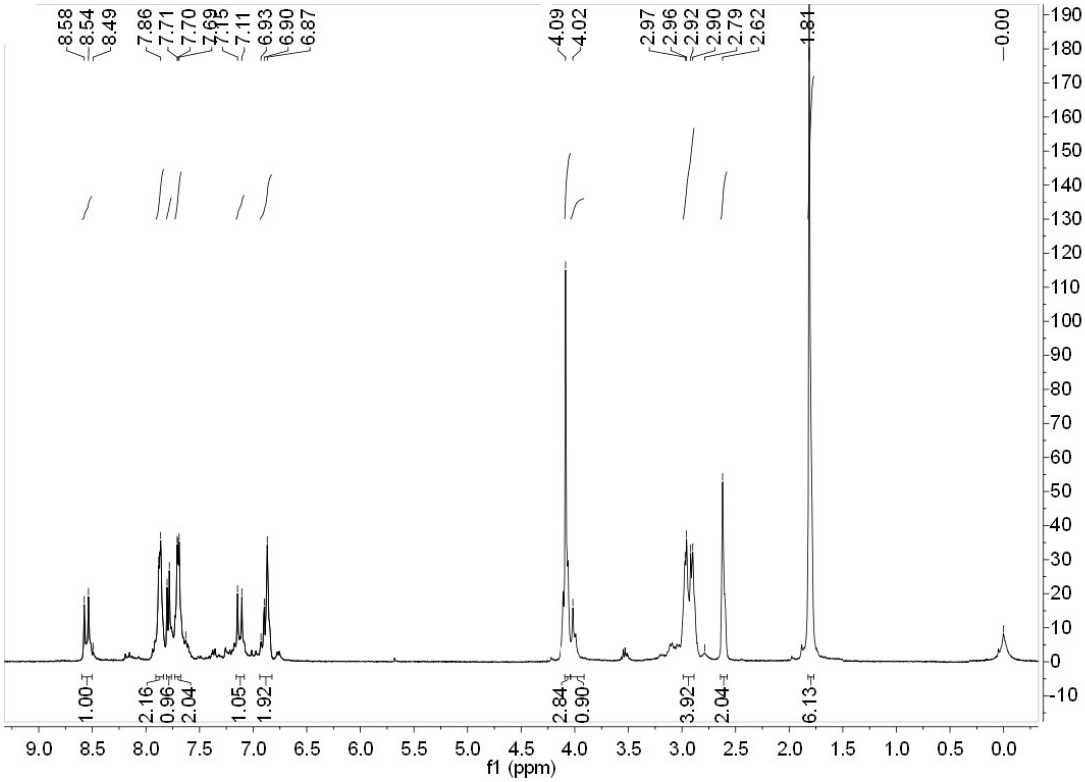


Figure. S7. ^{1}H NMR spectrum of the isolated product of the probe **FR-NIR-Cys** reacted with Cys.

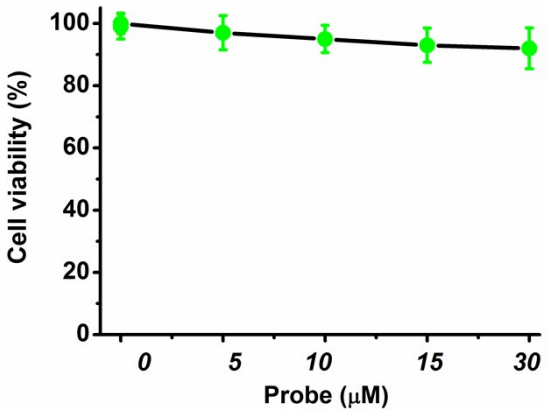


Figure S8. Cytotoxicity of the probe **FR-NIR-Cys** on A549 cells determined by MTT.

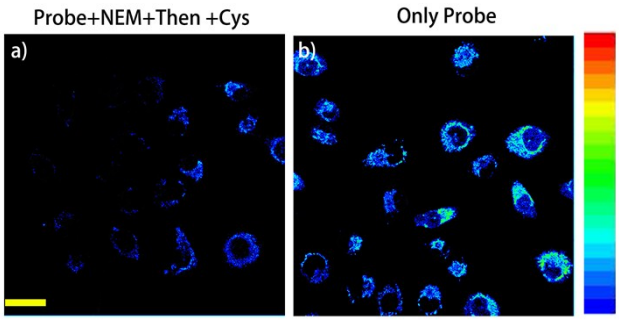


Figure S9. (a) Pseudocolored images (F_{green} / F_{red}) of the A549 cells pre-incubated with N-ethylmaleimide (1 mM) for 30 min, and then Cys and **FR-NIR-Cys** for 30 min; (b) Pseudocolored images (F_{green} / F_{red}) of the A549 cells stained only with **FR-NIR-Cys**. Scale bar =20 μm.

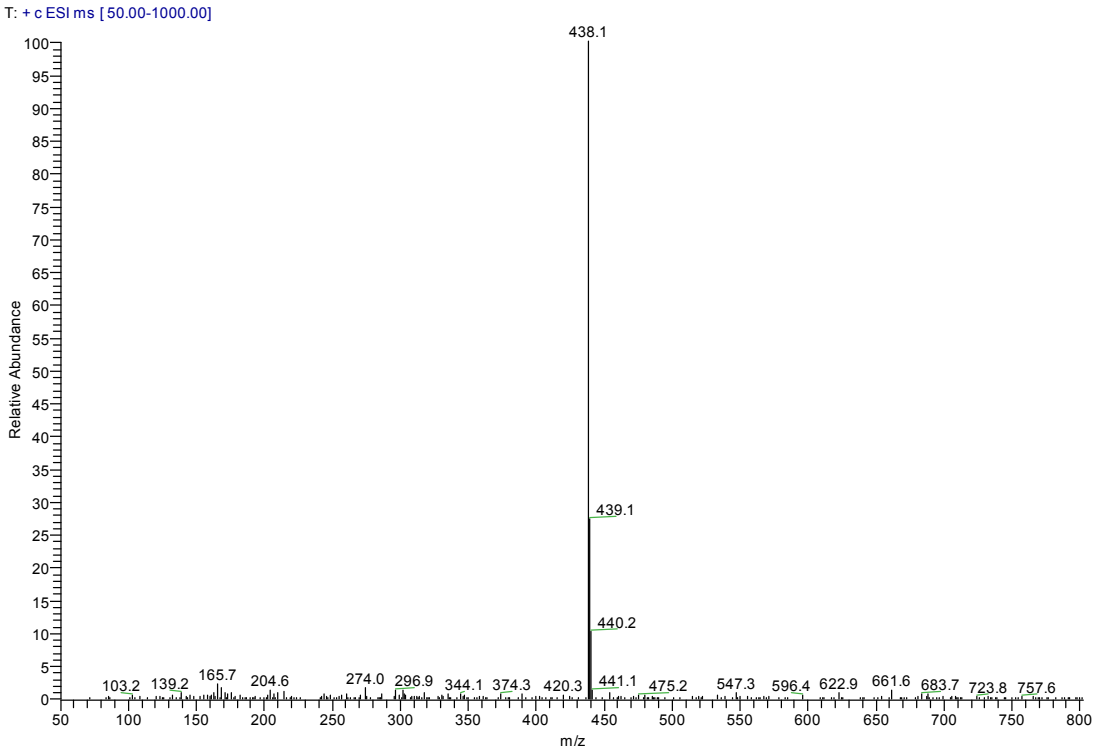


Figure S10. The MS of **FR-NIR-Cys.**

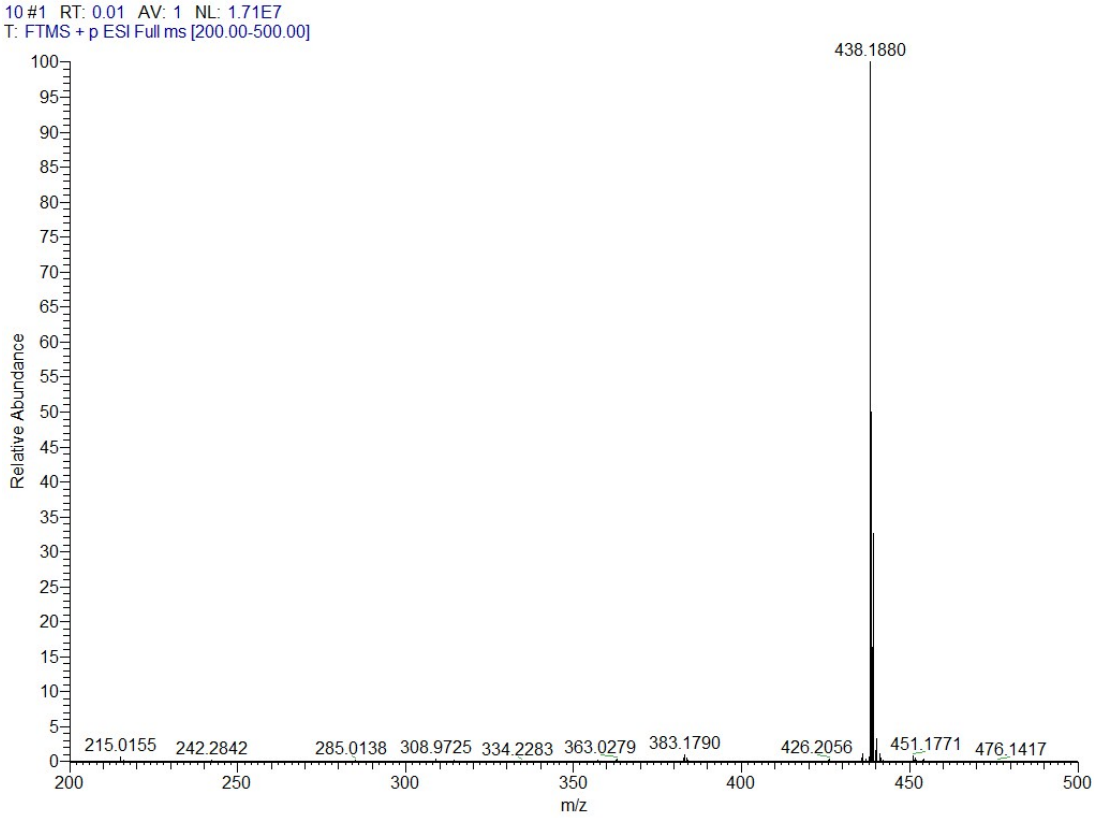


Figure S11. The HRMS of **FR-NIR-Cys.**

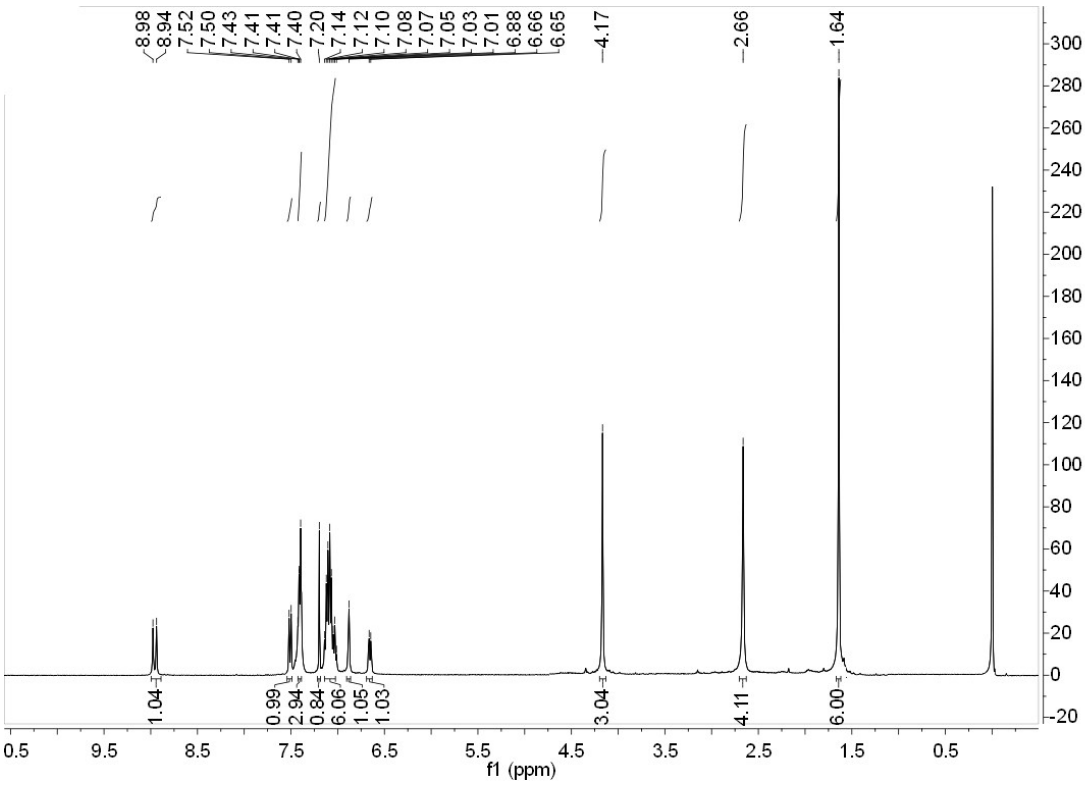


Figure S12. The ^{1}H NMR of **FR-NIR-Cys.**

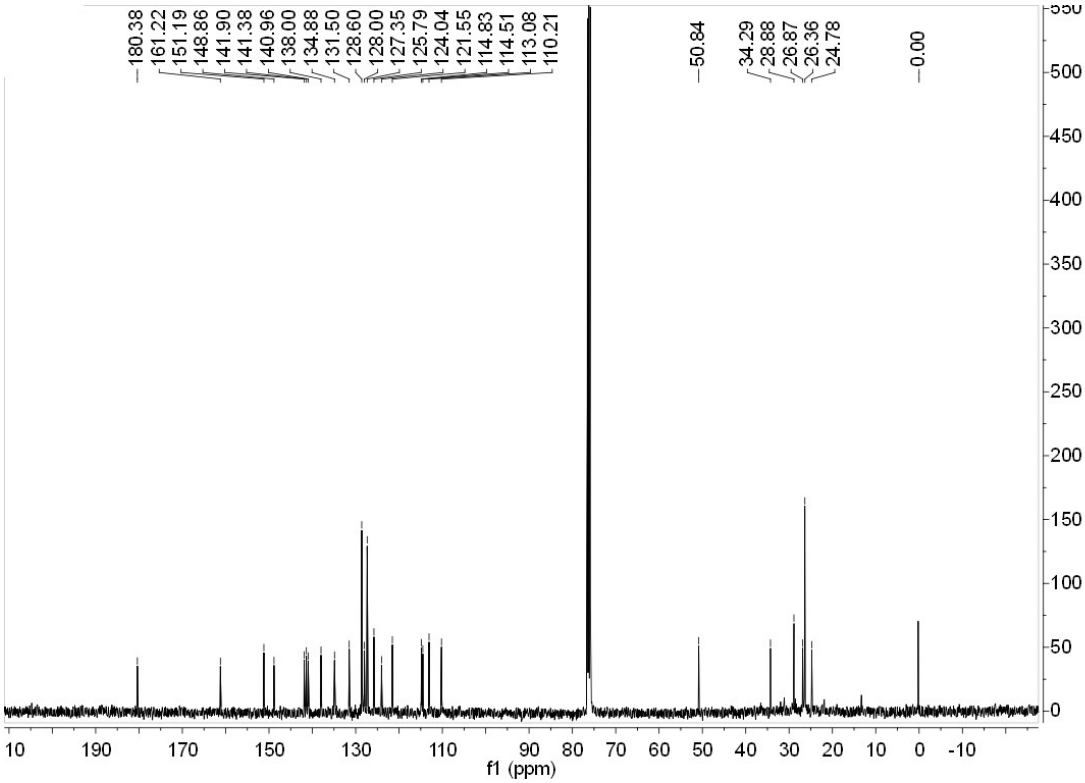


Figure S13. The ^{13}C NMR of **FR-NIR-Cys.**