Electronic Supplementary Material (ESI) for RSC Advances.

A Highly Selective Quinolizinium-based Fluorescent Probe for Cysteine Detection

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Supporting Information

General Procedure

All reagents were commercially available and used without further purification. Milli-Q® water used as solvent for preparation of stock solutions of analytes was deionized using a Milli-Q® Gradient A10 system (Millipore, Billerica, USA). Flash column chromatography was performed using silica gel 60 (230-400 mesh ASTM) with ethyl acetate/n-hexane or methanol/dichloromethane as eluent. ^{1}H and ^{13}C spectra were recorded on a Bruker DPX-400 or DPX-600 spectrometer. All chemical shifts are quoted on the scale in ppm using TMS or residual solvent as the internal standard. Coupling constants (J) are reported in Hertz (Hz) with the following splitting abbreviations: s = singlet, br s = broad singlet, d = doublet, dd = double doublet, t = triplet and m = multiplet. The absorption and emission spectra were obtained by the Agilent Cary 8454 UV-Visible spectrophotometer and Cary Eclipse Fluorescence Spectrophotometer respectively. High resolution mass spectra were obtained on an Agilent 6540 UHD Accurate-Mass Q-TOF LC/MS system equipped with an ion spray source in the positive ion mode.

Literature Reference

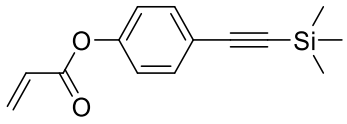
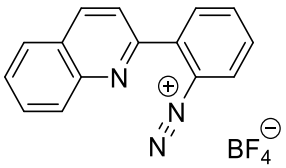
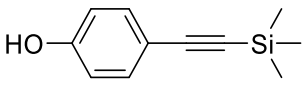
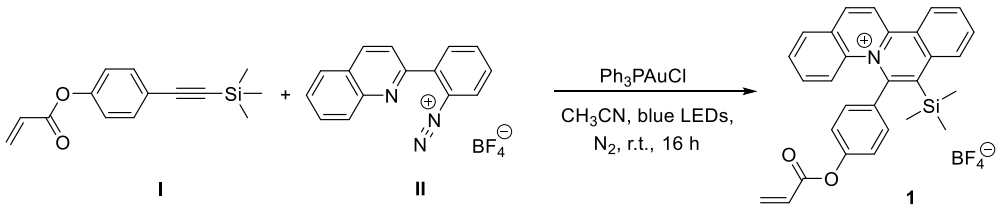
 **I**	X. Qin, Y. Si, Z. Wu, K. Zhang, J. Li, Y. Yin, *Anal. Chem.*, 2020, **92**, 924–931.
 **II**	J.-R. Deng, W.-C. Chan, N. C.-H. Lai, B. Yang, C.-S. Tsang, B. C.-B. Ko, S. L.-F. Chan, M.-K. Wong, *Chem. Sci.*, 2017, **8**, 7537–7544.
 **III**	J. Xie, S. Wang, P. Ma, F. Ma, J. Li, W. Wang, F. Lu, H. Xiong, Y. Gu, S. Zhang, H. Xu, G. Yang, R. A. Lerner, *iScience*, 2020, **23**, 101197.

Table S1. Examples of Fluorescent Probes for Cysteine Detection Reported by Literatures

		Mechanism	Scaffold	Response Time	Stoke shift (nm)	Detection Limit	Dynamic range
1.	*Biosens. Bioelectron.*, 2014, **59**, 35–39.	Turn-on	Coumarin	40 min	36	0.657 μM	0–40 μM
2.	*Sens. Actuators B Chem.*, 2017, **248**, 332–337.	Turn-on (PET)	Naphthol AS	50 min	130	0.5 μM	1–200 μM
3.	*Spectrochim. Acta A Mol. Biomol. Spectrosc.*, 2017, **173**, 918–923.	Turn-off (PET)	BODIPY	120 min	25	37 nM	0–100 μM
4.	*Ind. Eng. Chem. Res.*, 2017, **56**, 7650−7655.	Turn-off	Curcumin	10 min	82	0.19 μM	0–20 μM
5.	*Dyes Pigm.*, 2017, **139**, 381-387.	Turn-on (PET)	BODIPY	< 10 min	37	0.05 μM	0.2–30 μM
6.	*Anal. Methods*, 2017, **9**, 1891–1896.	Turn-on	Rhodamine	40 min	58	76 nM	0–30 μM
7.	*Spectrochim. Acta A Mol. Biomol. Spectrosc.*, 2018, **192**, 67–74.	Turn-on (PET)	1,8-naphthalimide	8 min	115	69 nM	0.15–10 μM
8.	*Dyes Pigm.*, 2019, **166**, 266–271.	Turn-on	Curcumin	80 min	95	1.55 μM	0–80 μM
9.	*Sens. Actuators B Chem.*, 2020, **304**, 127431.	Turn-on	Fluorescein	40 min	50	0.307 μM	40–200 μM
10.	***This work***	***Turn-off (PET)***	***Quinolizinium***	***100 min***	***75***	***0.18 μM***	***0–100 μM***

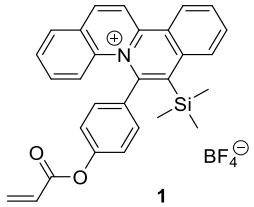
Procedure for the Synthesis of 1



Synthesis of 1

A mixture of **I** (1 equiv.), **II** (1.2 equiv.), Ph_3PAuCl (10 mol%) and 5 mL of CH_3CN was added into a 20 mL test tube. The test tube capped with a rubber septum was evacuated and refilled with nitrogen three times. After that, the tube containing the reaction mixture was irradiated with Blue LEDs for 16 h. After the reaction completed, the mixture was concentrated under reduced pressure. The resulting residue was purified by flash chromatography on silica gel using CH_2Cl_2/CH_3OH (19:1) as eluent to give the desired product.

Characterization Data of 1



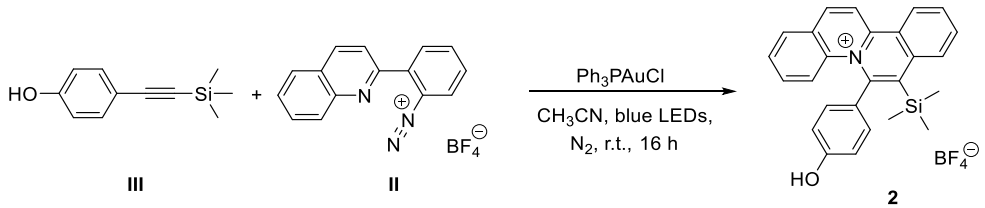
Yellow solid, 31% yield.

^{1}H NMR (400 MHz, CD_3CN) δ 8.97 (d, J = 8.9 Hz, 1H), 8.87 (dd, J = 12.7, 8.6 Hz, 2H), 8.41 (d, J = 7.5 Hz, 1H), 8.22 (d, J = 7.9 Hz, 1H), 8.15 (t, J = 7.0 Hz, 1H), 8.03 (t, J = 8.4 Hz, 1H), 7.71 (t, J = 7.1 Hz, 1H), 7.60 (d, J = 9.0 Hz, 1H), 7.51 (d, J = 8.6 Hz, 2H), 7.38 (d, J = 8.9 Hz, 1H), 7.28 (d, J = 8.8 Hz, 2H), 6.60 (dd, J = 17.3, 1.2 Hz, 1H), 6.40 (dd, J = 17.3, 10.4 Hz, 1H), 6.12 (dd, J = 10.4, 1.2 Hz, 1H), 0.10 (s, 9H).

^{13}C NMR (100 MHz, CD_3CN) δ 165.06, 153.46, 150.41, 146.75, 142.53, 138.02, 137.49, 137.28, 135.26, 134.23, 134.02, 131.18, 130.52, 130.18, 129.96, 128.42, 127.70, 126.43, 126.08, 123.92, 119.57.

HRMS (ESI): $[M\text{-}BF_4]^+$ Calcd. for $[C_{29}H_{26}NO_2Si]^+$ 448.1727, found 448.1762.

Procedure for the Synthesis of 2

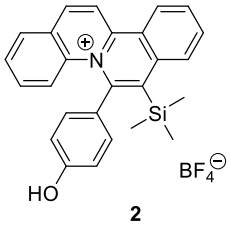


III II 2

Synthesis of 2

A mixture of **III** (1 equiv.), **II** (1.2 equiv.), Ph_3PAuCl (10 mol%) and 5 mL of CH_3CN was added into a 20 mL test tube. The test tube capped with a rubber septum was evacuated and refilled with nitrogen three times. After that, the tube containing the reaction mixture was irradiated with Blue LEDs for 16 h. After the reaction completed, the mixture was concentrated under reduced pressure. The resulting residue was purified by flash chromatography on silica gel using CH_2Cl_2/CH_3OH (19:1) as eluent to give the desired product.

Characterization Data of 2



2

Yellow solid, 30% yield.

^{1}H NMR (400 MHz, CD_2Cl_2) δ 8.86 (d, *J* = 9.0 Hz, 1H), 8.81 – 8.74 (m, 2H), 8.32 (d, *J* = 7.4 Hz, 1H), 8.18 – 8.08 (m, 2H), 8.01 (ddd, *J* = 8.4, 7.1, 1.2 Hz, 1H), 7.72 (ddd, *J* = 8.0, 7.0, 0.9 Hz, 1H), 7.65 (d, *J* = 9.0 Hz, 1H), 7.43 (ddd, *J* = 8.8, 7.1, 1.6 Hz, 1H), 7.36 (s, 1H), 7.19 (d, *J* = 8.6 Hz, 2H), 7.03 (d, *J* = 8.7 Hz, 2H), 0.15 (s, 9H).

^{13}C NMR (100 MHz, CD_2Cl_2) δ 160.03, 149.16, 147.35, 142.01, 137.88, 137.22, 134.98, 134.84, 133.38, 130.77, 130.66, 130.14, 129.84, 129.51, 129.04, 126.78, 125.23, 118.41, 117.42, 2.23.

HRMS (ESI): $[M\text{-}BF_4]^+$ Calcd. for $[C_{26}H_{24}NOSi]^+$ 394.1622, found 394.1633.

Measurements of Photophysical Properties of 1

The fluorescence quantum yield of **1** was determined by a relative method employing Coumarin 153 (Φ = 0.53 in ethanol) as standard and calculated with the following equation:

$$\Phi_{sample} = \Phi_{standard} \times \frac{F_{sample}}{F_{standard}} \times \frac{Abs_{sample}}{Abs_{standard}} \times (\frac{n_{sample}}{n_{standard}})^2$$

Absorption maximum λ_{abs} (ε) : 420 nm (4.64×10^3 dm^3mol^{-1} cm^{-1})
Emission maximum λ_{em} : 495 nm
Stokes shift : 75 nm
Quantum yield : 0.43

UV-Vis Absorption and Fluorescence Measurements of 1 upon Addition of Various Analytes

The stock solution of **1** (1 mM) was prepared in CH_3CN. Stock solutions of Cys, Hcy, GSH, alanine (Ala), arginine (Arg), asparagine (Asn), aspartic acid (Asp), glutamine (Gln), glutamic acid (Glu), glycine (Gly), histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), proline (Pro), threonine (Thr), tryptophan (Try), tyrosine (Tyr), serine (Ser) and valine (Val) (100 mM) were prepared in Milli-Q® water.

80 μL of **1** (1 mM in CH_3CN) and 16 μL of different analytes (100 mM in H_2O), 1920 μL of CH_3CN, 984 μL of H_2O and 1000 μL of PBS (50 mM, pH 7.4) was mixed in a 4 mL glass bottle. The reaction mixture was kept at 25 °C for 100 min. 1 mL of the reaction mixture was mixed with 3 mL of PBS for measurement of UV-Vis absorption and fluorescence signals. For control experiment, analyte was replaced by H_2O.

NMR Analysis of Crude Reaction Mixture of 1 with Cys

1000 μL of **1** (1 mM in CH_3CN) and 30 μL of L-cysteine (100 mM in H_2O), 470 μL of H_2O and 500 μL of PBS (50 mM, pH 7.4) was mixed in a 4 mL glass bottle. The reaction mixture was kept at 25 °C for 100 min. After the reaction, the solvent was removed by rotary evaporation to give residue which was dissolve in CD_3CN for NMR analysis.

Determination of Cys in Mouse Serum Samples

The serum samples were obtained from C57BL/6 mouse (source from The Chinese University of Hong Kong). Whole blood collected was allowed to clot by leaving it undisturbed for an hour at room temperature. The clotted blood was centrifuged at 1000 g for 10 min to remove the clot. Sera were separated and stored at -80 °C prior to the assay.

Mouse serum samples were diluted 1000-fold with PBS and Cys at different concentrations from 20 to 40 μM were added to the samples, respectively. 10 μL of **1** (1 mM in CH_3CN) and 490 μL of mouse serum spiked with different concentrations of Cys was mixed in a 1.5-mL Eppendorf tube at 25 °C for 100 min. After the reaction, the fluorescence signals of samples were measured.

For the linear curve for the detection of Cys in spiked mouse serum samples, please refer to Figure S8 on Page S10.

MS Analysis of Crude Reaction Mixture of 1 with Biothiols

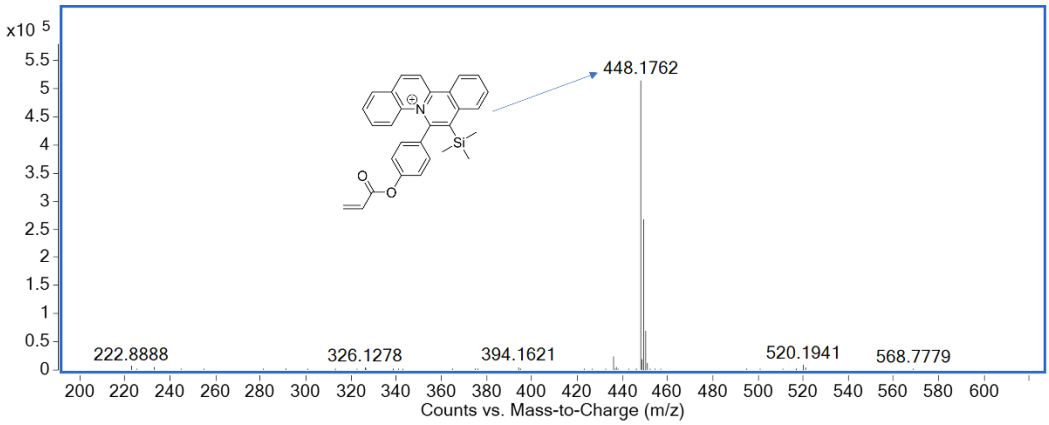


Figure S1 Mass spectrum of reaction mixture of control experiment

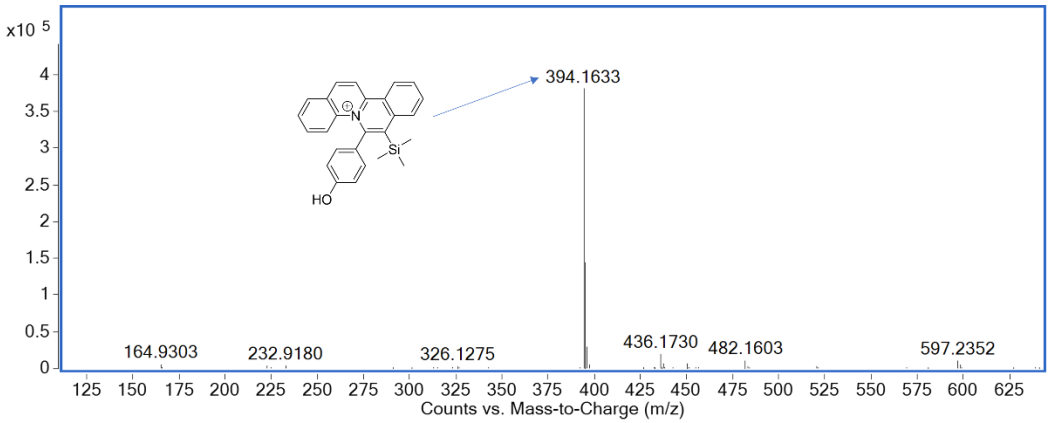


Figure S2 Mass spectrum of reaction mixture of **1** with Cys.

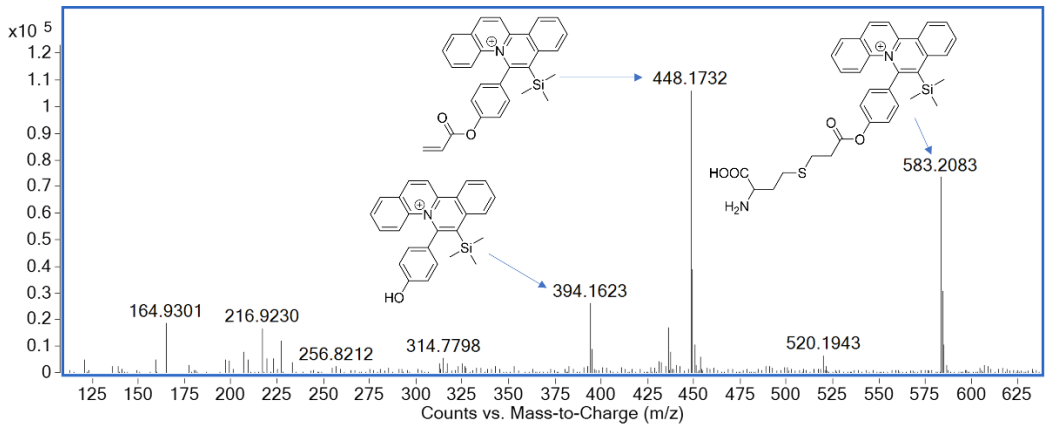


Figure S3 Mass spectrum of reaction mixture of **1** with Hcy.

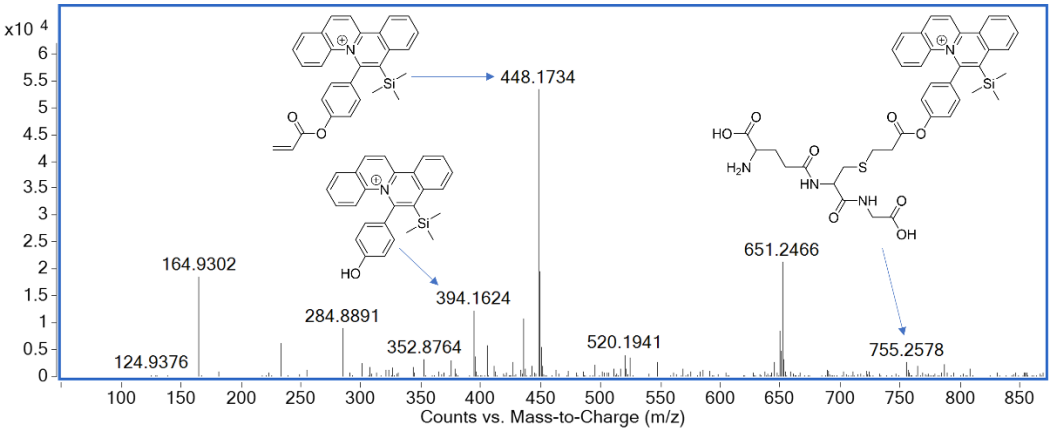


Figure S4 Mass spectrum of reaction mixture of **1** with GSH.

Cell Culture

HeLa cell lines (American Type Culture Collection) were cultured with Dulbecco's Modified Eagle's Medium (DMEM) (Gibco) supplemented with 44 mM sodium bicarbonate (Sigma-Aldrich), 10% v/v Fetal Bovine Serum (Gibco), and 100 U/mL penicillin (Gibco), 100 µg/mL streptomycin (Gibco) at 37 °C with 5% CO_2.

Cytotoxicity Assay

HeLa cells, 5 x 10^3 cells were seeded in 96 well plate one day before treatment. Cells were incubated with 0.4 µM to 100 µM of **1** for 72 h at 37 °C with 5% CO_2. After incubation, medium was removed and replaced with fresh medium. 10 µL MTS mixture (0.92mg/ mL PMS, Sigma-Aldrich: 2 mg/ mL MTS, Promega in 1:20) was added to each well. The plates were incubated at 37 °C with 5% CO_2 for 1 h. Absorbance at 490 nm was measured by EnSpire Multimode Plate Reader (PerkinElmer). IC_{50} value was calculated by GraphPad Prism.

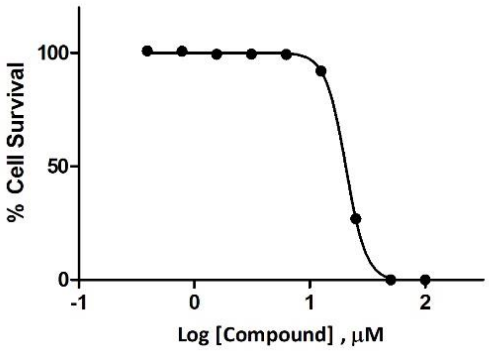


Figure S5 IC_{50} graph of HeLa cells incubated with **1**

Cellular Localization Study

HeLa cells were seeded on 35 mm glass bottom dishes (SPL) and allowed to adhere overnight in a 37 °C and 5% CO_2 cell culture incubator. Cells were incubated with dye alone or together with 50 nM MitoTracker™ Red FM (Invitrogen™) for 2 h. Images were examined under confocal microscope (Leica TCS SP8 MP).

Imaging of 1 in Live Cells

HeLa cells were seeded in confocal dishes one day before treatment. Cells were incubated with 100 μM L-cysteine or PBS for 30 min and then incubated with **1** for 2 h. Images were examined under confocal microscope (Leica TCS SP8 MP) with the excitation wavelength at 405 nm and the emission wavelength at 450-550 nm. Relative fluorescence was measured by ImageJ.

Probe **1** MitoTracker™ Red FM Merged

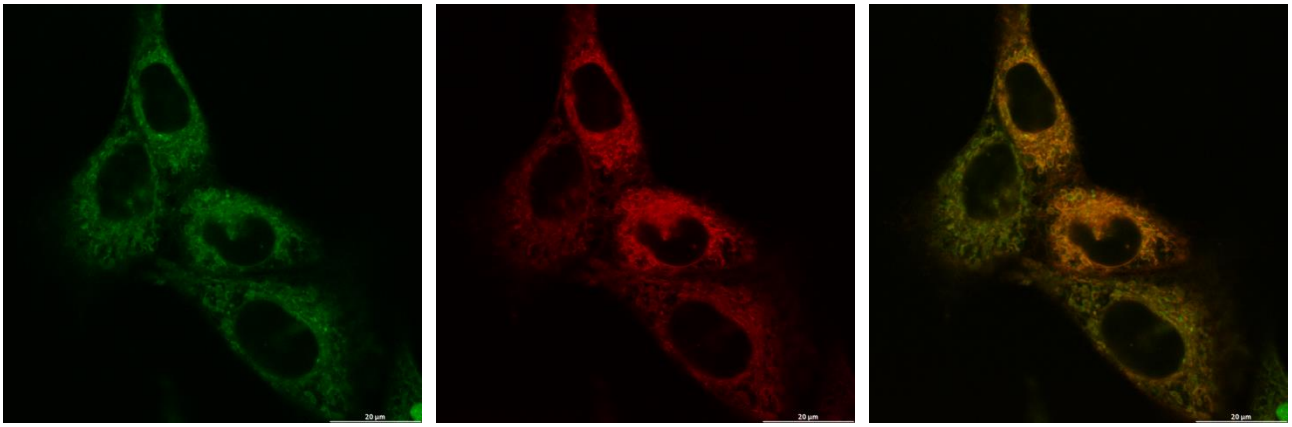


Figure S6 Confocal fluorescence microscopic images of HeLa cells with **1** colocalized with MitoTracker™ Red FM.

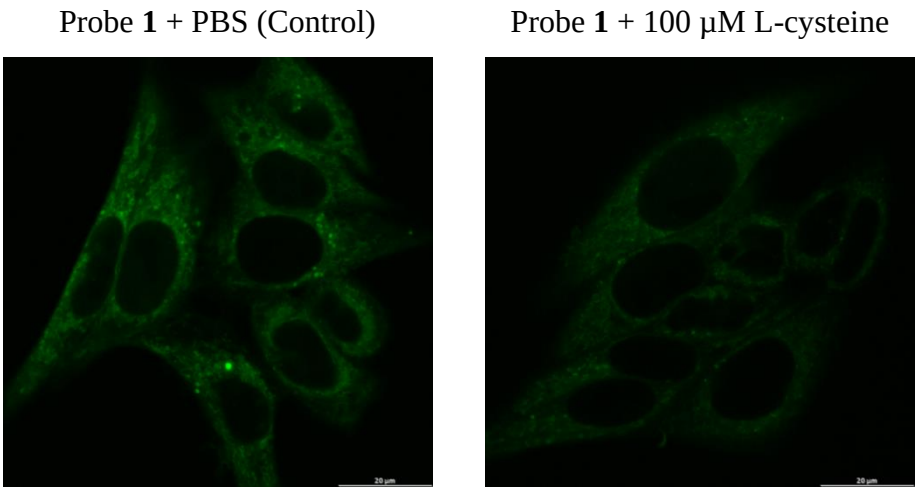


Figure S7 Confocal fluorescence microscopic images of HeLa cells with **1**

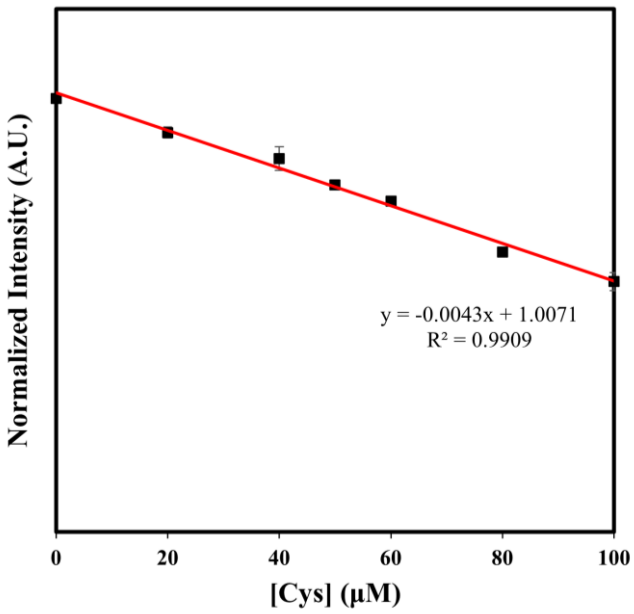
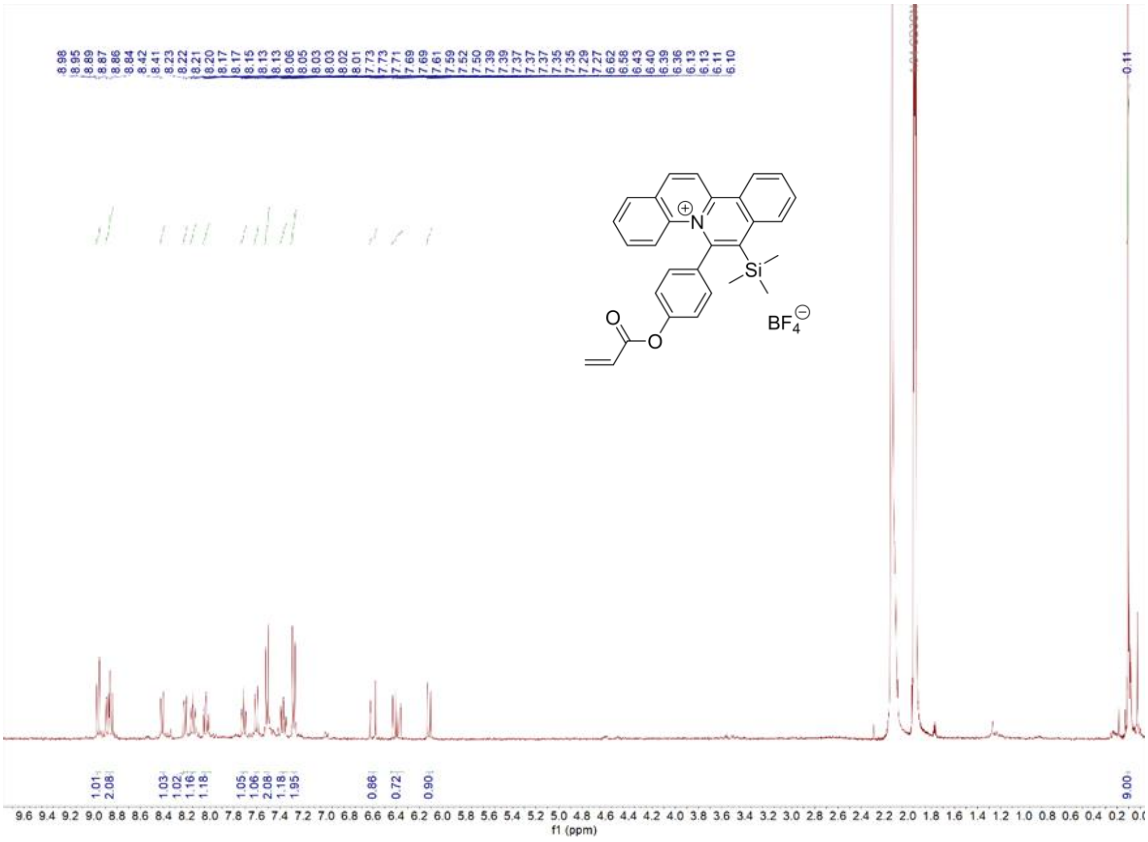
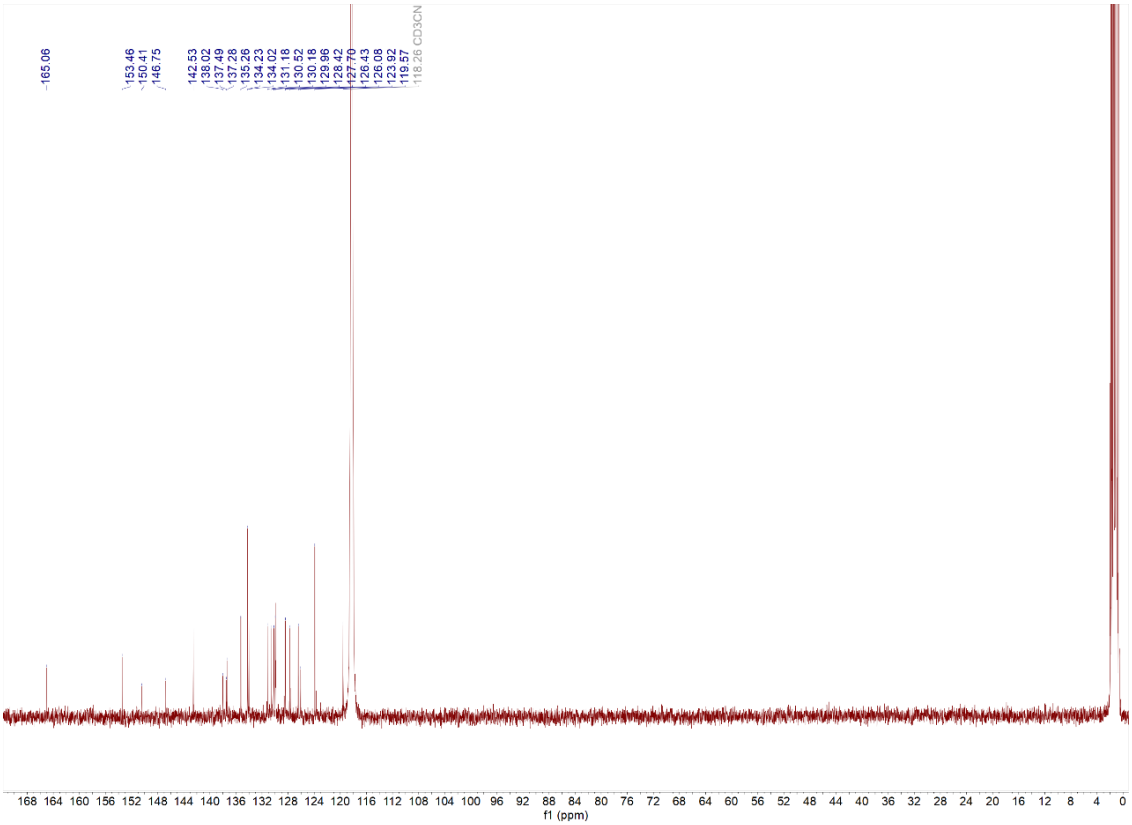


Figure S8 The linear curve for the detection of Cys in spiked mouse serum samples

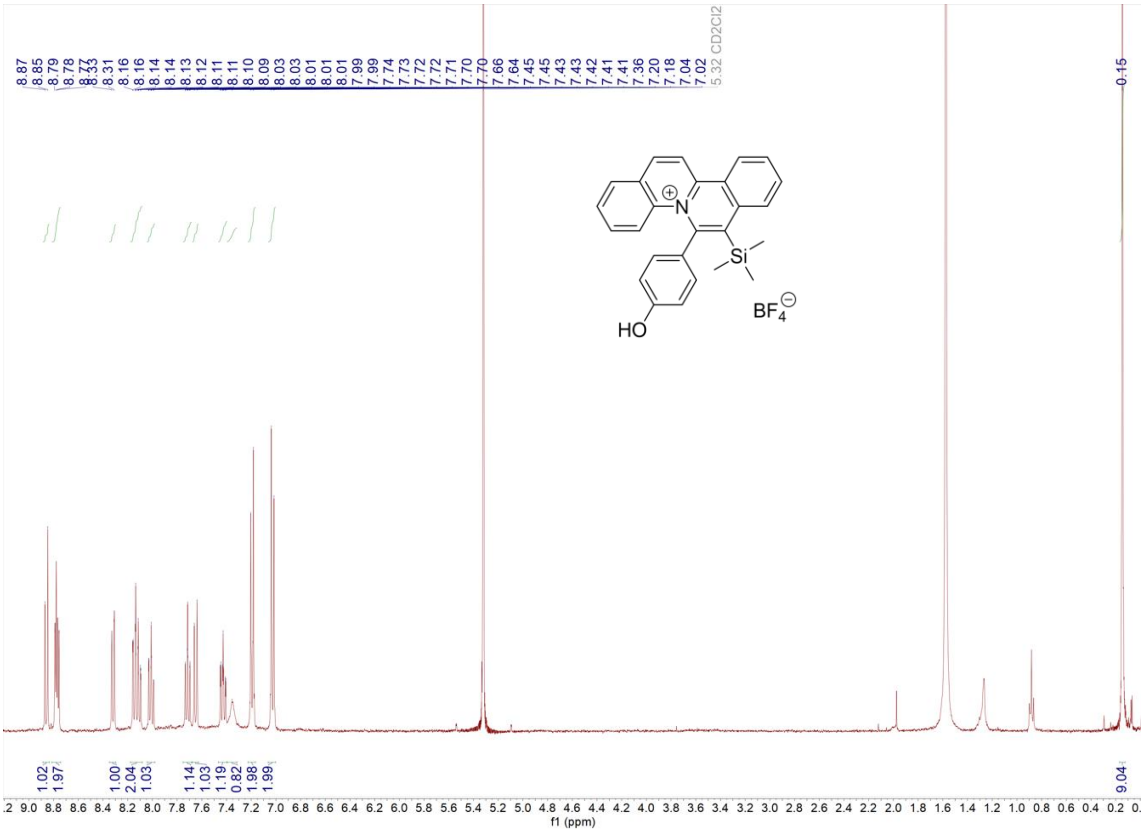
^{1}H NMR Spectrum of 1



^{13}C NMR Spectrum of 1



^{1}H NMR Spectrum of 2



^{13}C NMR Spectrum of 2