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Supporting Information

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Fig. S1 Fluorescence responses of NPSCY in the absence and presence of various analytes

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Reagents and apparatus.

All chemicals and reagents used in this work were purchased from commercial suppliers and used without further purification. All amino acids including alanine (Ala), arginine (Arg),

asparagine (Asn), aspartic acid (Asp), glutamine (Gln), glycine (Gly), histidine (His), lysine (Lys), phenylalanine (Phe), proline (Pro), threonine (Thr), tyrosine (Tye), threonine (Tyr), valine (Val), homocysteine (Hcy), glutathione (GSH), cysteine (Cys) were obtained from Sinopharm. Stock solution of probe (10^{-3} M) was prepared by DMSO and diluted to 10 μM in DMSO/ PBS buffer (5:5, v/v, pH = 7.4) as tested solutions by PBS (phosphate buffer solution). The concentrations of amino acids dissolved in PBS were 10^{-2} M.

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DMX600 NMR spectrometer DMSO-d_6 with tetramethylsilane (TMS) as standard. Mass spectra (MS) were obtained from the Ultraflex II (MALDI-TOF) spectrometer. UV–Vis absorption spectra were measured on a Shimadu UV-3600 spectrophotometer. Fluorescence spectra were measured on a HORIBA FL-4 Max spectrometer.

The detection of Cys in living cells and zebrafishes

For the imaging of Cys detection in living cells, firstly, Bel-7402 cells were incubated with NPSCY (5 μM) for 1 h. The Bel-7402 cells were washed with PBS (2×3 mL) and fluorescence imaging of NPSCY was set at wavelength range of from 430 to 470 nm and from 650 to 750 nm. Secondly, Bel-7402 cells were incubated with NPSCY (5 μM) for 1 h, and then treated with Cys (40 μM) for 1 h. The Bel-7402 cells were washed with PBS (2×3 mL) and fluorescence imaging of NPSCY was set at wavelength range of from 430 to 470 nm and from 650 to 750 nm.

For the imaging of Cys detection in zebrafishes, firstly, zebrafishes were incubated with NPSCY (5 μM) for 1 h. The zebrafishes cells were washed with PBS (2×3 mL) and fluorescence imaging of NPSCY was set at wavelength range of from 430 to 470 nm and from 650 to 750 nm. Secondly, zebrafishes were incubated with NPSCY (5 μM) for 1 h, then treated with Cys (40 μM) for 1 h. The Bel-7402 cells were washed with PBS (2×3 mL) and fluorescence imaging of NPSCY was set at wavelength range of from 430 to 470 nm and from 650 to 750 nm.

For the co-localization experiment of NPSCY, Bel-7402 cells were incubated with MitoRed (10 μM) for 30 min. Then, Bel-7402 cells were incubated with NPSCY (5 μM) for 1 h and Cys (40 μM) for 1h. The Bel-7402 cells were washed with PBS (2×3 mL) before fluorescence imaging. Fluorescence imaging of NPSCY was set at wavelength range of from 600 to 650 nm and from 650 to 750 nm.

The dark toxicity of NPSCY

A549 cells (10^5 cells per well) were cultured in a 96-well plate for 24 h in an atmosphere of 5% CO_2 at 37 °C. NPSCY (0.00, 1.25, 2.50, 3.75, 5.00 μM) were added and A549 cells were harvested for another 12 h. The solution of MTT (5-dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide, 0.5 mg/mL) in new culture medium was added and harvested for another 4 h. The culture medium of NPSCY was removed and 100 μL bioscale DMSO was added. At last, the absorbance of each plate at 490 nm was recorded and cell viability was calculated based on the equation: OD_{490} (sample)/OD_{490} (control).

The phototoxity of NPSCY

A549 cells (10^5 cells per well) were cultured in a 96-well plate for 24 h in an atmosphere of 5% CO_2 at 37 °C. NPSCY (0.00, 1.25, 2.50, 3.75, 5.00 μM) were added and the A549 cells were harvested for another 1 h. A549 cells were illuminated by 660 nm light for different time (0, 30, 60 min). Then, A549 cells were harvested for another 12 h. The solution of MTT (5-dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide, 0.5 mg/mL) in new culture medium was added and harvested for another 4 h. The culture medium of NPSCY was removed and 100 μL bioscale DMSO was added. At last, the absorbance of each plate at 490 nm was recorded and cell viability was calculated based on the equation: OD_{490} (sample)/OD_{490} (control).

Acridine orange (AO)/ethidium bromide (EB) staining assay of NPSCY

A549 cells were cultured for 24 h in an atmosphere of 5% CO_2 at 37°C. NPSCY (5 μM) were added and A549 cells were harvested for another 12 h. A549 cells were irradiated by 660 nm light for 0 and 60 min. Then, the A549 cells were harvested for for another 6 h and AO/ EB (10 μM) were added. Then, A549 cells were harvested for another 1 h. Fluorescence imaging of NPSCY was set at the wavelength range of from 570 to 670 nm for red channel and the wavelength range of from 500 to 540 nm for green channel.

Cell migration

A549 cells were incubated in a 6-well plate for 12 h, and then incubated with NPSCY for 12 h in the dark and spare.

The culture medium of A549 cells was removed and the cells were washed with PBS three times. A straight line on the well plate was draw and the new culture medium was added. Then, A549 cells were incubated in the dark for 0, 24 and 48 h and took pictures.

The culture medium of A549 cells was removed and the cells were washed with PBS three times. A straight line on the well plate was draw and the new culture medium was added. A549 cells were irradiated with 660 nm light for 60 min. Then, A549 cells were incubated for 24 and 48 h in the dark for 0, 24 and 48 h and took pictures.

Fig. S1 Fluorescence responses of NPSCY in the absence and presence of various analytes

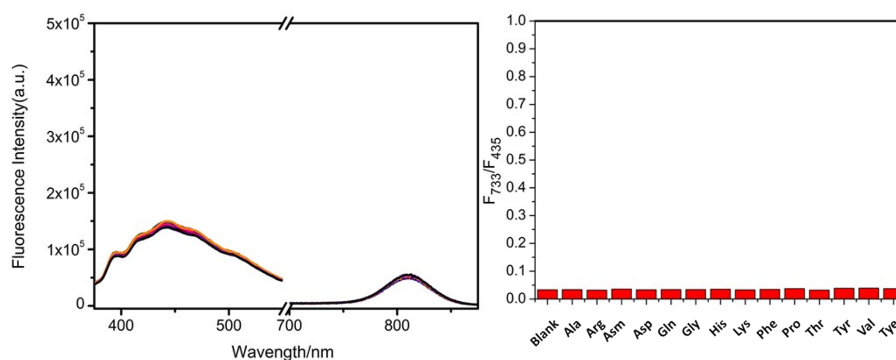


Fig. S1 Fluorescence responses of NPSCY in the absence and presence of various analytes (Blank, Ala, Arg, Asn, Asp, Gln, Gly, His, Lys, Phe, Pro, Thr, Tye, Tyr, Val, 20 μM) in DMSO/PBS buffer (5:5, v/v, pH = 7.4).

Fig. S2 Absorption responses of NPSCY towards increasing Cys

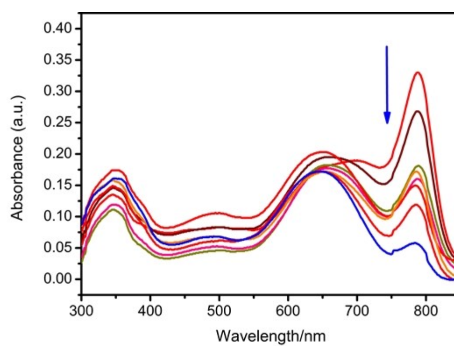


Fig. S3 Absorption responses of probe NPSCY (10 μM) in the presence of increasing concentration of Cys (2, 4, 6, 8, 10, 12, 15 and 20 μM) in DMSO/ PBS (5:5, v/v, pH = 7.4).

Fig. S4 Fluorescence responses of NPSCY towards increasing Hcy

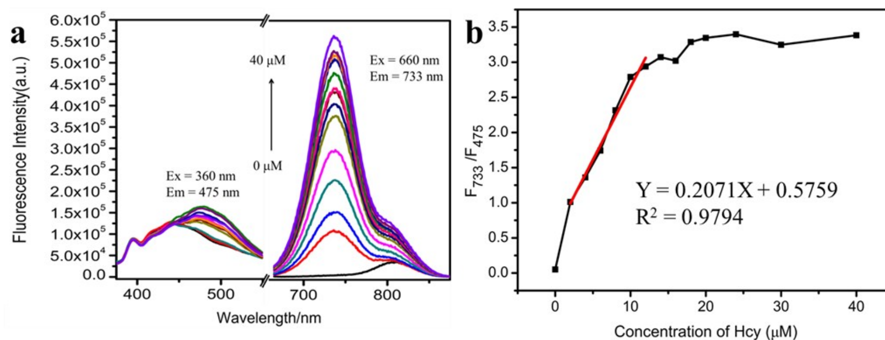


Fig. S3 (a) Fluorescence responses of NPSCY (10 μM) in the presence of increasing concentration of Hcy (0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 30 and 40 μM) in DMSO/PBS (5:5, v/v, pH = 7.4). (b) The linear relationship between the fluorescence intensities of NPSCY and the concentration of Hcy.

Fig. S4 pH-dependent experiment of NPSCY towards Hcy

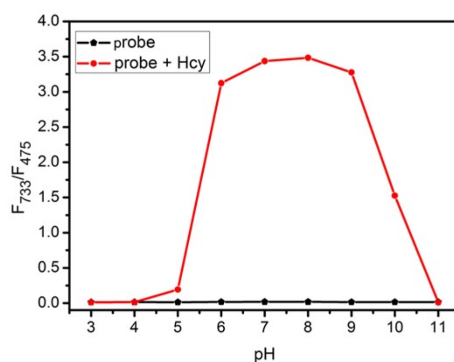


Fig. S4 pH-dependent fluorescence intensity ratio (F_{733}/F_{475}) of probe NPSCY (10 μM) in absence and presence of Hcy (40 μM) in DMSO/PBS buffer (5:5, v/v) under different pH values.

Fig. S5 Time-dependent experiment of NPSCY towards Hcy

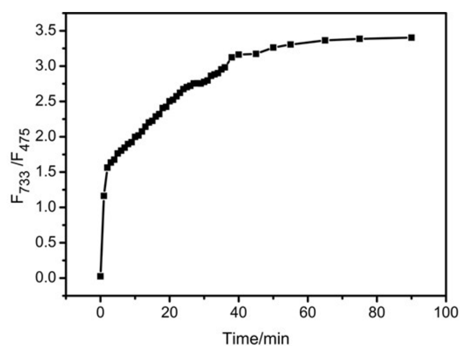


Fig. S5 Time-dependent fluorescence intensity ratio (F_{733}/F_{475}) of NPSCY (10 μM) in the presence

of Cys (40 μM) in DMSO/PBS (5:5, v/v, pH = 7.4).

Fig. S6 HRMS spectra of NPSCY in presence of Hcy

Fig. S6 HRMS spectra of NPSCY in presence of Hcy.

Fig. S7 pH-dependent experiment of NPSCY towards Cys

Fig. 7 pH-dependent fluorescence intensity ratio (F_{733}/F_{435}) of NPSCY (10 μM) in absence and presence of Cys (40 μM) in DMSO/PBS (5:5, v/v) under different pH values.

Fig. S8 Time-dependent experiment of NPSCY towards Cys

Fig. S8 Time-dependent fluorescence intensity ratio (F_{733}/F_{435}) of NPSCY (40 μM) in the presence of Cys (40 μM) in DMSO/PBS buffer (5:5, v/v, pH = 7.4).

Fig. S9 HRMS spectra of NPSCY in absence and presence of Cys

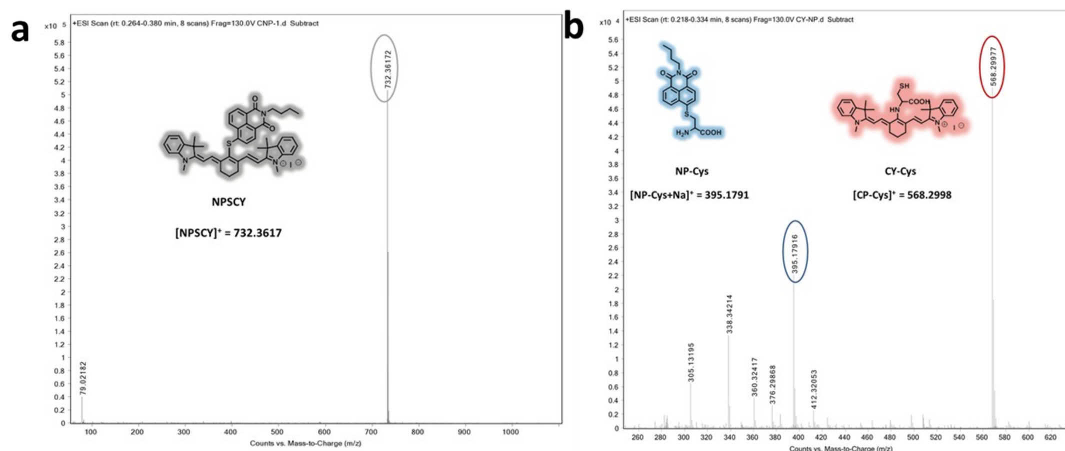


Fig. S9 HRMS spectra of NPSCY in absence and presence of Cys.

Fig. S10 The UV-Vis responses of NPSCY/DPBF towards 660 nm irradiation

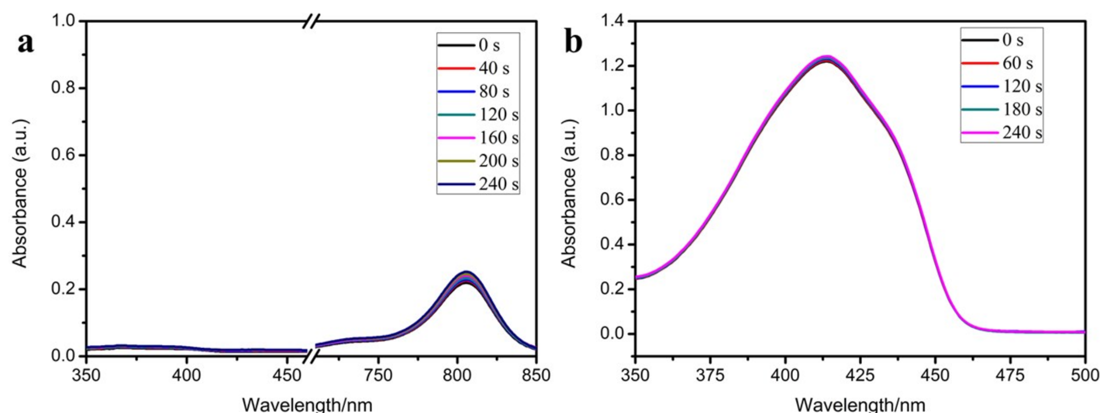


Fig. S10 (a) The UV-Vis responses of NPSCY towards the 660 nm irradiation in DCM. (b) The UV-Vis responses of DPBF towards the 660 nm irradiation in DCM.

Fig. S11 The ^{1}H NMR spectra of compound CY

Fig. S12 The ^{1}H NMR spectra of NPSCY

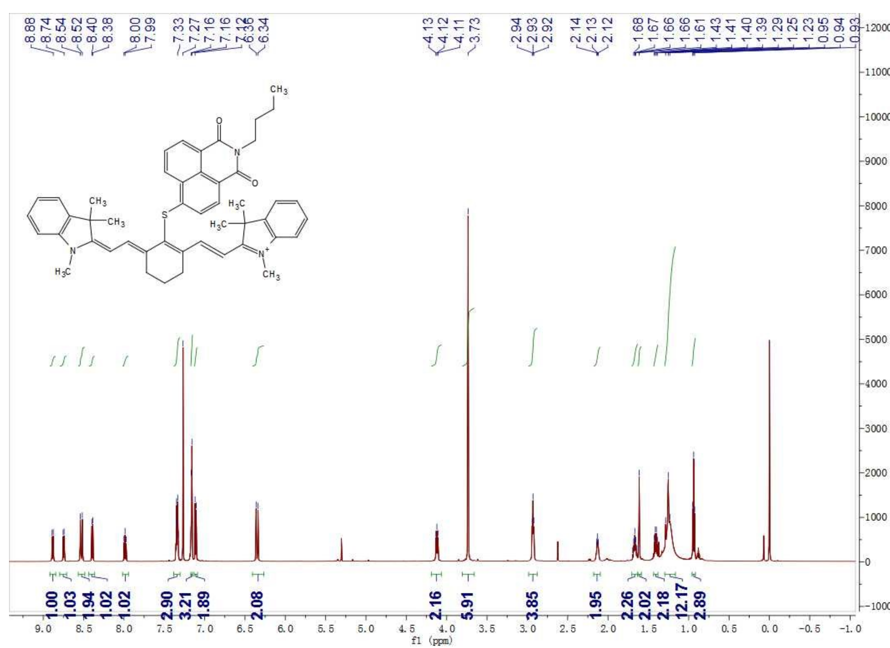


Fig. S13 The ^{13}C NMR spectra of NPSCY

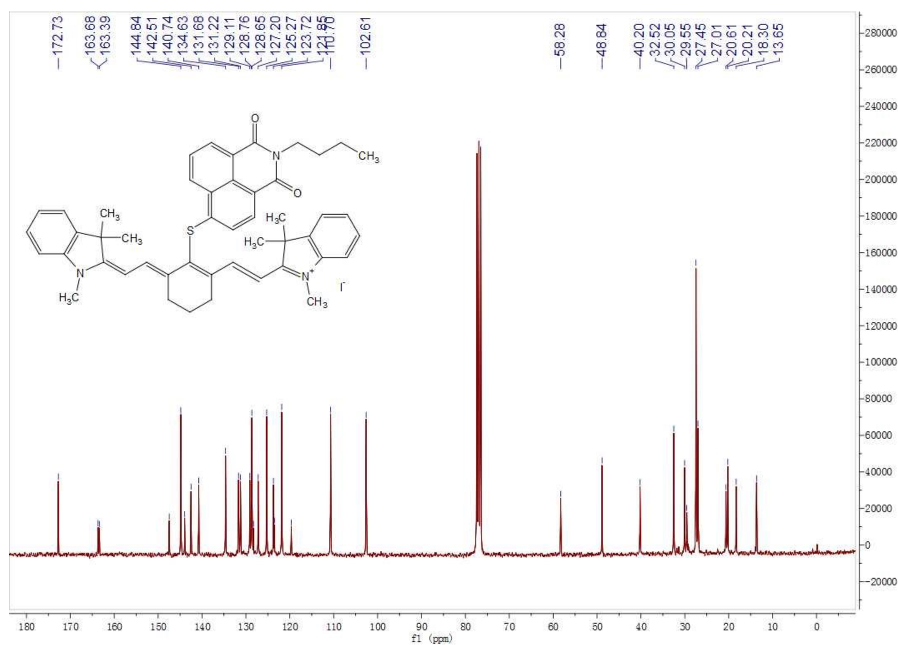


Fig. S14 The MS of NPSCY

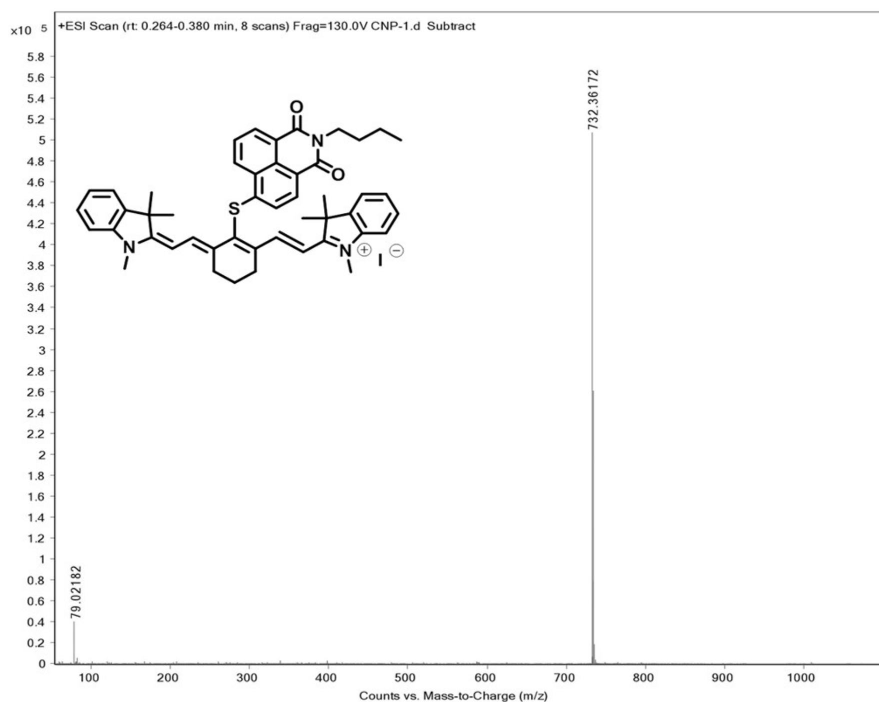
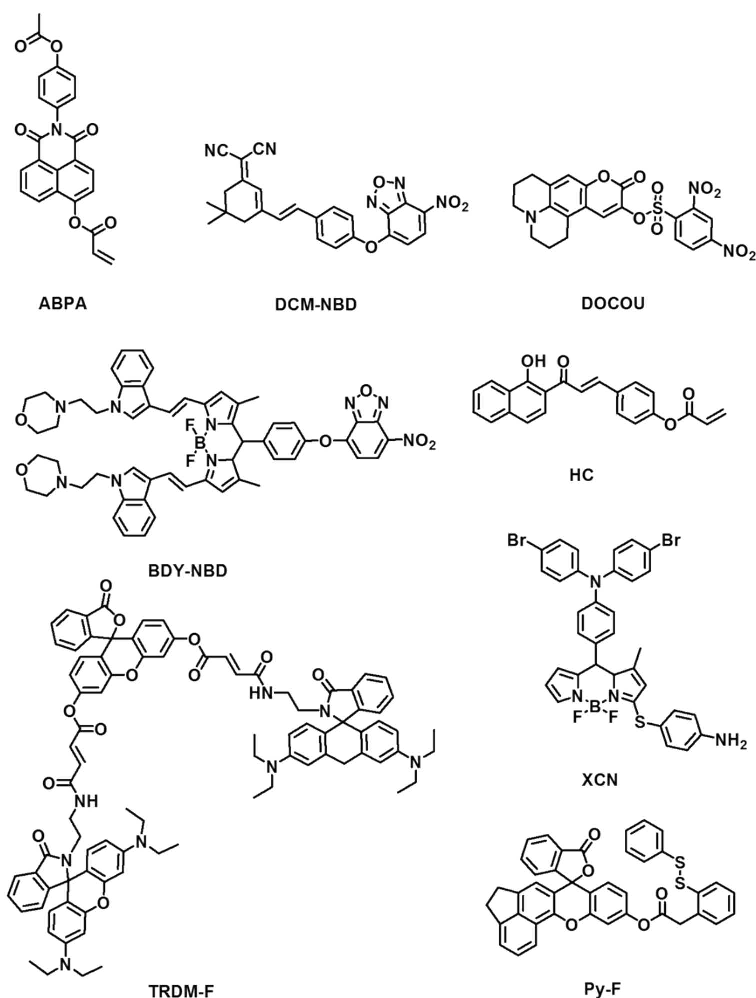


Table S1 The comparison with the reported Cys probe

Probe	Ex and Em /nm	Selectivity	Solvent (pH=7.4)	Detection Limit /nM	Time /min
ABPA[1]	Ex=391 nm, Em=559 nm	Cys	DMSO/H_2O(9/1,v/v)	120	10
DCM-NBD[2]	Ex=475 nm, Em=560 nm Ex=560 nm, Em=700 nm	Cys/Hcy	DMSO/H_2O(5/5,v/v)	15/15	21/17
DOCOU[3]	Ex=395 nm, Em=517 nm	Cys/Hcy/GSH	EtOH/H_2O(2/8,v/v)	17.1/14.5/24.0	16/16/24
BDY-NBD[4]	Ex=650 nm, Em=735 nm	Cys	DMSO/H_2O(3/7,v/v)	22	30
HC[5]	Ex=425 nm, Em=495, 620 nm	Cys	DMSO/H_2O(5/5,v/v)	91	10
TRDM-F[6]	Ex=492 nm, Em=512 nm	Cys	MeCN/H_2O(2/8,v/v)	88	20
Py-F[7]	Ex=520 nm, Em=552 nm	Cys/GSH	MeCN/H_2O(3/7,v/v)	120/130	10/40
XCN[8]	Ex=501 nm, Em=549 nm	Cys/Hcy	DMSO/H_2O(2/1,v/v)	3100/1600	5
This work	Ex=365 nm, Em=435nm Ex=655nm, Em=733nm	Cys	DMSO/H_2O(5/5v/v)	27.9	50

Scheme S1 The structure of Cys fluorescent probe mentioned in Table S1



Scheme S1 The structure of Cys fluorescent probe mentioned in Table S1

Reference

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