Supplementary Information (SI) For

Detection and Differentiation of Cys, Hcy and GSH mixtures by ¹⁹F NMR Probe

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Characterization of compound 2 and 1



¹H NMR of compound 2 recorded in DMSO-d₆.



¹³C NMR of compound 2 recorded in DMSO-d₆.



HR MS-ESI of compound 2.









Color changes of probe 1 with Cys, Hcy and GSH:



Figure S1. Color changes of probe 1 (10 μ M) in 20 mM HEPES buffer (pH 7.4) solution with 30% acetonitrile and 5% D₂O as cosolvent at 25 °C. The solutions were incubated with 10 equiv. Cys, 10 equiv. Hcy and 10 equiv. GSH for 90 min, respectively.

Fluorescence scan kinetics of probe 1 with Cys:



Figure S2. Time-dependent of fluorescence kinetics spectra of probe 1 (10 μ M) upon addition of different concentrations of Cys in HEPES (20 mM, pH 7.4) solution with 30% acetonitrile and 5% D₂O at 25 °C. The reactions were monitored at 615 nm.

Uv-vis spectra changes of probe 1 upon addition of Hcy and GSH:



Figure S3. Uv-vis spectra changes of probe 1 (10 μ M) upon addition of 10 equiv. of (a) Hcy and (b) GSH. The Spectra were collected from 0 to 90 min. All the spectra were carried out in 20 mM HEPES buffer (pH 7.4) with 30% acetonitrile and 5% D₂O at 25 °C.



Figure S4. Fluorescence spectra changes of probe 1 (10 μ M) in the presence of (a) 10 equiv. Hcy and (b) 10 equiv. GSH. The Spectra were collected from 0 to 90 min. All the experiments were carried out in HEPES buffer solution (pH 7.4, 20 mM) with 30% acetonitrile and 5% D₂O at 25 °C.



Apparent rate constant for the reaction of probe 1 with Cys, Hcy and GSH:

Figure S5. (a) Fluorescence scan kinetics spectra of probe 1 ($10 \mu M$) in the presence of 10 equiv. Cys, 10 equiv. Hcy and 10 equiv. GSH, respectively. (b) The apparent rate constant for the reaction of probe 1 with Cys, Hcy and GSH

were calculated by fitting (green solid line) the scanning kinetics data to the pseudo first-order equation: $Ln((I_{max}-I_t)/I_{max}) = -kt$, where I_{max} and I_t are the final fluorescence intensity and the fluorescence intensity at the maximum emission wavelength at time t, and k is the apparent rate constant. All the experiments were carried out in HEPES buffer solution (pH 7.4, 20 mM) with 30% acetonitrile and 5% D₂O at 25 °C.



Absorption and fluorescence selectivity experiments of probe 1 towards other species:

Figure S6. The response of absorption and fluorescence intensity of probe 1 (10 μ M) toward various other species. Bars represent the relative intensity after addition of 10 equiv of a single species (from left to right: 1. probe 1, 2. Cys, 3. Hcy, 4. GSH, 5. Na₂S₂O₃, 6. Threonine, 7. Serine, 8. Leucine, 9. Sodium Erythorbate, 10. K₂SO₈, 11. Glutamine, 12. Tyrosine, 13. Lysine, 14. Glycine, 15. Methionine, 16. Aspartic acid, 17. Tryptophan, 18. Pyroglutamic acid, 19. Phenylalanine, 20. Phenylglycine). All experiments were performed in HEPES buffer solution (pH 7.4) with 30% acetonitrile and 5% D₂O at 25 °C and each spectrum was obtained at 90 min after addition of analyte.

Absorption and fluorescence spectra changes of probe 1 towards the mixture of Cys, Hcy and GSH:

Figure S7. (a) Absorption and (b) fluorescence spectra changes of probe 1 (10 μ M) upon addition of the mixture of Cys (100 μ M), Hcy (200 μ M) and GSH (200 μ M). All the experiments were carried out in HEPES buffer solution (pH 7.4, 20 mM) with 30% acetonitrile and 5% D₂O at 25 °C.

¹⁹F NMR spectra changes of probe 1 upon addition of 3 and 1 equivalents Cys:

(a)127.6 ppm	0 min	(b) <u>\</u> -127.6 ppm	0 min
-128.0 ppm -128.3 ppm	6 min -135.4 ppm	-128.3 ppm	5.5 min
	9 min	~~ /	8 min
	12 min	~ ^ ~	12 min -135.4 ppm
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	16 min	~~ .	16 min
*****	20 min	~~~	
	28 min	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	20 min
	40 min.		
	60 min /		40 min
	90 min /	~~	60 min
	277 min ^	~~	90 min
	compound 2 A	~	compound 2 A
-127 -128 -129 -130 -131 -132	-133 -134 -135 -136 -13	-127 -128 -129 -130 -131 -132	-133 -134 -135 -136 -137

Figure S8. ¹⁹F NMR spectra changes of probe 1 (200  $\mu$ M) upon addition of (a) 3 equiv. and (b) 1 equiv. Cys. All the spectra were measured in HEPES buffer solution (pH 7.4) with 30% acetonitrile and 5% D₂O at 25 °C by 24 scans.

(a)127.6 ppm	0 min	(b) 127.6 ppm 0 min
-128.5 ppm	5 min	-128.4 ppm 6 min
-128.3 ppm	7 min	-128.1 ppm 8 min
	10 min	10 min
- 4	13 min	<u>13 min</u>
	16 min	16 min
	20 min	20 min
	20 1111	30 min
	28 min	40 min
	40 min	50 min
<u>_</u>	60 min	60 min
	100 min	. Λ 10h25 min
	653 min -135.4 ppm	-135.4 ppm compound 2
-127 -128 -129 -130 -131	-132 -133 -134 -135 -136 -137 -138	-127 -128 -129 -130 -131 -132 -133 -134 -135 -136 -137 -138

¹⁹F NMR spectra changes of probe 1 upon addition of 5 equivalents Hcy and GSH:

Figure S9. ¹⁹F NMR spectra changes of probe 1 (200  $\mu$ M) upon addition of 5 equiv. (a) Hcy and (b) GSH. All the spectra were measured in HEPES buffer solution (pH 7.4) with 30% acetonitrile and 5% D₂O at 25 °C by 24 scans.

#### ¹⁹F NMR spectra changes of probe 1 upon addition of 10 and 20 equivalents Hcy:

(a)	≜ -127.6 ppm	0 min						(b)		<u>∫</u> -127.6 p	pm		0 min					
<u></u>	-128.5 ppm	7 min									-128.5 ppn	n	5 min					-
	-128.3 ppm								-1	28.3 ppm			7 min					
		9 min								٨			9 min					
		12 min											10 min					
	Λ	16 min							~~~~~		~~~~~~		12 000					
		00 min											16 min					
		20 11111			~~~~~					.Λ.			20 min					
		25 min								. ^			25 min					
	·····	30 min								. A			31 min					
	. Λ	40 min								٨			40 min					
		50 min								^			50 min		~~~~~			~~~~~
		60 min								Λ			60 min					
	<b>/</b>	75 min											75 min					
	٨	90 min								٨			90 min					
	Δ	122 mir	1										120 min					
-127	-128 -129	-130 -131 -1 ō/p	32 -133 pm	-134	-135	-136	-137	-126	-127	-128	-129	-130	-131 ō/ppm	-132	-133	-134	-135	-136

Figure S10. ¹⁹F NMR spectra changes of probe 1 (200  $\mu$ M) upon addition of (a) 10 equiv. and (b) 20 equiv. Hcy. All the spectra were measured in HEPES buffer solution (pH 7.4) with 30% acetonitrile and 5% D₂O at 25 °C by 24 scans.

#### ¹⁹F NMR spectra changes of probe 1 upon addition of 10 and 20 equivalents GSH:

(a) ∧ -127.6 ppm	0 min	(b) A-127.6 ppm	0 min	
-128.1 ppm -128.4 ppm	6 min	128.4 ppm	5 min	
	0 min	-128.1 ppm	7 min	
	311111		10 min	
	12 min		15 min	
	16 min		20 min	
	20 min		05 min	
	28 min		20 min	
٨	40 min			
			40 min	
	80 Min	A	50 min	
······	90 min	A	70 min	
	120 min		160 min	
	compound 2 -135.4 ppm		compound 2	-135.4 ppm
-127 -128 -129 -130 -1	31 -132 -133 -134 -135 -136 -137 δ/ppm	-127 -128 -129 -130 -13	11 -132 -133 -134 5/ppm	-135 -136 -137

Figure S11. ¹⁹F NMR spectra changes of probe 1 (200  $\mu$ M) upon addition of (a) 10 equiv. and (b) 20 equiv. GSH. All the spectra were measured in HEPES buffer solution (pH 7.4) with 30% acetonitrile and 5% D₂O at 25 °C by 24 scans.

Chemical shift (ppm) Apparent rate Analyte before constant (min-1)a after 90 min upon addition  $\Delta \, \delta$ addition Cys -127.6 -128.0, -128.3, -135.4 -135.4 7.8 0.049 Hcy -127.6 -128.5, -128.3 -128.3 0.7 0.0046 GSH -127.6 -128.4, -128.1 -128.1 0.5 0.0023

Data of ¹⁹F NMR chemical shift changes of probe 1 with Cys, Hcy and GSH:

Table S1. The ¹⁹F NMR chemical shift changes data of probe 1 when respond to Cys, Hcy and GSH. ^a calculated from the data of fluorescence scan kinetics.

#### HPLC spectra of the reaction between probe 1 and Cys:

![](_page_8_Figure_7.jpeg)

Figure S12. (a) Time course (0-90 min) of HPLC-UV profile of the reaction of probe 1 with 10 equiv. Cys in 20 mM HEPES buffer solution (pH 7.4) with 30% acetonitrile and 5% D₂O at 25 °C. The spectra were monitored at 398 nm.

![](_page_9_Figure_0.jpeg)

HR-MS spectra of reaction mixture between probe 1 and 10 equiv. of Cys:

Figure S13. HR MS spectrum of reaction mixture between probe 1 and 10 equiv. of Cys in HEPES buffer solution (pH 7.4, 20 mM) with 30% acetonitrile and 5% D₂O at 25 °C. The peaks were consistent with the molecular weight of (a) [1-Cys+H⁺] intermediate (calcd 497.1289), (b) compound [2 + H⁺] (calcd 322.0986) and (c) probe [1 + H⁺].

![](_page_9_Figure_3.jpeg)

![](_page_9_Figure_4.jpeg)

Figure S14. (a) HPLC spectra of the reaction of probe 1 with 10 equiv. Hcy and GSH in HEPES buffer solution (pH 7.4, 20 mM) with 30% acetonitrile and 5%  $D_2O$  at 25 °C.

![](_page_10_Figure_0.jpeg)

![](_page_10_Figure_1.jpeg)

Figure S15. HR MS spectrum of reaction mixtures of probe 1 with 10 equiv. of (a) Hcy and (b) GSH in HEPES buffer solution (pH 7.4, 20 mM) with 30% acetonitrile and 5% D₂O at 25 °C. The peaks were consistent with the molecular weight of [1-Hcy+H⁺] intermediate (calcd 511.1446) and compound [1-GSH+H⁺] (calcd 683.1930), respectively.

Sensitivity of probe 1:

![](_page_10_Figure_4.jpeg)

Figure S16. 4800 scans ¹⁹F NMR spectrum of 10  $\mu$ M probe 1 after treatment with 50  $\mu$ M Cys in 20 mM HEPES buffer solution (pH 7.4) with 30% acetonitrile and 5% D₂O as cosolvent.

Stability experiment in bovine serum containing solution:

![](_page_11_Figure_1.jpeg)

Figure S17. ¹⁹F NMR spectrum of probe 1 (200  $\mu$ M) in 20 mM HEPES buffer solution (pH 7.4) with 30% acetonitrile and 5% D₂O (containing 10% bovine serum) after 90 min.

Probe 1	in resp	onse to	the indiv	idual Cv	. Hev and	l GSH in	bovine serum	solution:
					· · · · · · · · · · · · · · · · · · ·			~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~

![](_page_11_Figure_4.jpeg)

![](_page_12_Figure_0.jpeg)

Figure S18. ¹⁹F NMR spectra of probe 1 (200  $\mu$ M) in the presence of (a) 1000  $\mu$ M Cys, (b) 1000  $\mu$ M Hcy and (c) 2000  $\mu$ M GSH in 20 mM HEPES buffer (pH 7.4) solution with 30% acetonitrile and 5% D₂O (containing 10% bovine serum).

#### Probe 1 in response to the mixture of Cys, Hcy and GSH in bovine serum solution:

![](_page_13_Figure_1.jpeg)

Figure S19. ¹⁹F NMR spectra change of probe 1 (600  $\mu$ M) upon addition of the mixture of Cys (200  $\mu$ M), Hcy (400  $\mu$ M) and GSH (400  $\mu$ M) in 20 mM HEPES buffer (pH 7.4) solution with 30% acetonitrile and 5% D₂O (containing 10% bovine serum) at 25 °C.