*Supporting information for*

# Dual response site fluorescent probe for highly sensitive detection of Cys/Hcy and GSH in vivo through two different emission channels

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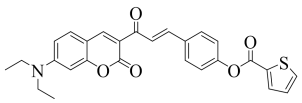
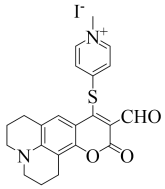
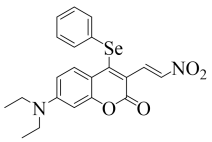
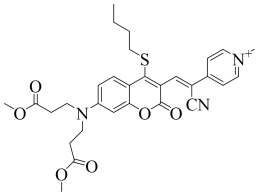
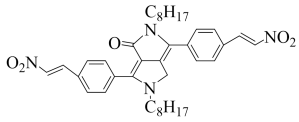
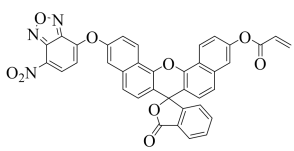
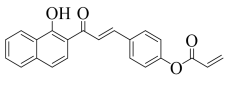
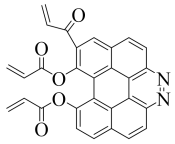
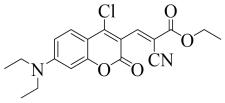
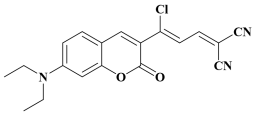
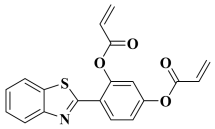
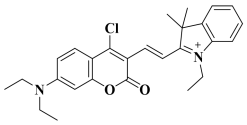
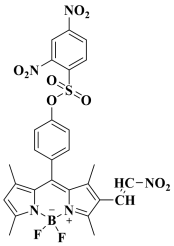
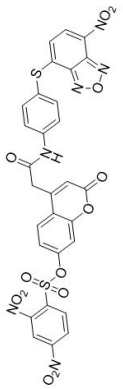
**Table S1.** The reported fluorescent probes based on dual-site for thiols.

| Probe | Stokes shift | Test system | Detection limit | Response time | Application | Literature |
|---|---|---|---|---|---|---|
|  | 51nm | HEPES: DMSO= 6:4 | Cys:0.24μM | Cys:10min | HeLa cells BHK-21 cells | Dyes Pigments 2022, 197, 109823 |
|  | 104nm/ 61nm | PBS buffer | Cys: 132nM Hcy: 105nM GSH :62nM | Cys: 190s Hcy: 155s GSH: 80s | zebrafish embryos; HeLa cells | Dyes Pigments 2022, 199, 110058 |
|  | Cys: 95nm Hcy: 92nm GSH: 62nm | PBS: DMSO= 9:1 | Cys: 39.2nM Hcy: 126.7nM GSH: 839.5nM | Cys: 12min Hcy: 12min GSH: 20min | HepG2 cells | Dyes Pigments 2022, 203, 110312 |
|  | Cys: 96nm Hcy: 97nm GSH: 90nm | PBS: DMSO= 8:2 | Cys: 27.3nM Hcy: 45.9nM GSH: 13.7nM | ------- | HepG2 cells ; zebrafish | Chinese Chem. Lett. 2022, 33, 1609-1612 |
|  | 59nm | PBS: THF= 2:3 | $6.14\times10^{-8}$M | Cys: 1min Hcy: 3min GSH: 16min | HeLa cells | Sensor Actua B-Chem. 2017, 244, 531-540 |
|  | Cys: 87nm Hcy: 73nm | PBS with 1.0 mM CTAB | Cys: $1.5\times10^{-7}$M Hcy: $1.1\times10^{-7}$M | 60min | HeLa cells | Tetrahedron Lett. 2018, 59, 2232-2237 |
|  | 140nm | PBS: DMSO= 1:1 | Cys： $9.1\times10^{-8}$M | Cys:10min | U87 cells | Biosens. Bioelectron 2017, 92, 583-588 |

| | | | | | | |
|---|---|---|---|---|---|---|
|  | 65nm | PBS buffer | 0.11μM | 5min | HeLa cells | Sensor Actua B-Chem. 2020, 320, 128304 |
|  | Hcy: 78nm Cys: 80nm GSH: 81nm | PBS: DMSO= 7:3 | Hcy: 3nM, Cys:6nM GSH: 200nM | 15min | A375 cells | Talanta 2020, 219, 121353 |
|  | 60nm | PBS: DMSO= 1:1 | 0.3μM | 7min | A549 cell | Sensor Actua B-Chem .2 017, 253, 42-49 |
|  | 113nm | HEPES: DMSO= 9:1 | 0.8μM | ------- | HeLa cells | Dyes Pigments 2017, 139, 73-78 |
|  | 76nm | $CH_3CN$: HEPES= 1:9 | $4.63\times10^{-7}$M | ------ | Hi5 cell C. eleg-an s | Chinese Chem. Lett. 2017, 28, 2023-2026 |
|  | ------- | $CH_3CN$: HEPES= 1:1 | Hcy: 87nM Cys: 147nM GSH: 129nM | Cys: 6.7min Hcy: 13.5min GSH: 79.4min | HeLa cells | Dyes Pigments 2018, 152, 29-35 |
|  | **Cys/ Hcy/GS H: 117 nm** | **PBS: DMSO= 8:2** | **Cys: 32nM Hcy: 45nM GSH :22nM** | **Cys: 300s Hcy: 350s GSH: 350s** | **HeLa cells Zebrafish** | **This work** |

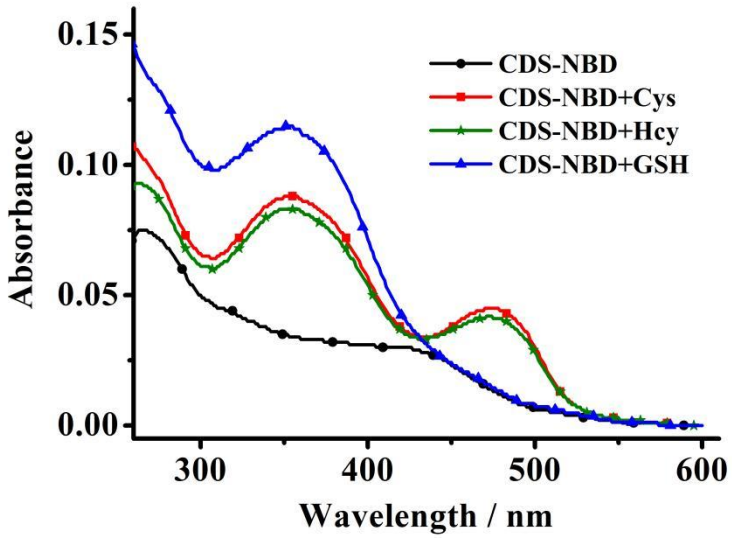



**Figure S1** UV-vis absorption spectra of probe **CDS-NBD** (black) and reacted with Cys (red), Hcy (green) and GSH (blue) in PBS buffer.

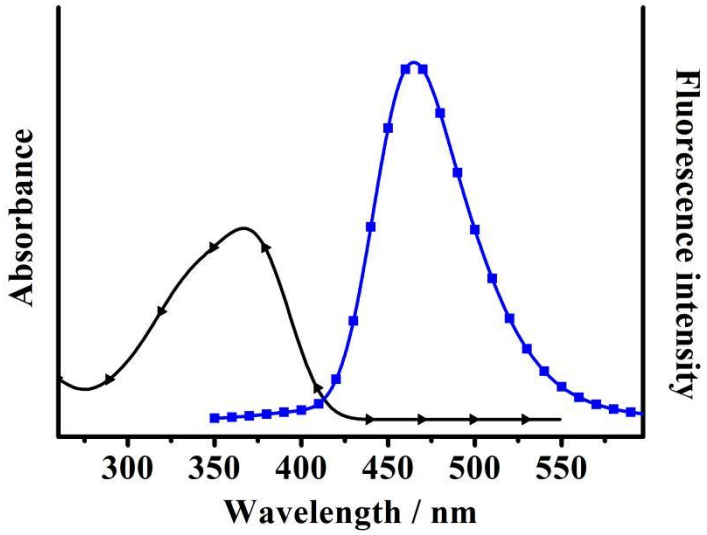



**Figure S2** UV-vis absorption (black) and fluorescence (blue) spectra of **7-HCA** in PBS buffer.

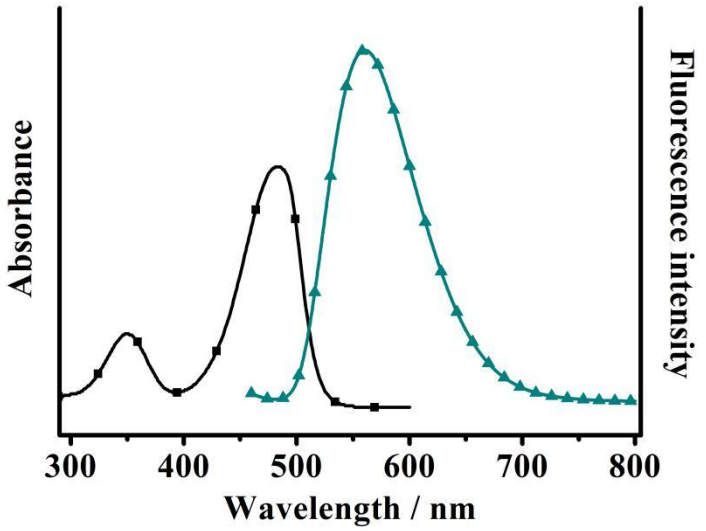



**Figure S3** UV-vis absorption (black) and fluorescence (green) spectra of **NBD-N-Bu** in PBS buffer.

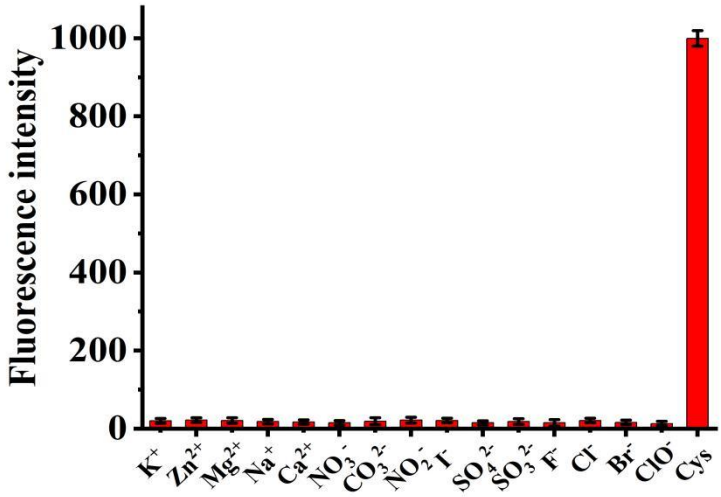



**Figure S4** The change of fluorescence intensity of the probe **CDS-NBD** in PBS with the addition of 100.00 μM biologically relevant ions at 470 nm of emission.

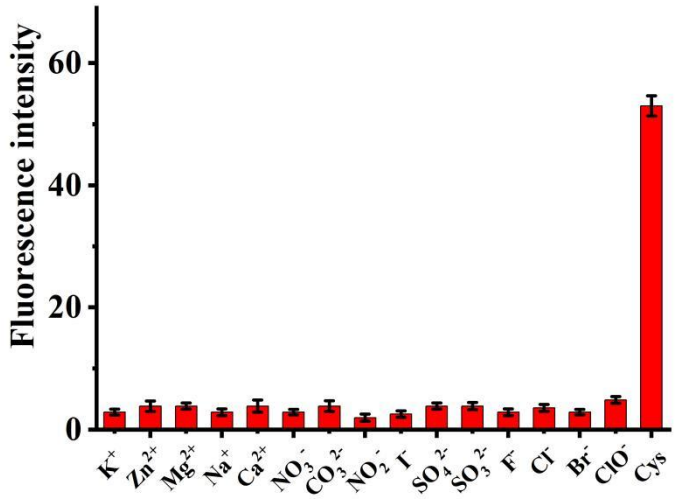



**Figure S5** The change of fluorescence intensity of the probe **CDS-NBD** in PBS with the addition of 100.00 μM biologically relevant ions at 557 nm of emission.

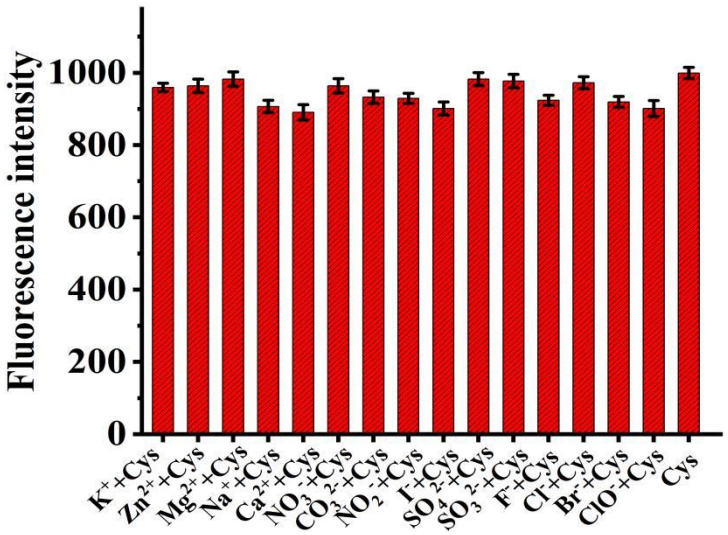



**Figure S6** Fluorescence responses of probe **CDS-NBD** toward Cys in the present of various coexistence substances (100.00 μM ) at 470 nm of emission.

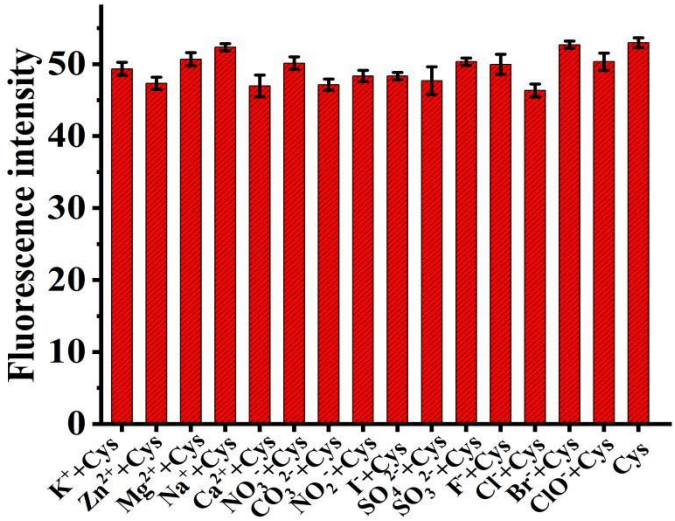



**Figure S7** Fluorescence responses of probe **CDS-NBD** toward Cys in the present of various coexistence substances (100.00 μM ) at 557 nm of emission.

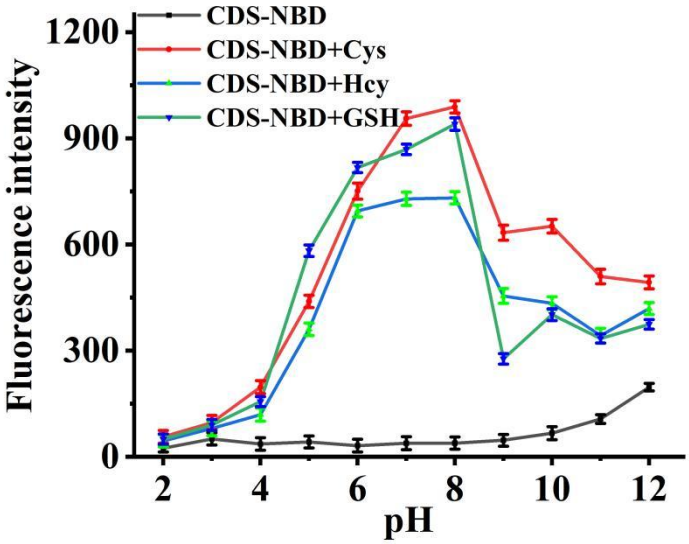



**Figure S8** pH effect on the fluorescence intensity of probe **CDS-NBD** (10.0 μM) without (blue) and with (60.0 μM) biothiols (red: Cys, green: Hcy, blue: GSH) at 470 nm.

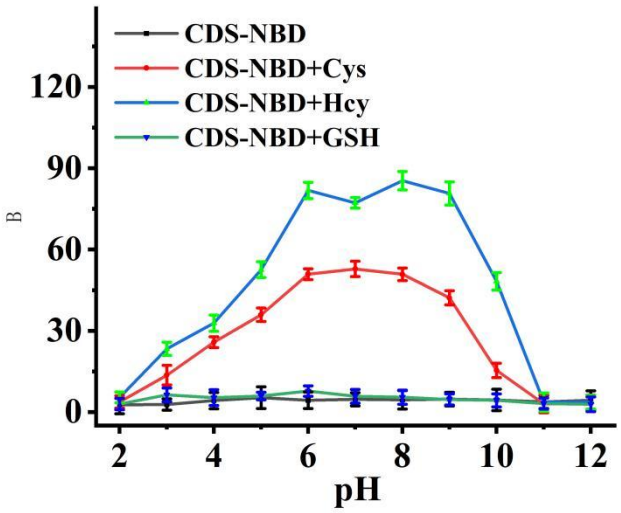



**Figure S9** pH effect on the fluorescence intensity of probe **CDS-NBD** (10.0 μM) without (blue) and with (60.0 μM) biothiols (red: Cys, green: Hcy, blue: GSH) at 557 nm.

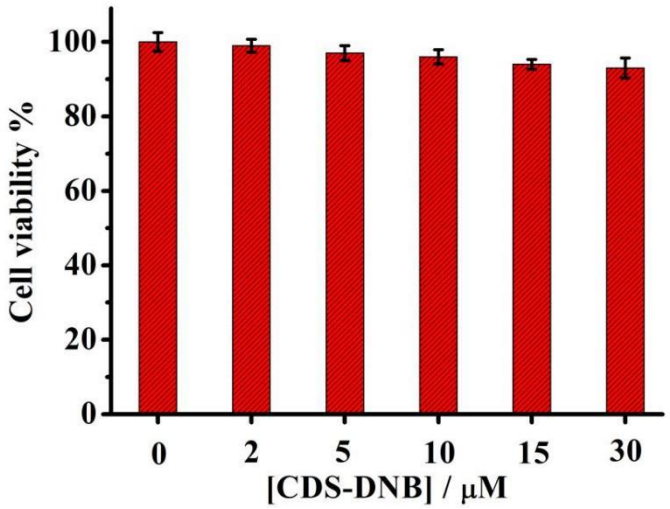



**Figure S10** Percentage of viable HeLa cells after treatment with indicated concentrations of probe **CDS-NBD** after 24 hours.

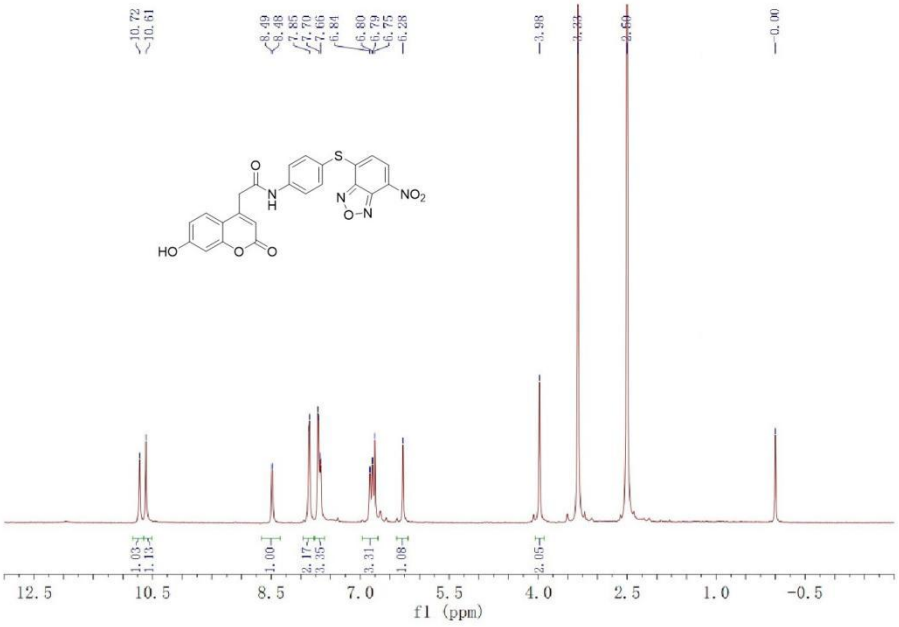



**Figure S11** $^{1}$H NMR spectrum of compound **1** in DMSO-$d_6$.

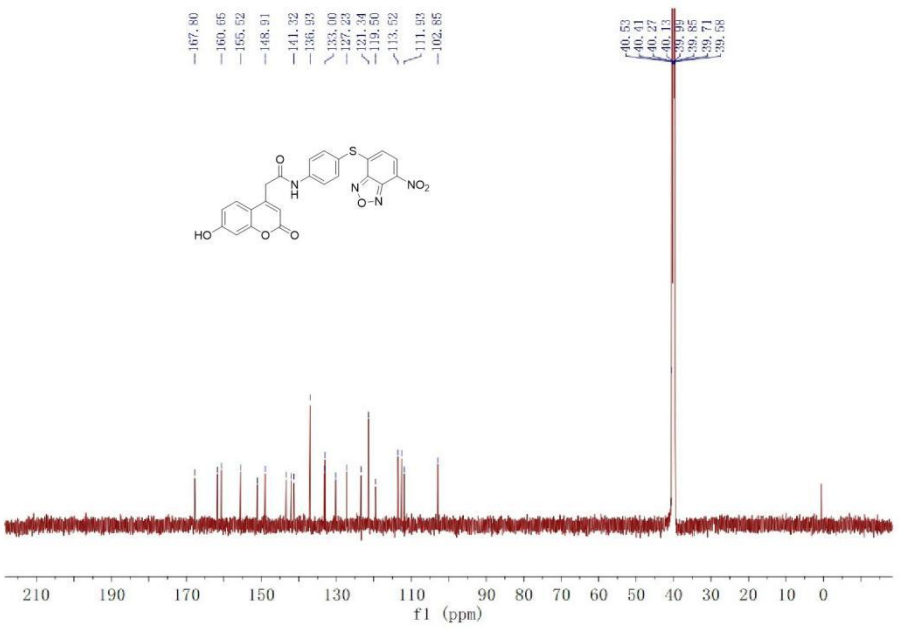



**Figure S12** $^{13}$C NMR spectrum of compound **1** in DMSO-$d_6$.

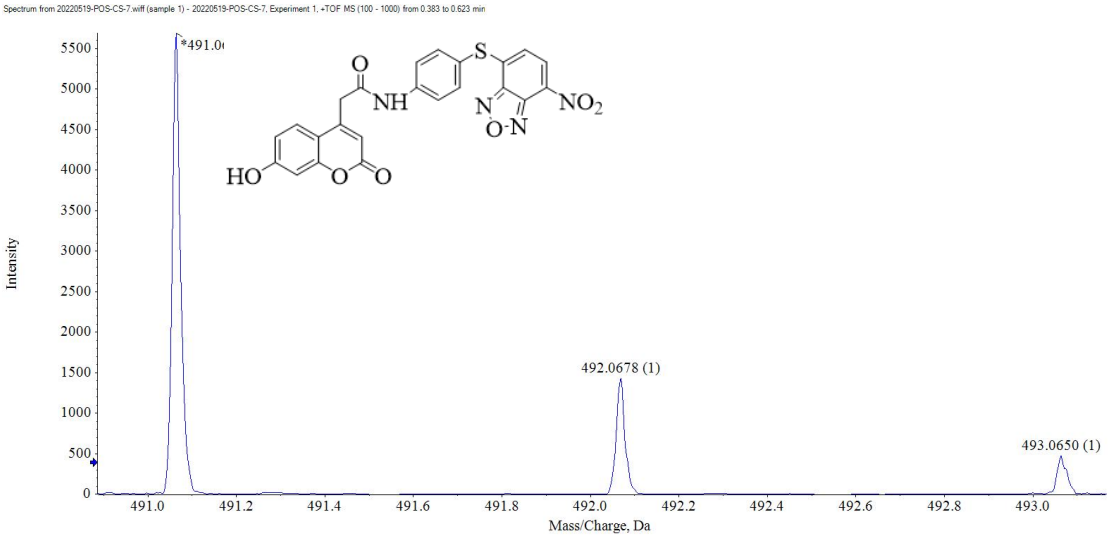



**Figure S13** Mass spectrum of compound **1**.

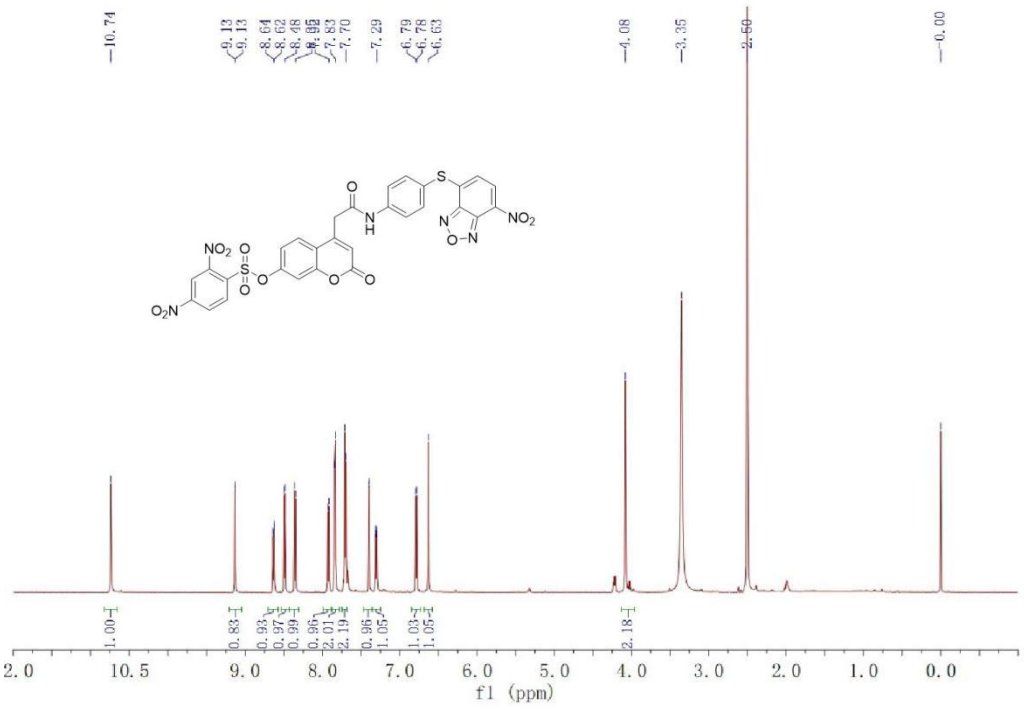



**Figure S14** $^{1}$H NMR spectrum of probe **CDS-NBD** in DMSO-$d_6$.

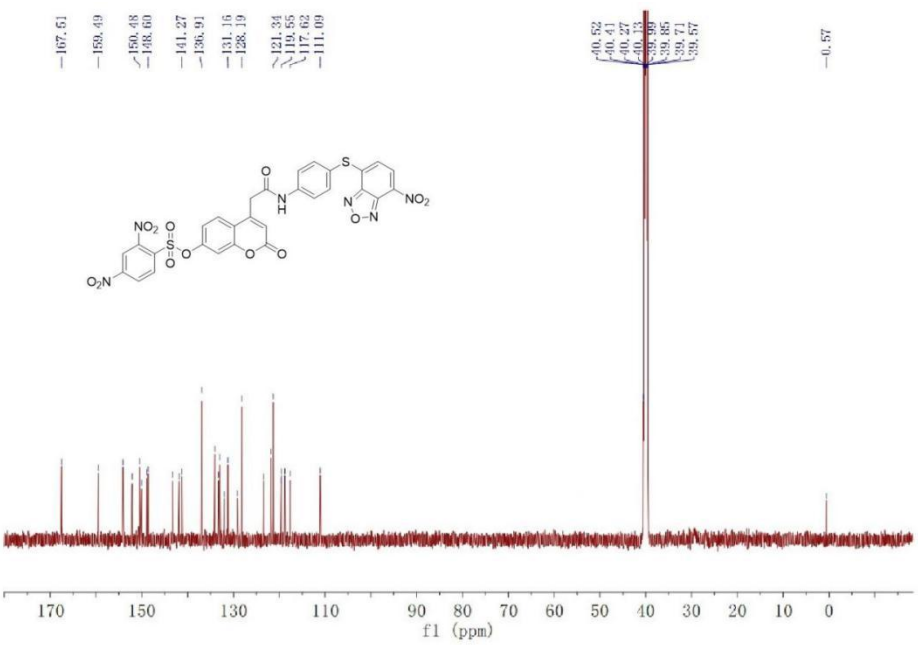



**Figure S15** $^{13}$C NMR spectrum of probe **CDS-NBD** in DMSO-$d_6$.

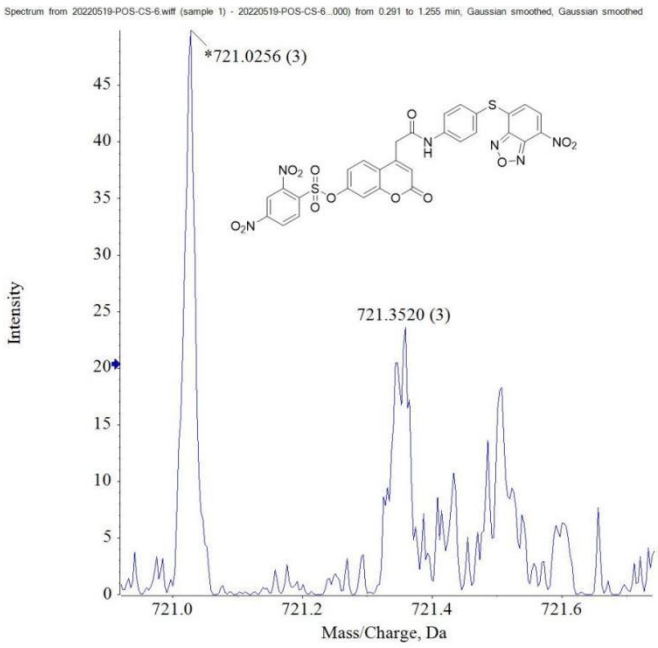



**Figure S16** Mass spectrum of probe **CDS-NBD**.

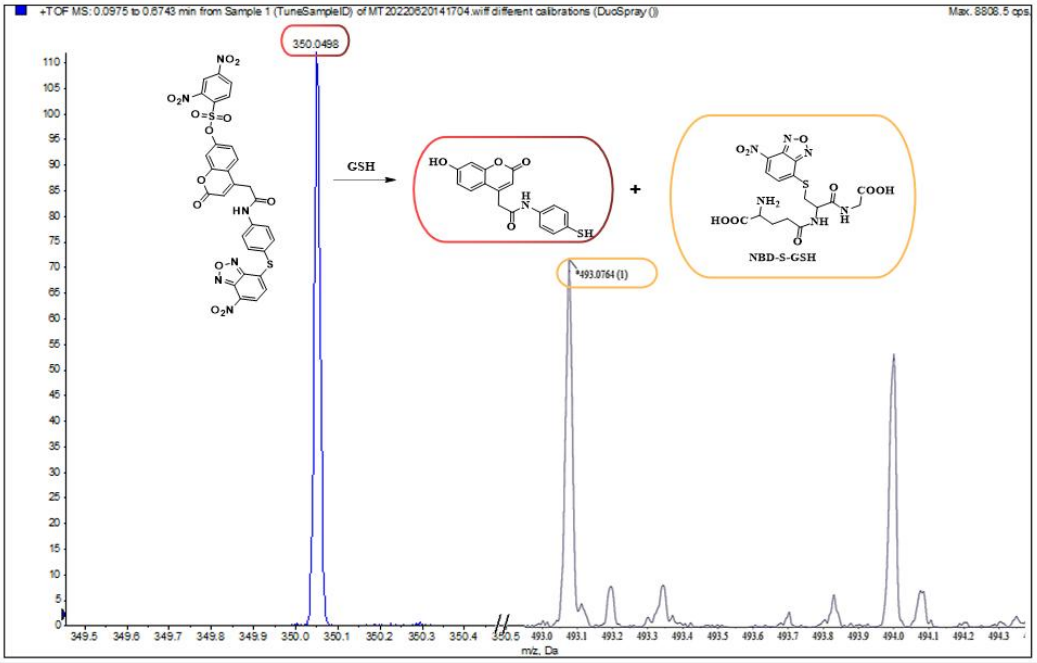



**Figure S17** Mass spectrum of probe **CDS-NBD** with GSH.

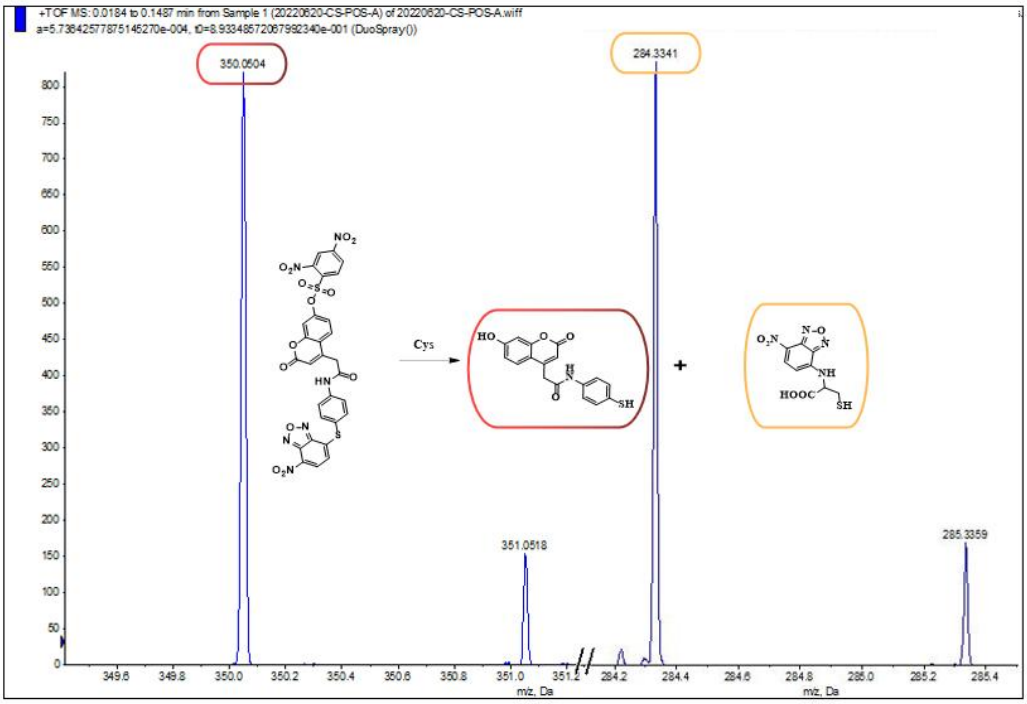



**Figure S18** Mass spectrum of probe **CDS-NBD** with Cys.