Supporting Information

A lysosome-targeted fluorescent probe based on BODIPY structure for Cys/Hcy detection

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Synthesis of 4-bromo-N, N-bis (4-(2-morpholinoethoxy) phenyl) aniline (2)

The started compound **1** (500 mg, 1.41 mmol), 4-(2-chloroethyl)morpholine (577 mg, 3.10 mmol), Cs_2CO_3 (2.74g, 8.41 mmol) and KI (50 mg, 0.28 mmol) were dissolved in anhydrous acetonitrile (15 mL). The reaction mixture was stirred and refluxed for 12 h under the protection of Ar at 105 ℃. After the reaction was completed, the organic solvent was removed under reduced pressure, and the residue was separated by column chromatography (eluent: DCM/MeOH = 40/1, v/v). The desired product **2** (350 mg) was achieved as yellowish oily liquid. Yield: 43%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24 (d, J = 8.9 Hz, 2H), 7.00 (d, J = 8.9 Hz, 4H), 6.80 (dd, J = 11.2, 8.9 Hz, 6H), 4.13 (t, J = 5.2 Hz, 4H), 3.82 – 3.74 (m, 8H), 2.87 (s, 4H), 2.67 (s, 8H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.59, 147.69, 141.03, 131.86, 126.45, 122.41, 115.52, 112.78, 65.91, 65.01, 57.26, 53.58. HRMS (ESI): Calculated for $C_{30}H_{37}BrN_3O_4$ $[M+H]^+$: 582.1967, found: 582.1964.

Synthesis of 4-(2-morpholinoethoxy)-N-(4-(2-morpholinoethoxy) phenyl)-N-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)aniline (3)

Compound **2** (450 mg, 0.78 mmol), boronate (406 mg, 1.60 mmol), potassium acetate (230 mg, 2.34 mmol) and $Pd(dppf)_2Cl_2$ (57 mg, 0.078 mmol) were dissolved in 1,4-Dioxane (15 mL). The mixture was stirred and refluxed for 12 h under the protection of Ar at 100 ℃. The mixture derived from the reaction was separated by column chromatography (eluent: DCM/MeOH = 30/1, v/v), the desired product **3** (350 mg) was obtained as white oily liquid. Yield: 62%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (d, J = 8.5 Hz, 2H), 7.03 (d, J = 8.9 Hz, 4H), 6.86 (d, J = 8.5 Hz, 2H), 6.82 (d, J = 8.9 Hz, 4H), 4.08 (t, J = 5.7 Hz, 4H), 3.75 – 3.71 (m, 8H), 2.79 (t, J = 5.6 Hz, 4H), 2.59 – 2.55 (m, 8H), 1.31 (s, 12H). ^{13}C NMR (100 MHz, MeOD-*d*$_4$) δ 155.57, 151.47, 140.41, 135.47, 127.07, 126.01, 117.94, 115.26, 66.22, 65.31, 57.38, 53.75, 23.89. HRMS (ESI): Calculated for $C_{36}H_{49}BN_3O_6$ $[M+H]^+$: 630.3714, found: 630.3715.

Synthesis of B-T-OH

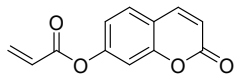
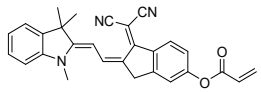
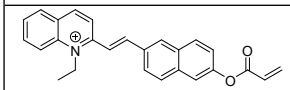
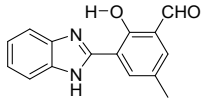
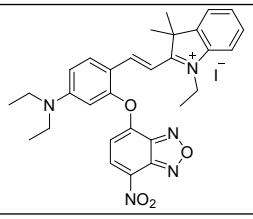
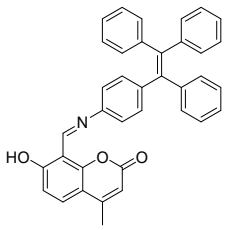
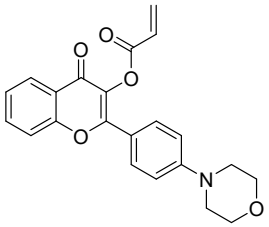
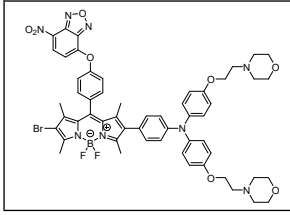
BODIPY-OH (238 mg, 0.48 mmol), compound **3** (300 mg, 0.48 mmol), $Pd(PPh_3)_4$ (50 mg, 0.043 mmol) and K_2CO_3 aqueous solution (2 M, 1.44 mL) were dissolved in toluene (20 mL). The reaction mixture was stirred and refluxed for 12 h under the

protection of Ar at 100 ℃. The mixture derived from the reaction was separated by column chromatography (eluent: DCM/MeOH = 40/1, v/v) and the product B-T-OH (66 mg) was obtained as a violet solid. Yield: 15%, M. p. = 134–136 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.10 (d, J = 8.5 Hz, 2H), 7.02 (d, J = 8.9 Hz, 4H), 6.94 (d, J = 8.5 Hz, 2H), 6.87 (s, 4H), 6.81 (d, J = 9.0 Hz, 4H), 4.13 (t, J = 5.3 Hz, 4H), 3.80 – 3.76 (m, 8H), 2.89 (s, 4H), 2.69 (s, 8H), 2.59 (s, 3H), 2.52 (s, 3H), 1.43 (s, 3H), 1.37 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.34, 154.94, 150.87, 147.76, 142.12, 141.05, 140.88, 138.49, 134.59, 132.32, 130.65, 130.49, 129.43, 126.82, 126.50, 124.83, 119.96, 116.58, 115.50, 110.53, 77.48, 77.16, 76.84, 66.59, 65.60, 57.76, 54.07, 14.26, 13.79, 13.57, 13.44. HRMS (ESI): Calculated for $C_{49}H_{54}BBrF_2N_5O_5$ $[M+H]^+$: 920.3369, found: 920.3379.

Synthesis of NBD-B-T

B-T-OH (42 mg, 0.046 mmol), NBD-Cl (10 mg, 0.05 mmol) and Et_3N (0.1 mL) were dissolved in anhydrous dichloromethane (10 mL). The reaction mixture was stirred and refluxed for 6 h under the protection of Ar. After the solvent was removed under reduced pressure, the residue product was separated by column chromatography (eluent: DCM/MeOH = 40/1, v/v). The target product NBD-B-T (15 mg) was obtained as violet solid. Yield: 30%, M. p. = 135–137 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.49 (d, J = 8.3 Hz, 1H), 7.51 (d, J = 8.4 Hz, 2H), 7.43 (d, J = 8.4 Hz, 2H), 7.07 (d, J = 8.8 Hz, 4H), 6.92 (s, 4H), 6.85 (d, J = 8.8 Hz, 4H), 6.61 (d, J = 8.3 Hz, 1H), 4.13 (s, 4H), 3.77 (s, 8H), 2.85 (s, 4H), 2.62 (d, J = 4.0 Hz, 11H), 2.56 (s, 3H), 1.50 (s, 3H), 1.44 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.47, 155.25, 153.77, 153.36, 151.62, 148.09, 145.10, 144.21, 140.72, 140.11, 139.32, 137.76, 135.29, 134.14, 133.05, 131.81, 131.30, 130.89, 130.49, 129.73, 126.92, 123.96, 121.96, 119.59, 115.51, 110.93, 108.44, 66.90, 66.01, 57.72, 54.10, 41.05,13.83, 13.57, 13.40. HRMS (ESI): Calculated for $C_{55}H_{55}BBrF_2N_8O_8$ $[M+H]^+$: 1083.3387, found: 1083.3383.

Table S1 Comparison of probe NBD-B-T with reported Cys/Hcy probes.

Cys/Hcy probe	Response type	Reaction Time	LOD	λ_{em}/nm	Organelle targeting	Ref
	turn on	Hcy: 8 h Cys: 60 min	Hcy:79 nM Cys:65 nM	455 nm	none	[1]
	turn on	Hcy: 20 min Cys: 20 min	Hcy: 3.46 μM Cys: 1.92 μM	625 nm	none	[2]
	turn off	Cys: 90 min	Cys: 1.97 μM	531 nm	none	[3]
	turn on	Hcy: 80 min	Hcy: 9.02 μM	468 nm	none	[4]
	turn on	Hcy: 6 min Cys: 10 s	Hcy: 2.81 μM Cys: 2.33 μM	592 nm	none	[5]
	ratiometric	Hcy: 30 min Cys: 30 min	Hcy: 0.554 μM Cys: 1.816 μM	452 nm 558 nm	none	[6]
	turn on	Cys: 5 min	Cys: 0.16 μM	510 nm	lysosome	[7]
	turn on	Hcy: 20 min Cys: 60 min	Hcy: 76.0 nM Cys: 97.6 nM	550 nm	lysosome	This work

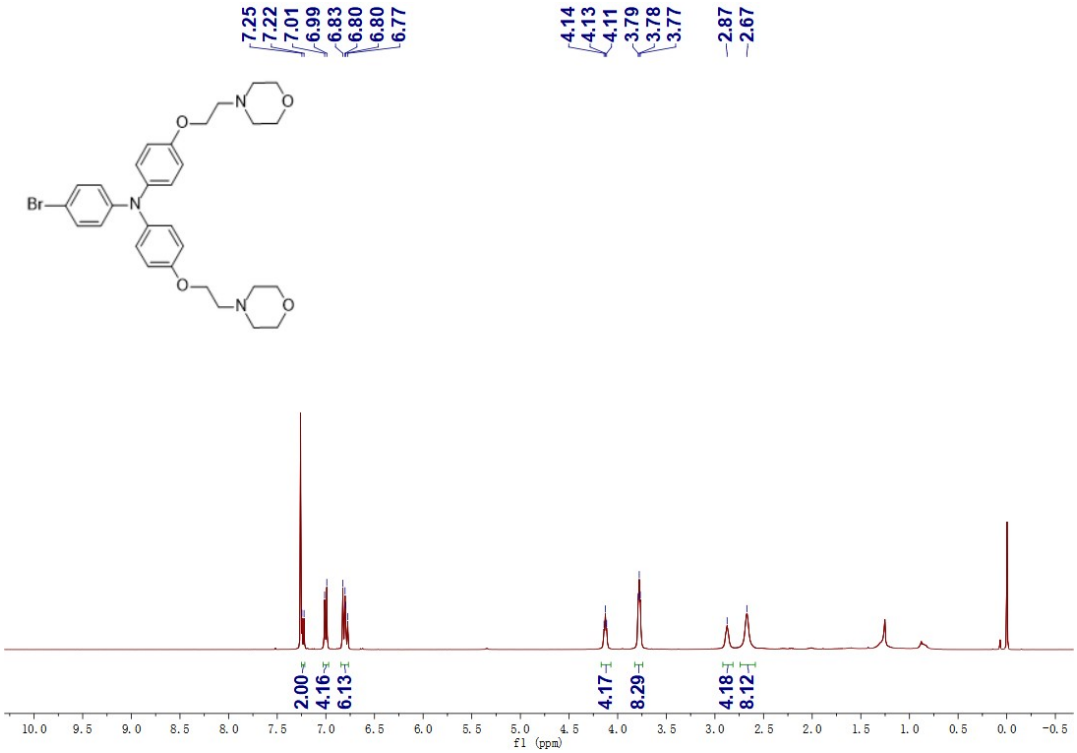


Fig. S1. ^{1}H NMR of compound **2**.

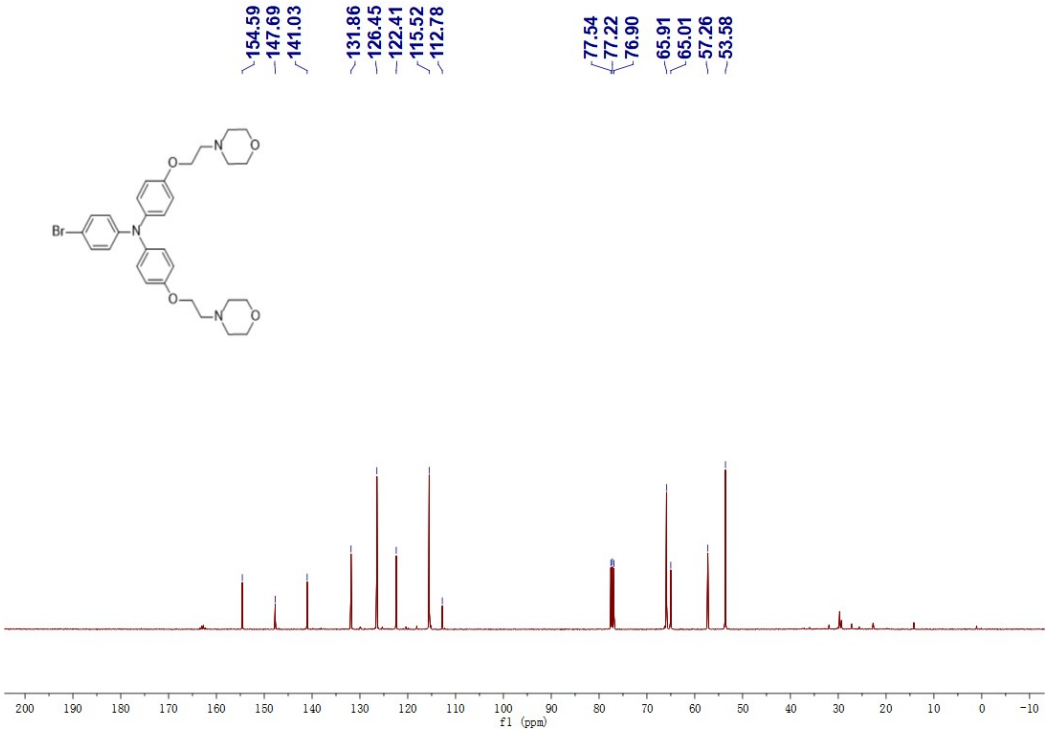


Fig. S2. ^{13}C NMR of compound **2**.

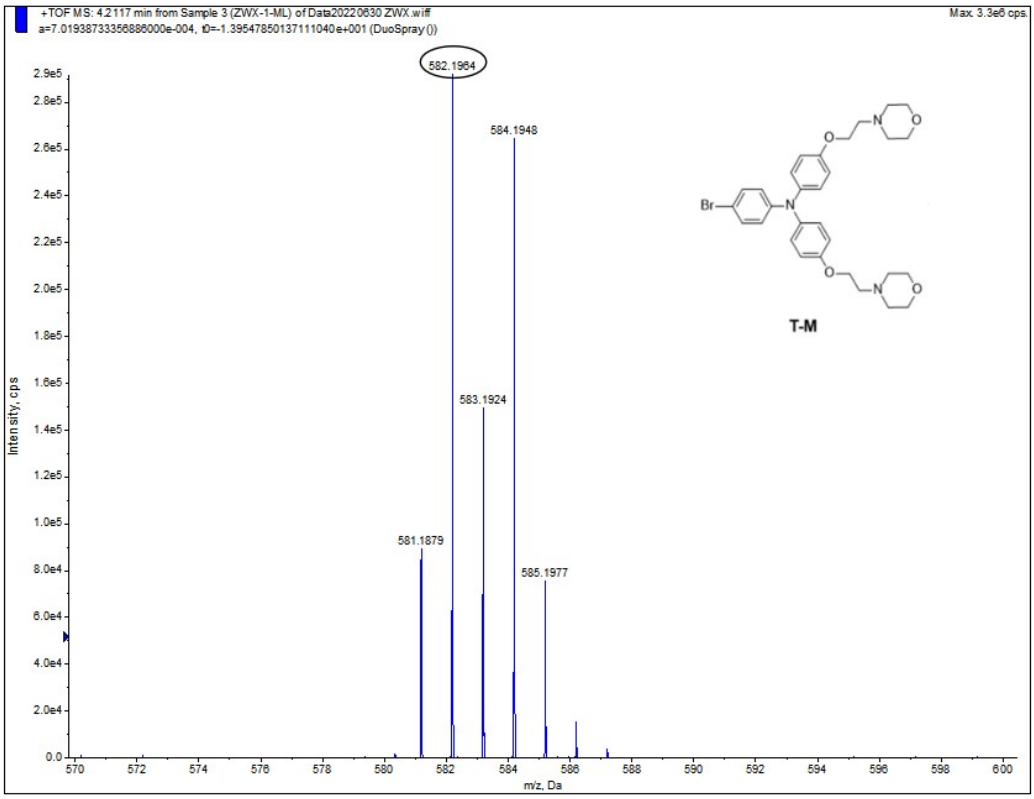


Fig. S3. HR-MS spectrum of compound **2**.

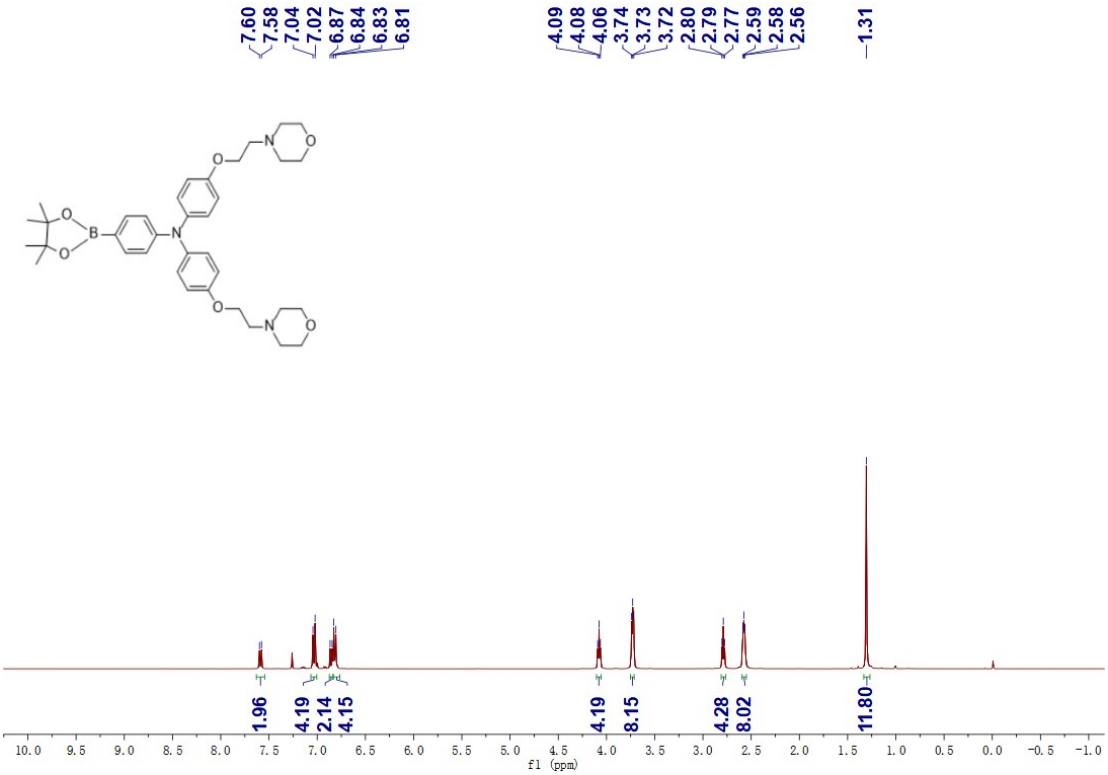


Fig. S4. ^{1}H NMR of compound **3**.

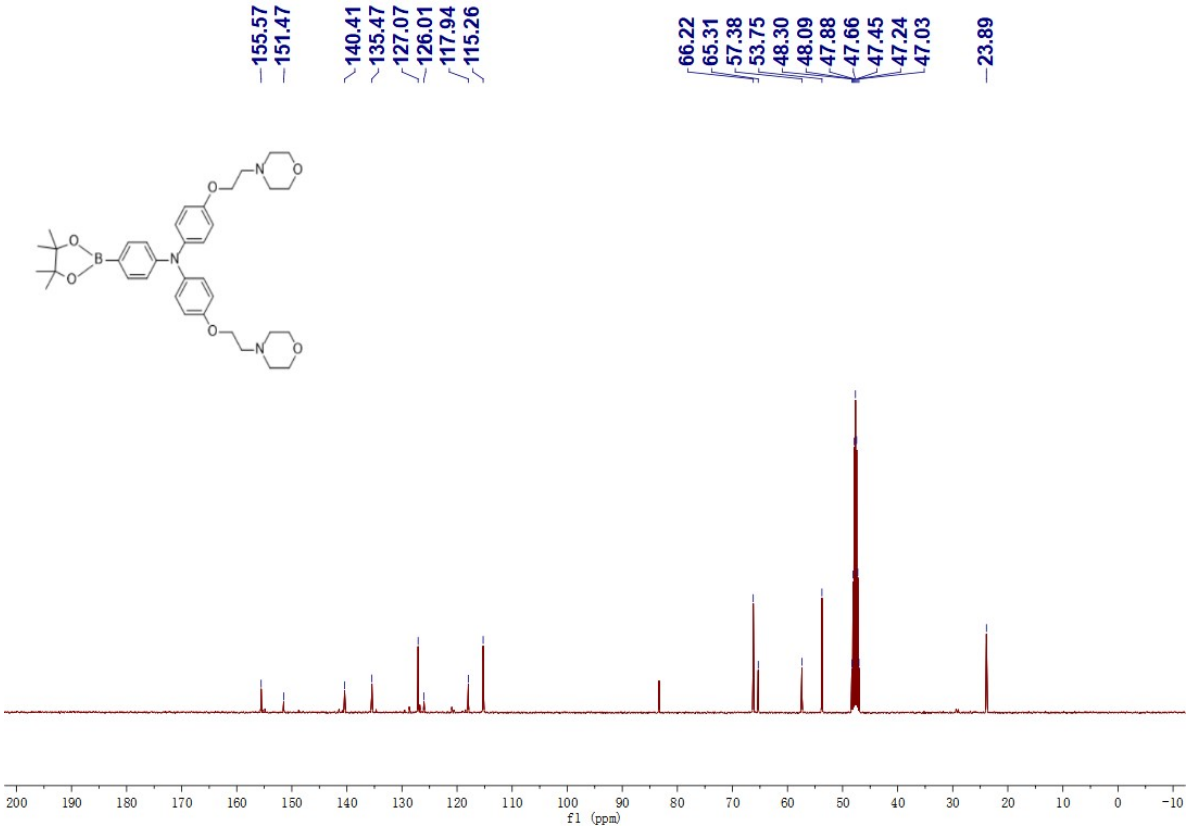


Fig. S5. ^{13}C NMR of compound **3**.

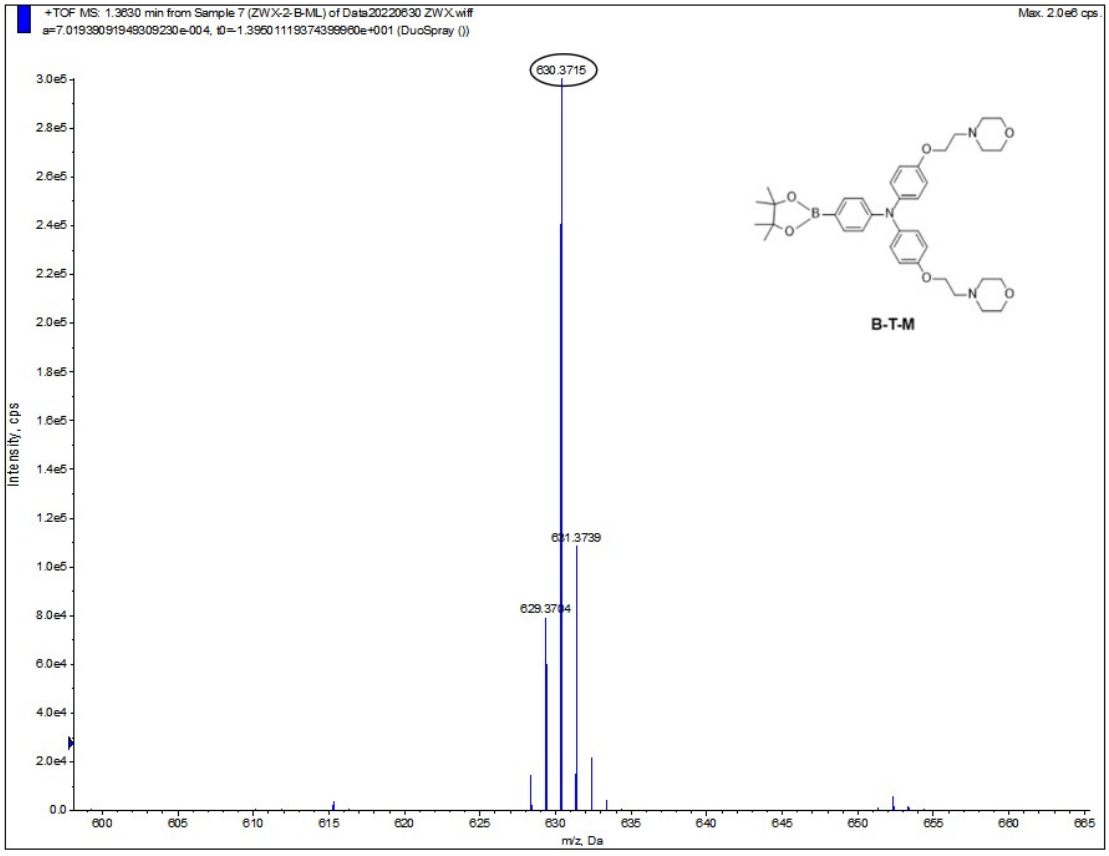


Fig. S6. HR-MS spectrum of compound **3**.

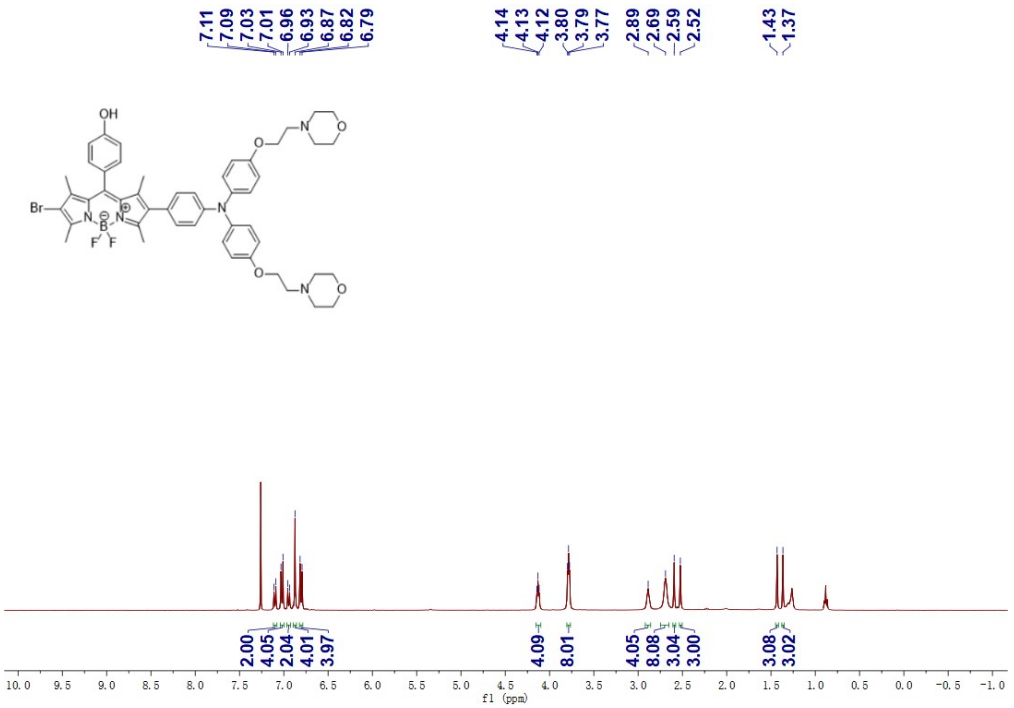


Fig. S7. ^{1}H NMR of compound B-T-OH.

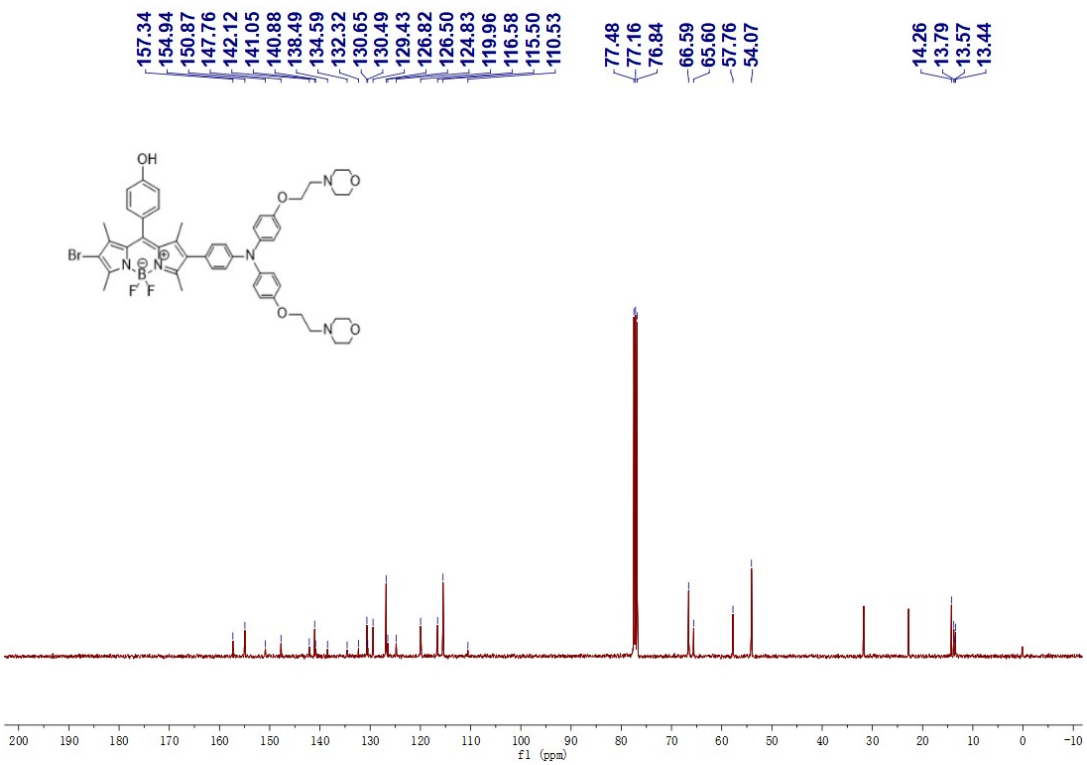


Fig. S8. ^{13}C NMR of compound B-T-OH.

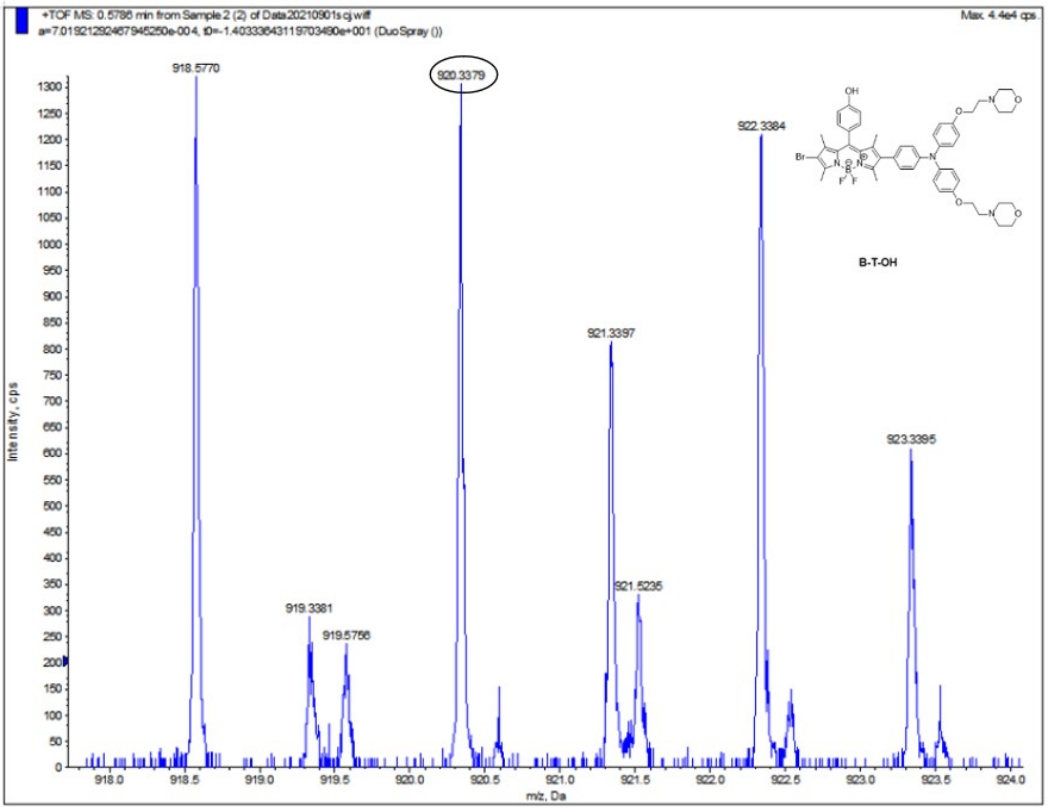


Fig. S9. HR-MS spectrum of compound B-T-OH.

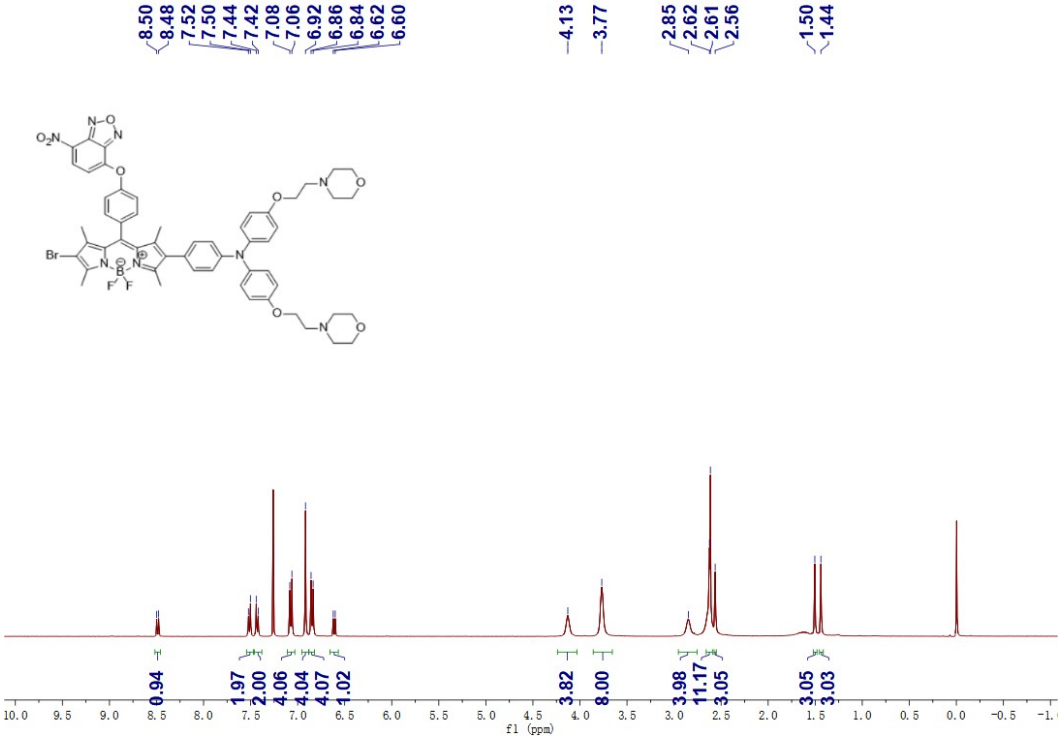


Fig. S10. ^{1}H NMR of compound NBD-B-T.

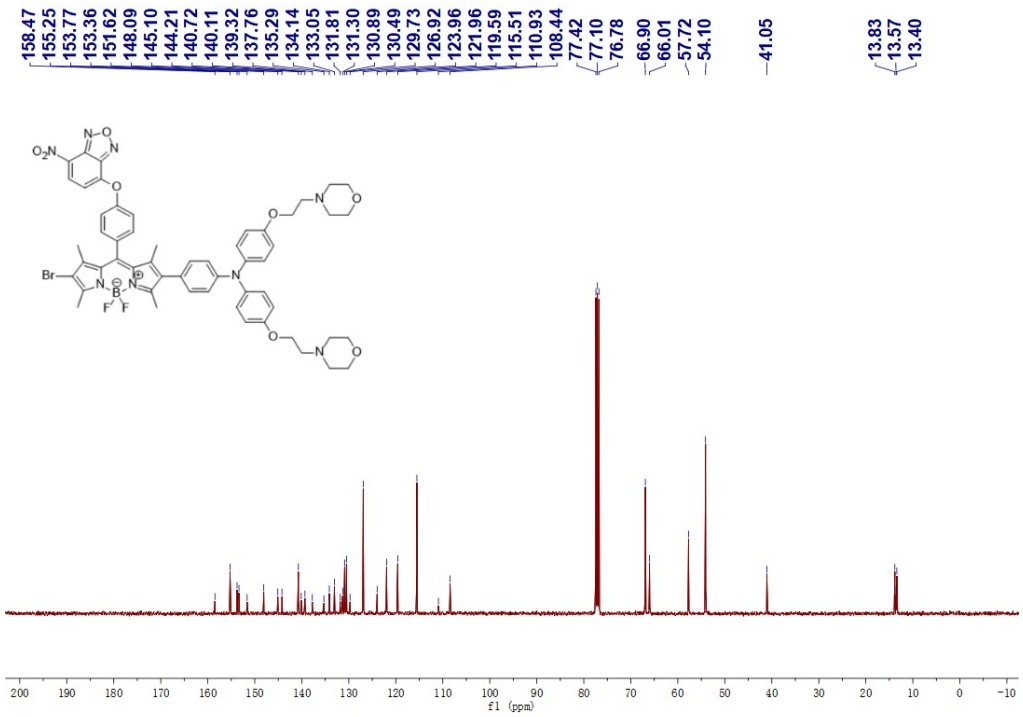


Fig. S11. ^{13}C NMR of compound NBD-B-T.

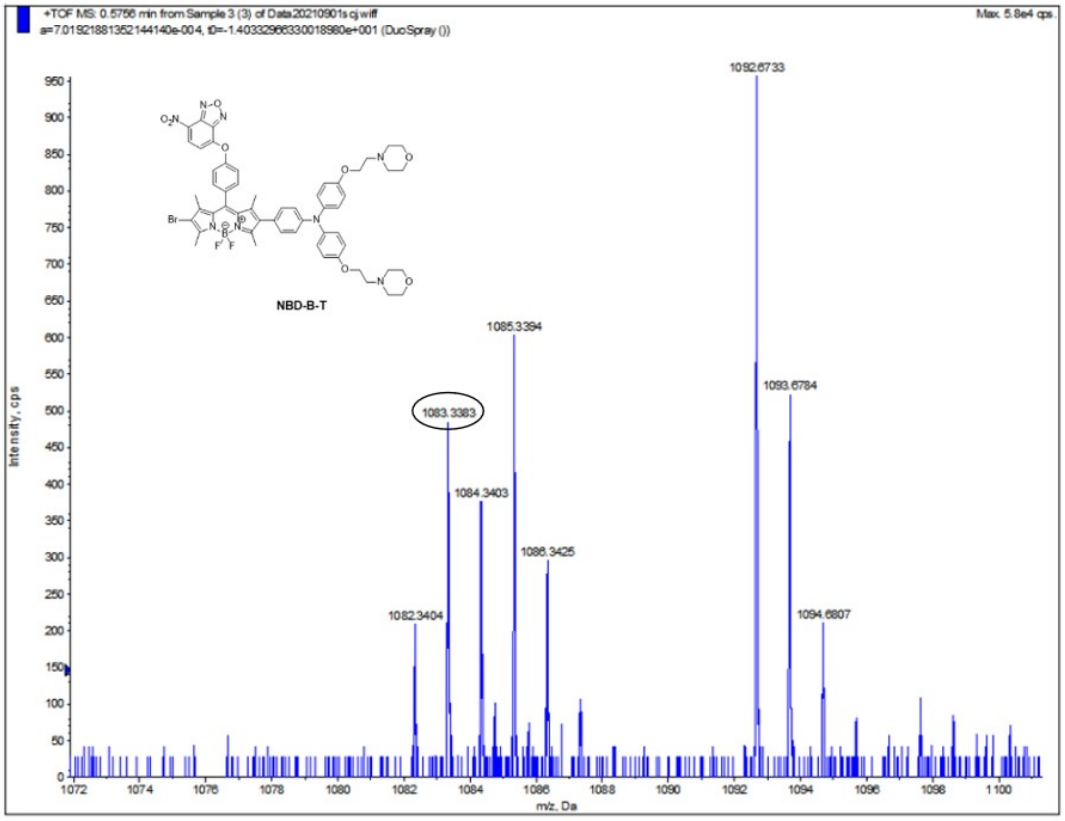


Fig. S12. HR-MS spectrum of compound NBD-B-T.

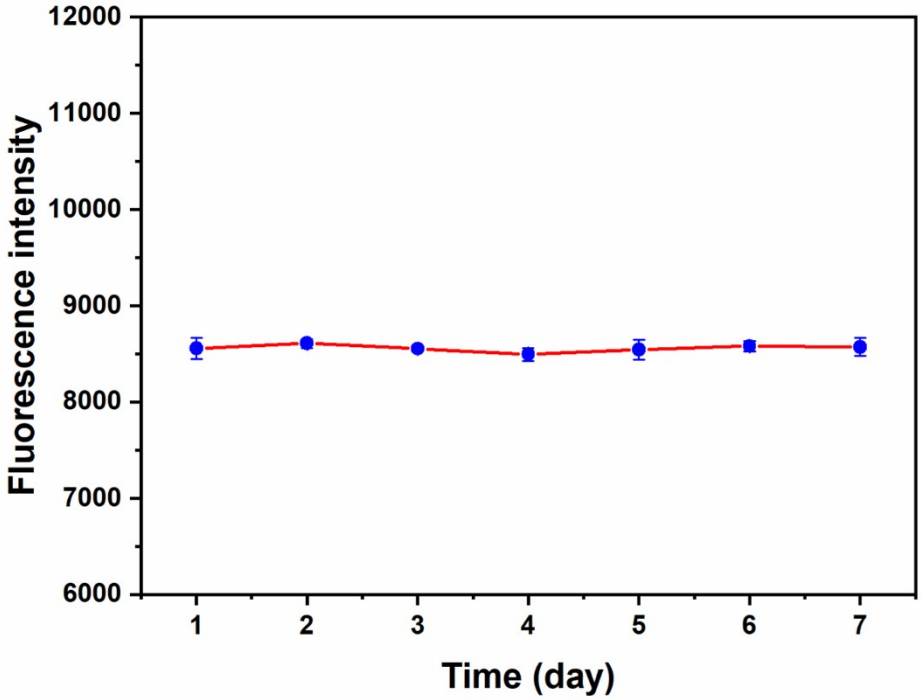


Fig. S13. Fluorescence intensity of NBD-B-T for seven days.

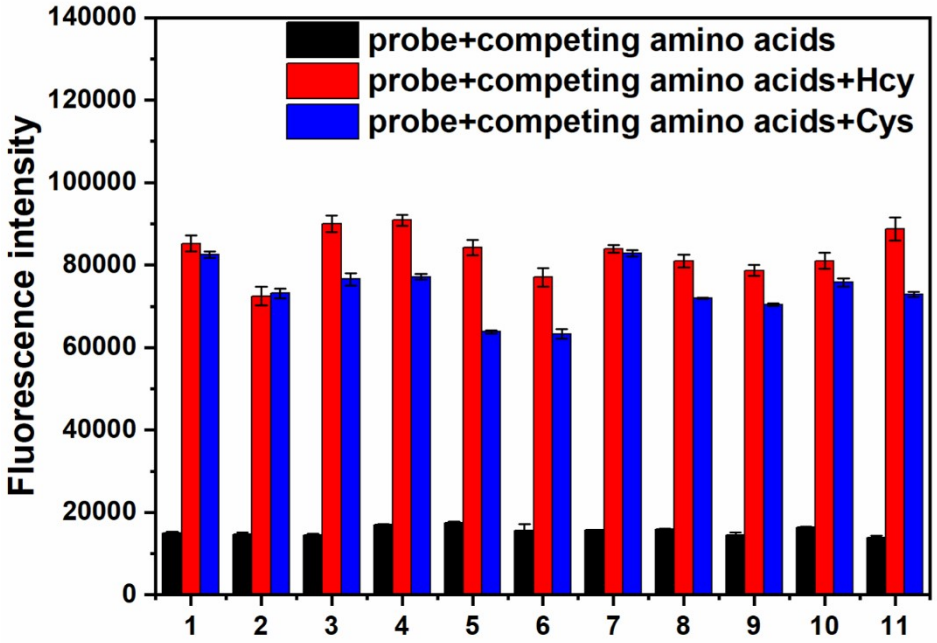


Fig. S14. The fluorescence response of NBD-B-T (10 μM) toward Cys (blue) and Hcy (red) and various analytes (500 μM) in DMF/PBS (v/v =1/1, pH = 7.4). λ_{ex} = 365 nm. Black bars represent the solution of NBD-B-T (10 μM) in the presence of various analytes (500 μM). Red bars, blue bars represent the addition of Hcy and Cys (500 μM) to the above solution, respectively. Analytes: 1. Glu, 2. His, 3. Leu, 4. Met, 5. Phe, 6. Pro, 7. Ser, 8. Thr, 9. Val, 10. Gly, 11. Sar.

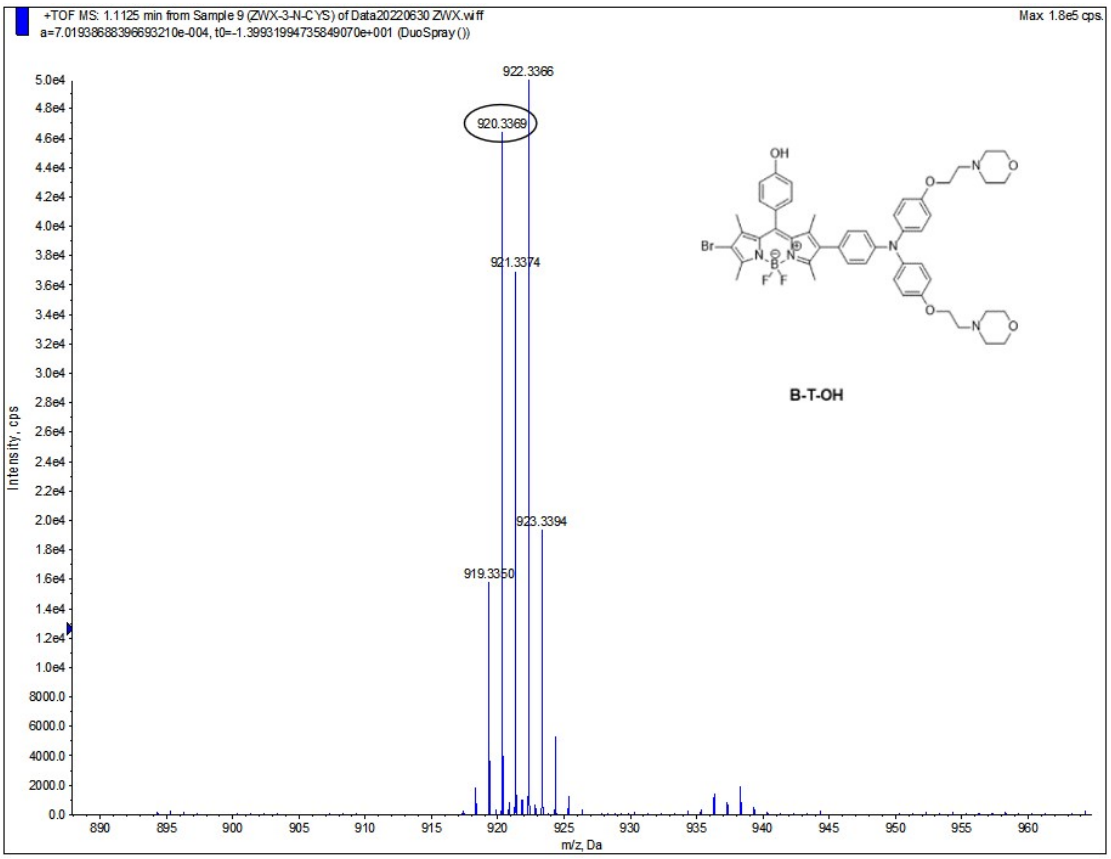


Fig. S15. HR-MS of NBD-B-T + Cys.

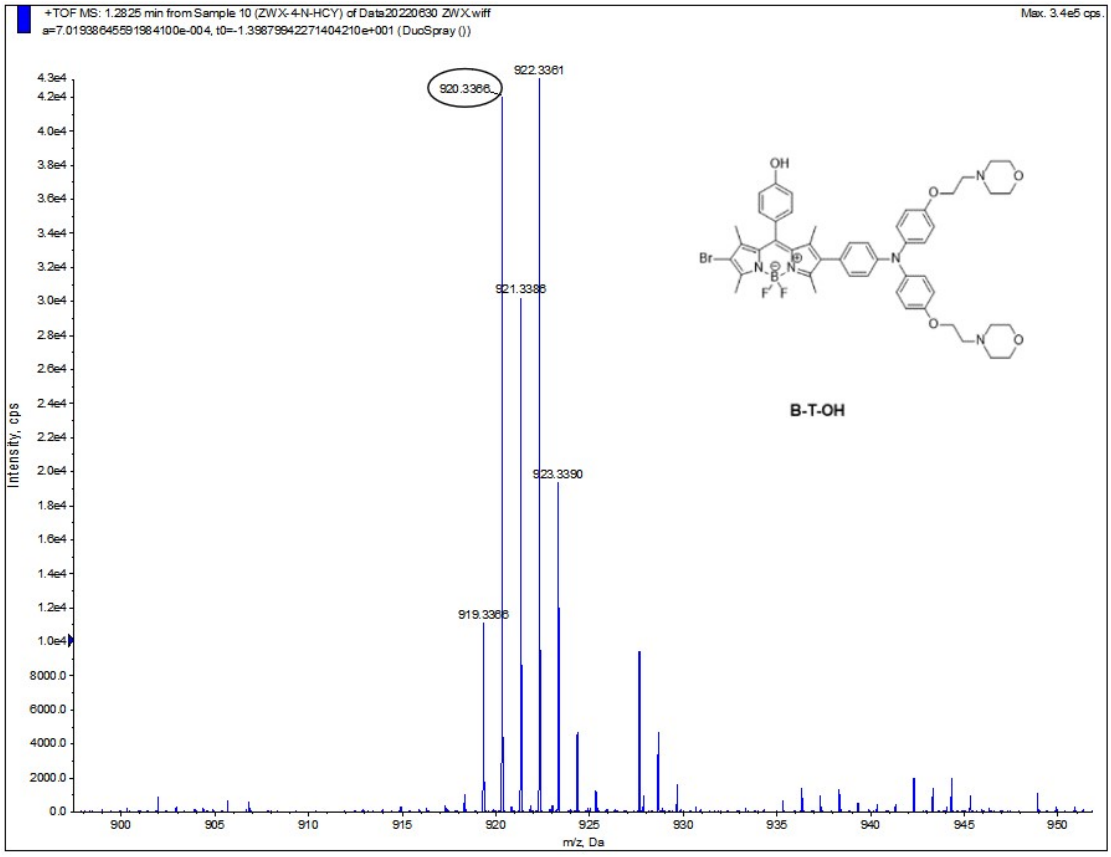


Fig. S16. HR-MS of NBD-B-T + Hcy.

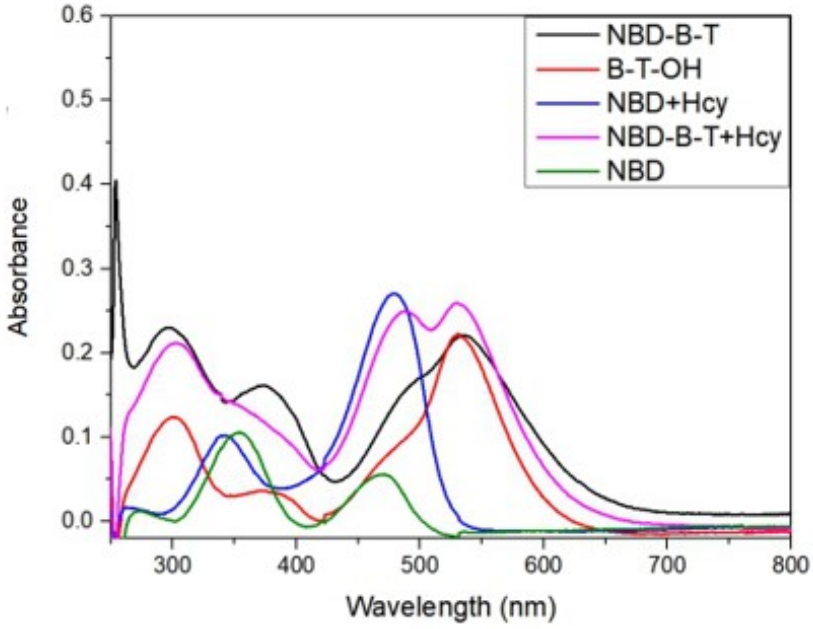


Fig. S17. Absorption spectra of probe NBD-B-T (10 μM), B-T-OH (10 μM), NBD-B-T (10 μM) +Hcy (500 μM), NBD (10 μM) +Hcy (500 μM) and NBD (10 μM) in DMF/PBS (v/v =1/1, pH = 7.4).

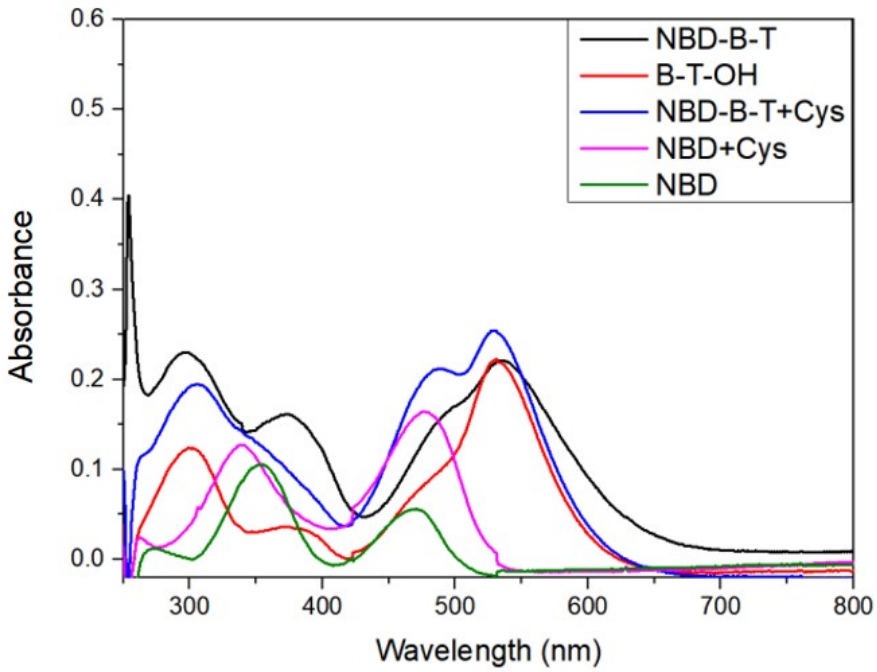


Fig. S18. Absorption spectra of probe NBD-B-T (10 μM), B-T-OH (10 μM), NBD-B-T (10 μM) +Cys (500 μM), NBD (10 μM) +Cys (500 μM) and NBD (10 μM) in DMF/PBS (v/v =1/1, pH = 7.4).

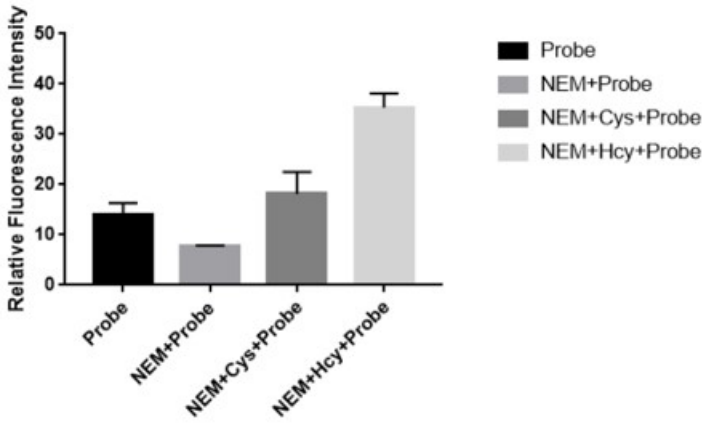


Fig. S19. Relative fluorescence intensity of Fig. 9a2-9d2.

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