

A broadly applicable strategy for the fluorescence based detection and differentiation of glutathione and cysteine/homocysteine: Demonstration in-vitro and in-vivo

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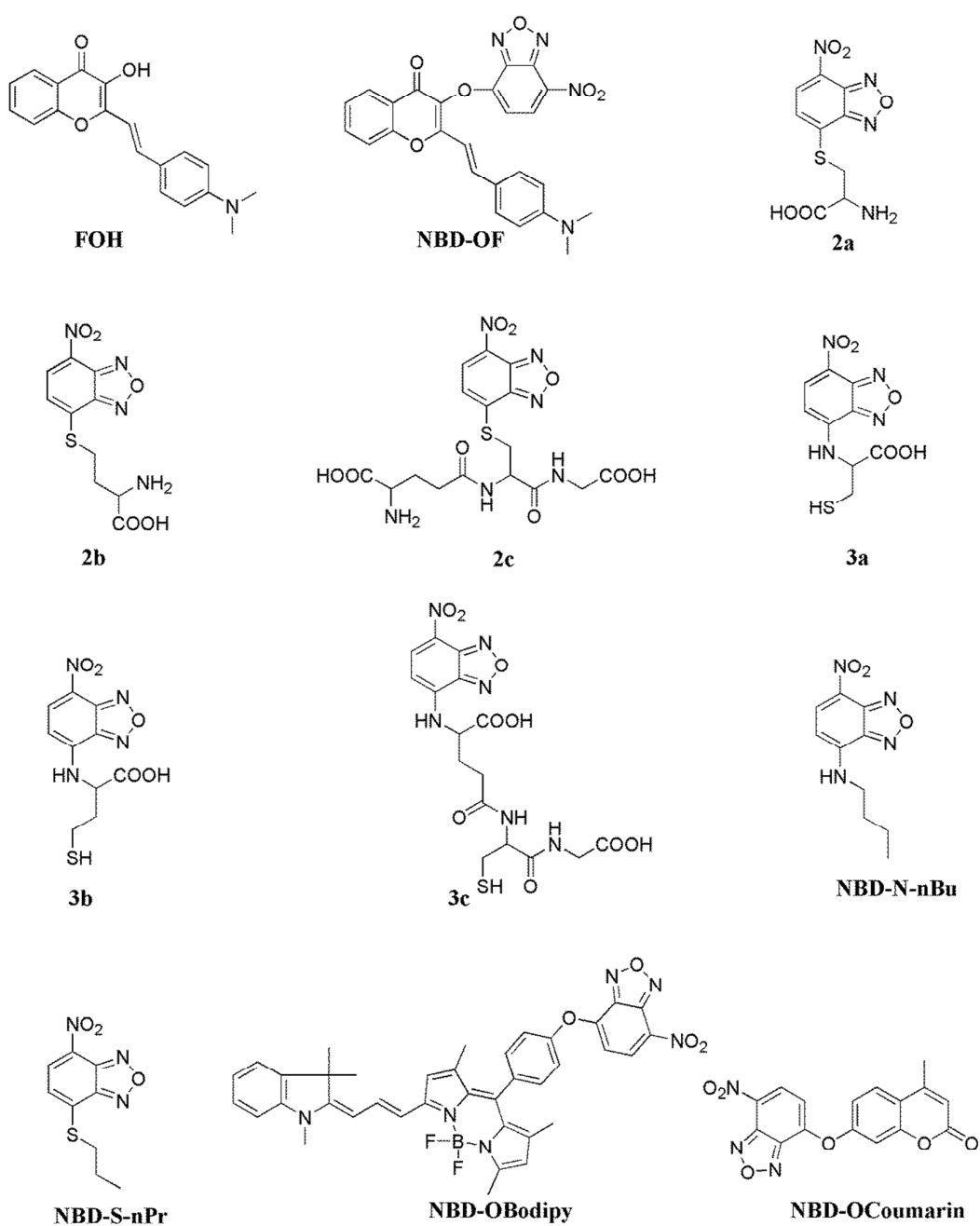


Figure S1. Chemical structures and the corresponding names of the compounds in this work.

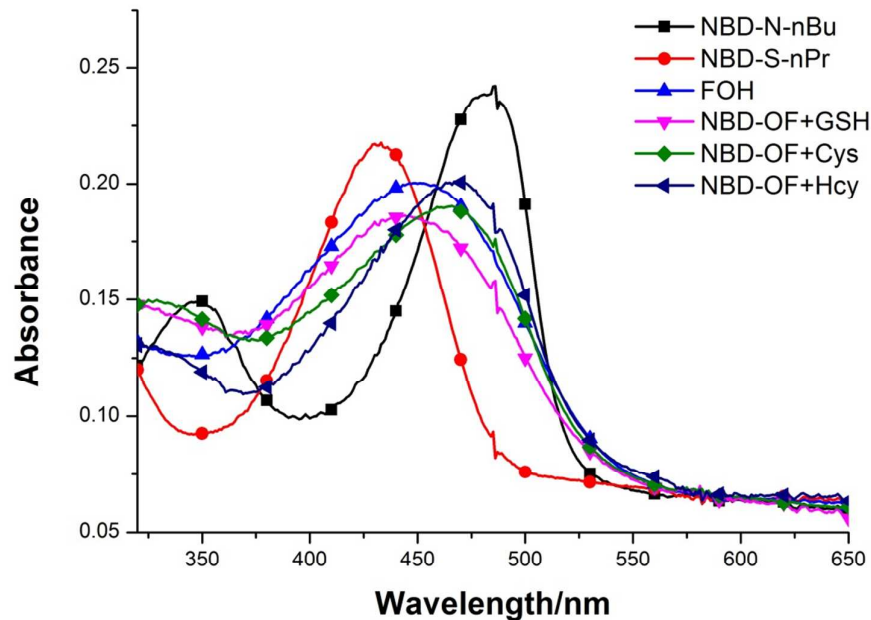


Figure S2. UV-vis absorption spectra of **NBD-N-nBu** (10.0 μ M), **NBD-S-nPr** (10.0 μ M), **FOH** (10.0 μ M) and **NBD-OF** (10.0 μ M) with 100 equiv. of Cys, Hcy and GSH in PBS buffer (10 mM, pH 7.4, containing 30% CH₃CN, v/v).

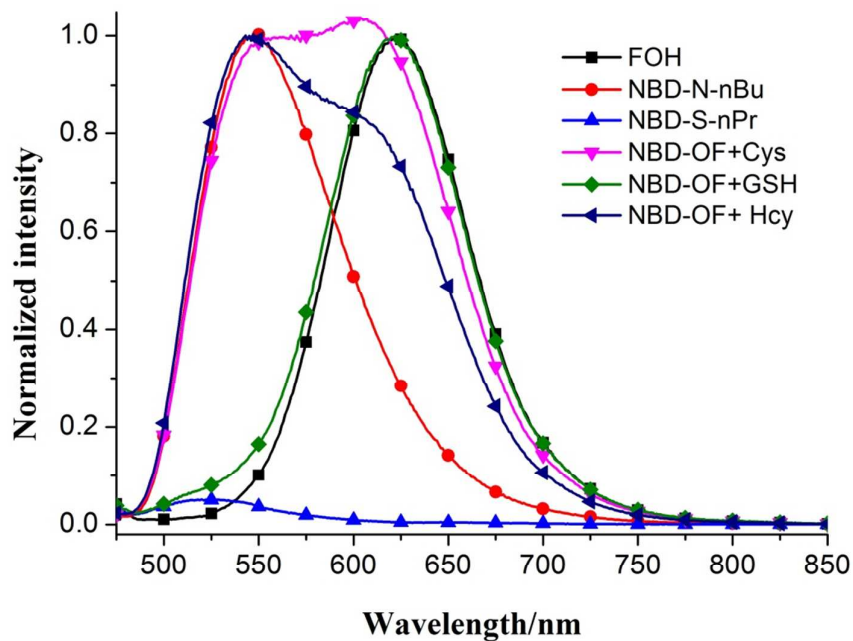


Figure S3. Normalized fluorescence spectra of **FOH**, **NBD-N-nBu**, **NBD-S-nPr**, **NBD-OF** with 100 equiv. of Cys, GSH and Hcy in PBS buffer (10 mM, pH 7.4, containing 30% CH₃CN, v/v)

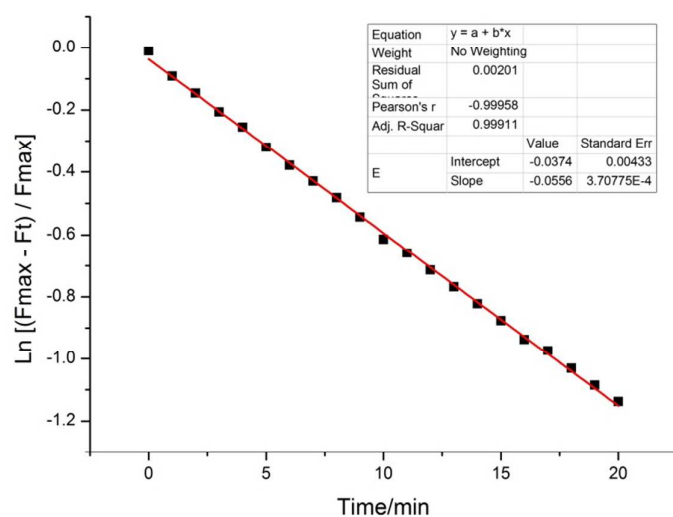


Figure S4. The natural log of fluorescence intensity change plotted as a function of time for the reaction of **NBD-OF** (5.0 μM) with Cys (100 equiv.) in PBS buffer (10.0 mM, pH 7.4, containing 30% CH_3CN , v/v). Slope = $5.56 \times 10^{-2} \text{ min}^{-1}$.

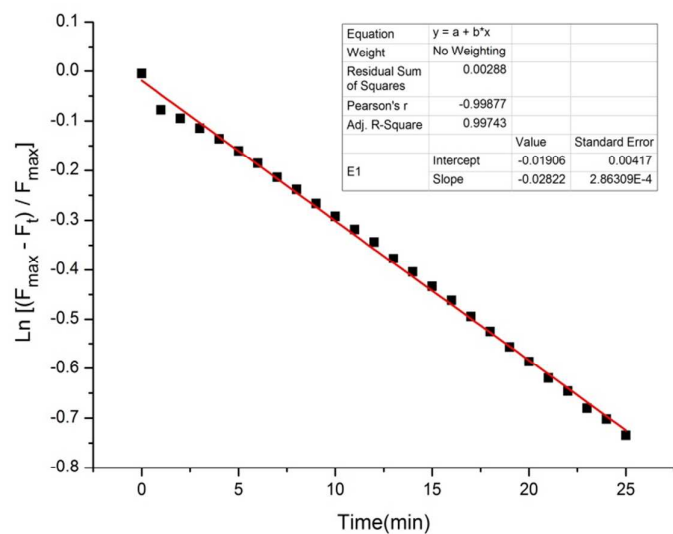


Figure S5. The natural log of fluorescence intensity change plotted as a function of time for the reaction of **NBD-OF** (5.0 μM) with Hcy (100 equiv.) in PBS buffer (10.0 mM, pH 7.4, containing 30% CH_3CN , v/v). Slope = $2.82 \times 10^{-2} \text{ min}^{-1}$.

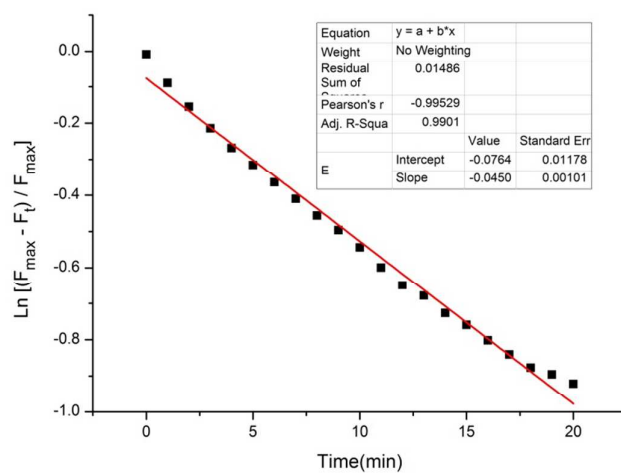


Figure S6. The natural log of fluorescence intensity change plotted as a function of time for the reaction of **NBD-OF** ($5.0 \mu\text{M}$) with GSH (100 equiv.) in PBS buffer (10.0 mM, pH 7.4, containing 30% CH_3CN , v/v). Slope = $4.50 \times 10^{-2} \text{ min}^{-1}$.

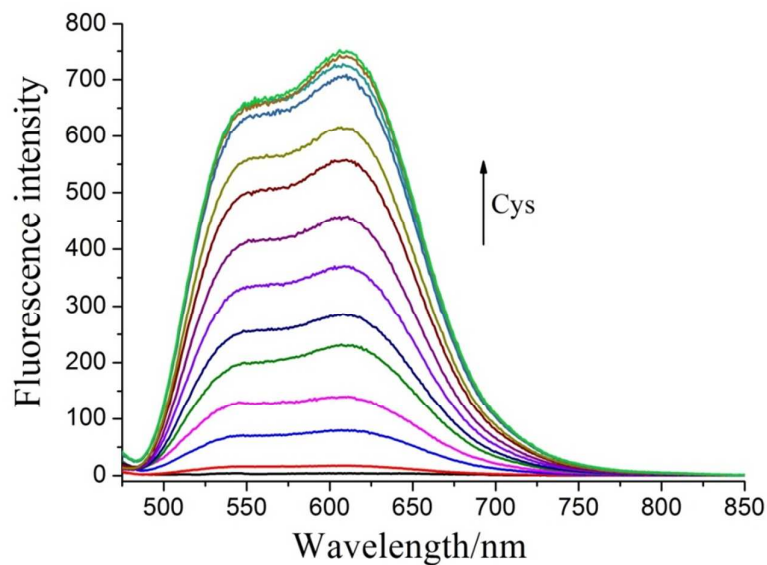


Figure S7. Fluorescence spectra of **NBD-OF** ($5.0 \mu\text{M}$) with different concentrations of Cys (0.0-600.0 μM) in PBS buffer (10 mM, pH 7.4, containing 30% CH_3CN , v/v).

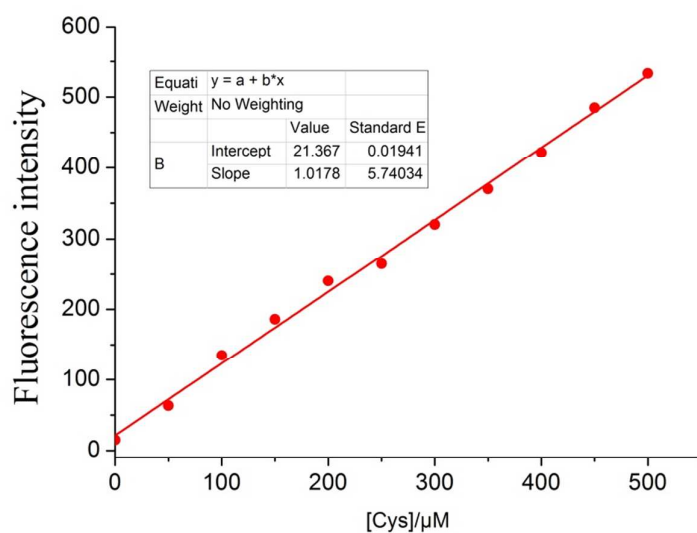


Figure S8. The linear relationship between the fluorescence intensity of **NBD-OF** (5.0 μM) at 545 nm and the concentration of Cys (0.0–500.0 μM) in PBS buffer (10 mM, pH 7.4, containing 30% CH_3CN , v/v).

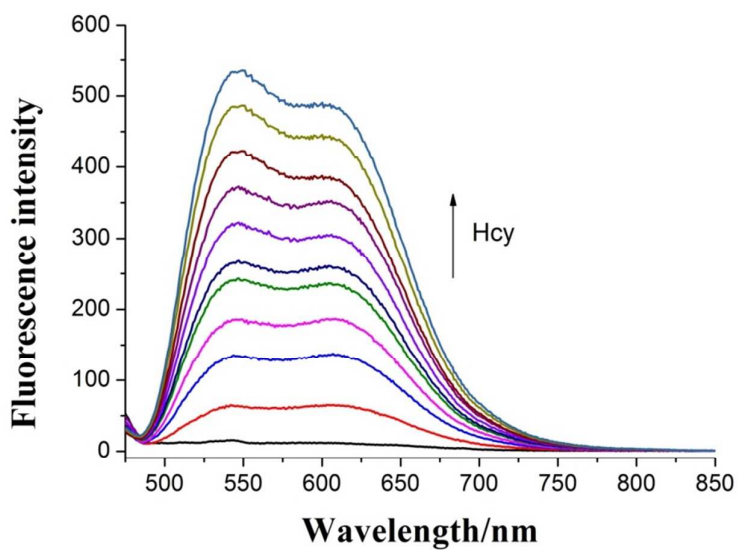


Figure S9. Fluorescence spectra of **NBD-OF** with different concentrations of Hcy (0.0–500.0 μM) in PBS buffer (10 mM, pH 7.4, containing 30% CH_3CN , v/v).

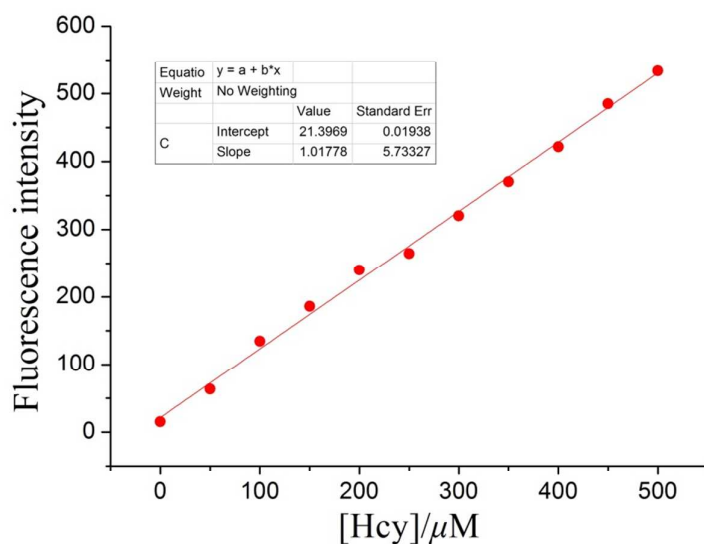


Figure S10. The linear relationship between the fluorescence intensity of **NBD-OF** (5.0 μM) at 545 nm and the concentrations of Hcy (0.0–500.0 μM) in PBS buffer (10 mM, pH 7.4, containing 30% CH_3CN , v/v).

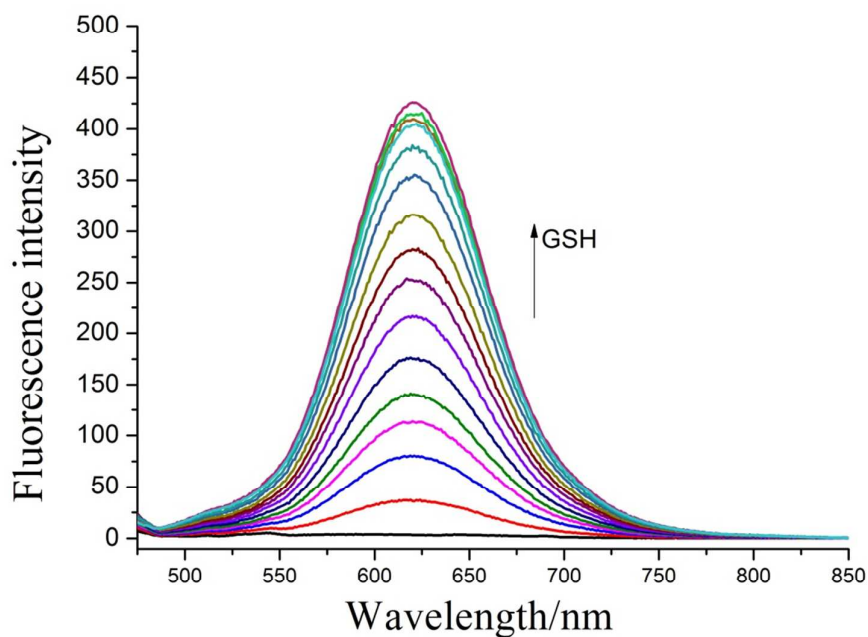


Figure S11. Fluorescence spectra of **NBD-OF** after addition of increasing concentrations of GSH (0.0–700.0 μM) in PBS buffer (10 mM, pH 7.4, containing 30% CH_3CN , v/v).

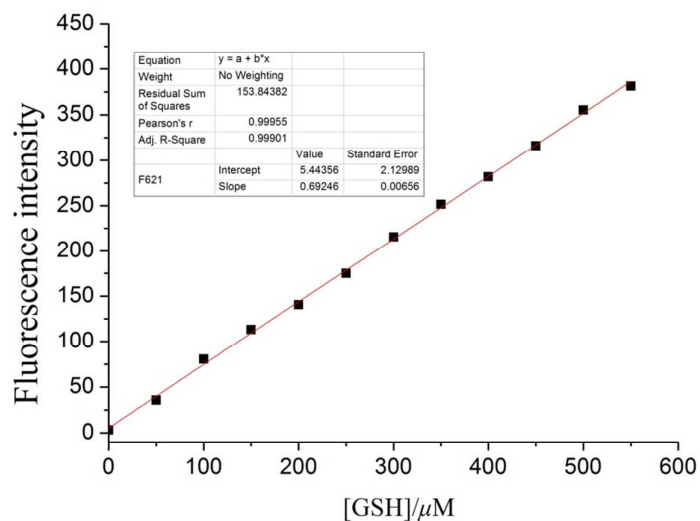


Figure S12. The linear relationship between the fluorescence intensity of **NBD-OF** (5.0 μM) at 545 nm and the concentrations of GSH (0.0–550.0 μM) in PBS buffer (10 mM, pH 7.4, containing 30% CH_3CN , v/v).

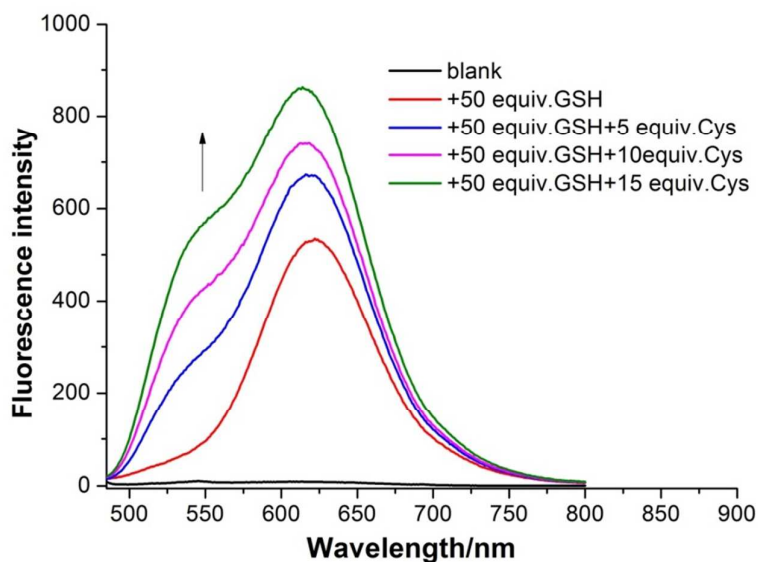


Figure S13. The fluorescence spectra of **NBD-OF** (5 μM) after incubation with 50 equiv. of GSH and different concentrations of Cys (0.0 μM , 25.0 μM , 50.0 μM , 75.0 μM) in PBS buffer (10.0 mM, pH 7.4, containing 30% CH_3CN , v/v) for 2 hours.

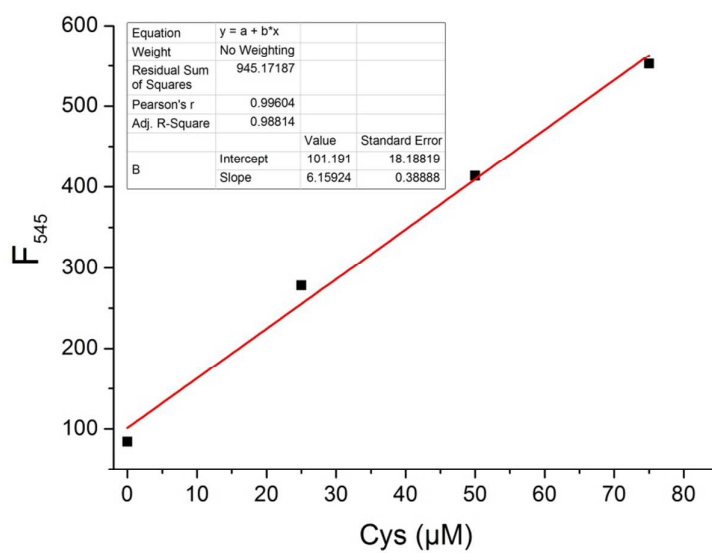


Figure S14. The linear relationship between the fluorescence intensity of **NBD-OF** (5.0 μM) at 545 nm and the concentrations of Cys (0.0–75.0 μM) in PBS buffer (10 mM, pH 7.4, containing 30% CH₃CN, v/v).

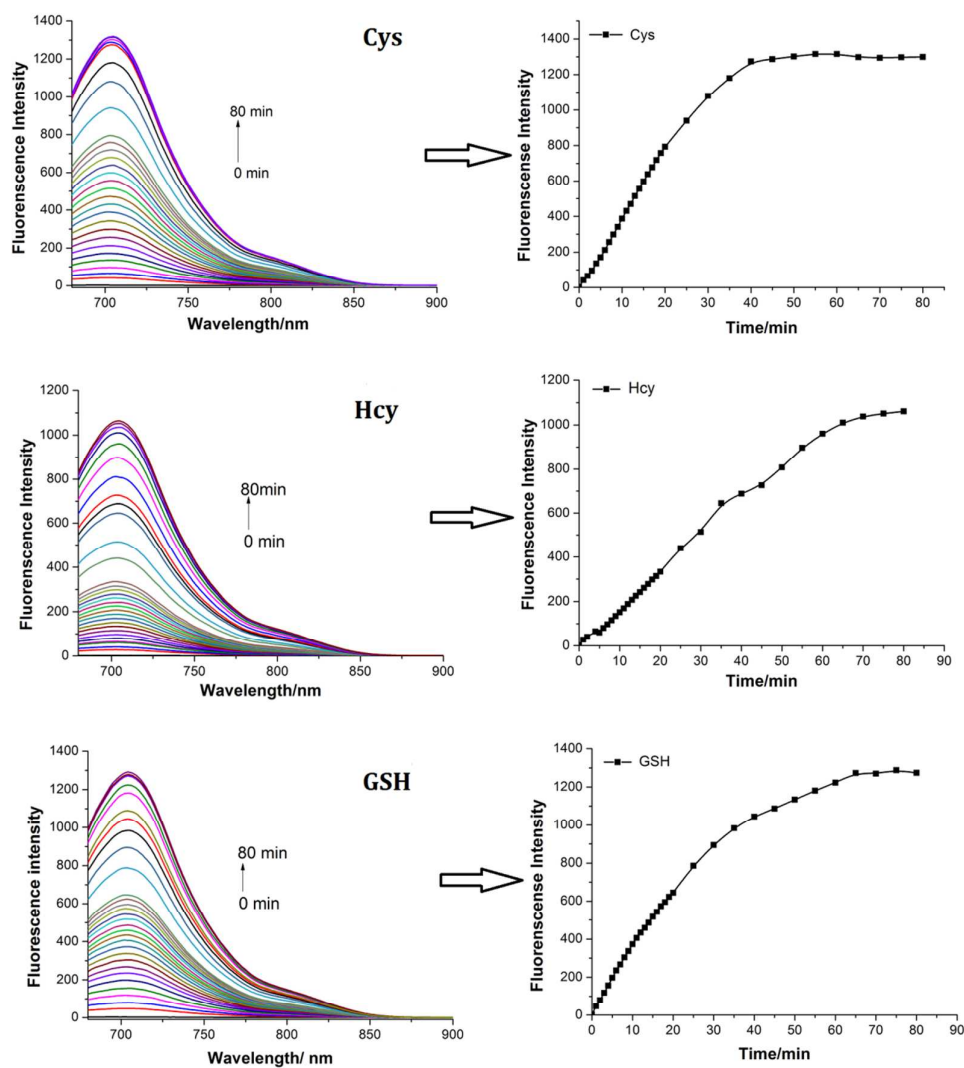


Figure S15. Time-dependent fluorescence spectra (left rows) and the corresponding fluorescence intensity changes at 705nm (right rows) of **NBD-OBodipy** (5.0 μ M) in the presence of 10.0 equiv. of Cys, Hcy and GSH in PBS buffer (10 mM, pH 7.4, containing 1.0 mM CTAB). Excitation wavelength is at 650 nm.

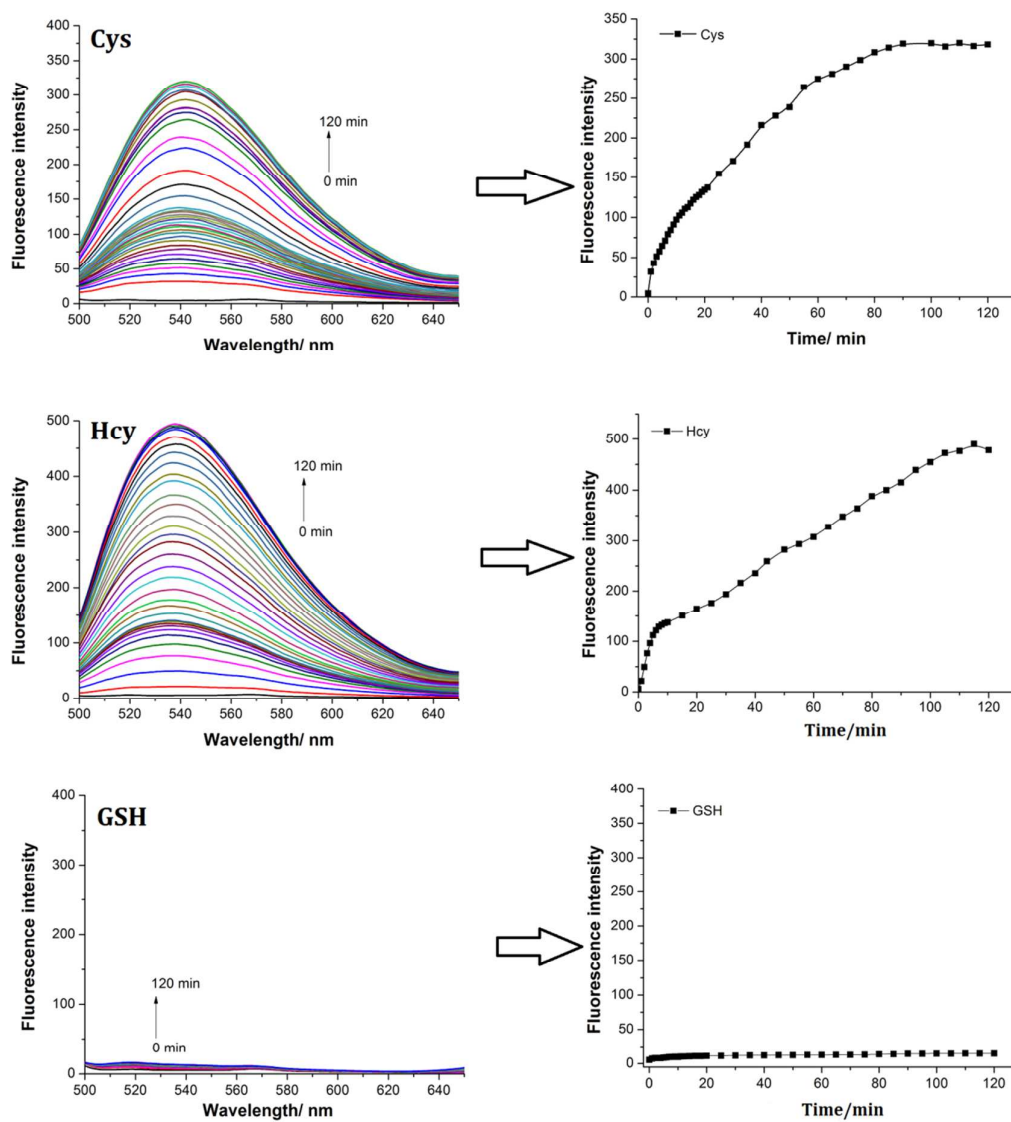


Figure S16. Time-dependent fluorescence spectra (left rows) and the corresponding fluorescence intensity changes at 545nm (right rows) of **NBD-OBodipy** (5.0 μM) in the presence of 10.0 equiv. of Cys, Hcy and GSH in PBS buffer (10 mM, pH 7.4, containing 1.0 mM CTAB). Excitation wavelength is at 476 nm.

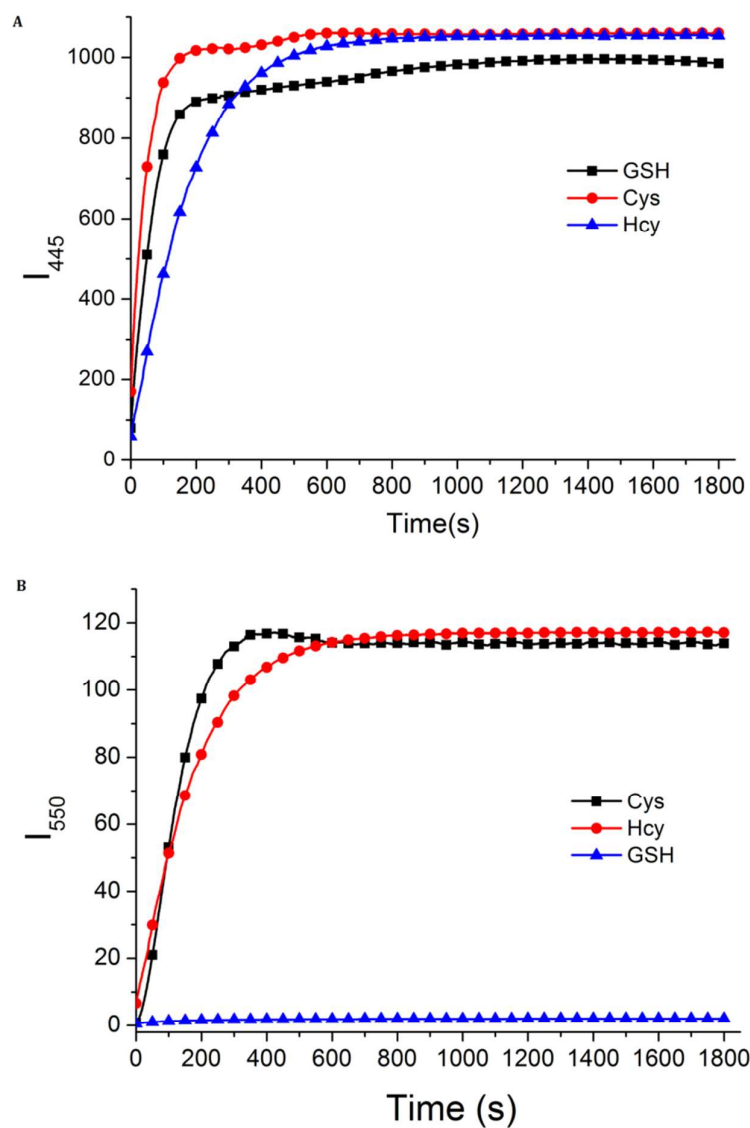


Figure S17. Time-dependent fluorescence intensity changes of **NBD-OCoumarin** toward 20.0 equiv. of Cys, Hcy and GSH when excited at 380 nm (A) and 476 nm (B) in PBS buffer (10 mM, pH 7.4, containing 30% CH₃CN, v/v).

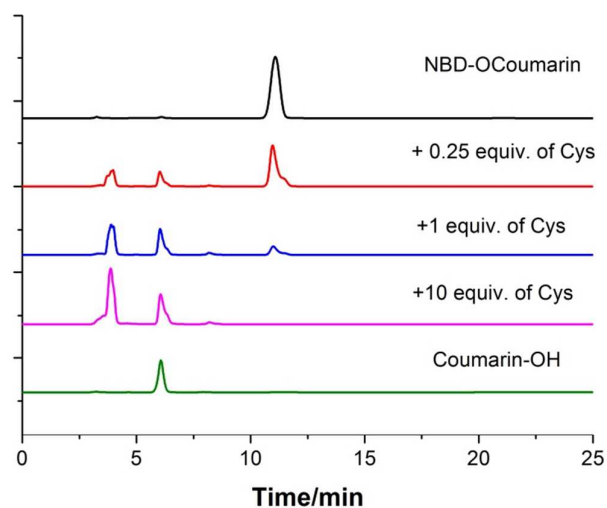


Figure S18. HPLC chromatograms of **NBD-OCoumarin**, **NBD-OCoumarin** with different amounts of Cys measured after 30 min mixing, and **Coumarin-OH**. The concentrations of both **NBD-OCoumarin** and **Coumarin-OH** were 100.0 μM . Conditions: eluent, $\text{CH}_3\text{CN}/\text{H}_2\text{O}$; gradient, 70% CH_3CN ; flow rate, 0.5 mL/min; temperature, 25 $^\circ\text{C}$; detection wavelength, 350 nm; injection volume, 20.0 μL .

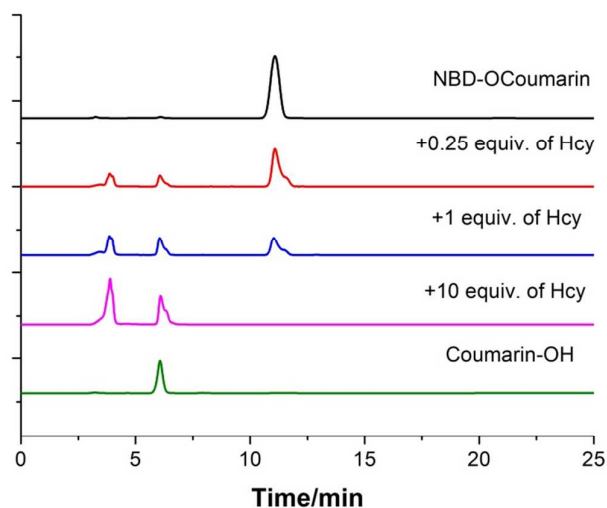


Figure S19. HPLC chromatograms of **NBD-OCoumarin**, **NBD-OCoumarin** with different amounts of Hcy measured after 30 min mixing, and **Coumarin-OH**. The concentrations of both **NBD-OCoumarin** and **Coumarin-OH** were 100.0 μM . Conditions: eluent, $\text{CH}_3\text{CN}/\text{H}_2\text{O}$; gradient, 70% CH_3CN ; flow rate, 0.5 mL/min; temperature, 25 $^\circ\text{C}$; detection wavelength, 350 nm; injection volume, 20.0 μL .

temperature, 25 °C; detection wavelength, 350 nm; injection volume, 20.0 μ L.

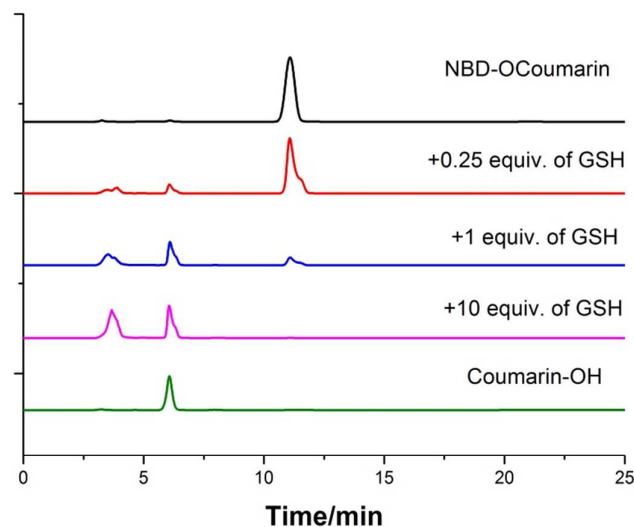


Figure S20. HPLC chromatograms of **NBD-OCoumarin**, **NBD-OCoumarin** with different amounts of GSH measured after 30 min mixing, and **Coumarin-OH**. The concentrations of both **NBD-OCoumarin** and **Coumarin-OH** were 100.0 μ M. Conditions: eluent, CH₃CN/H₂O; gradient, 70% CH₃CN; flow rate, 0.5 mL/min; temperature, 25 °C; detection wavelength, 350 nm; injection volume, 20.0 μ L.

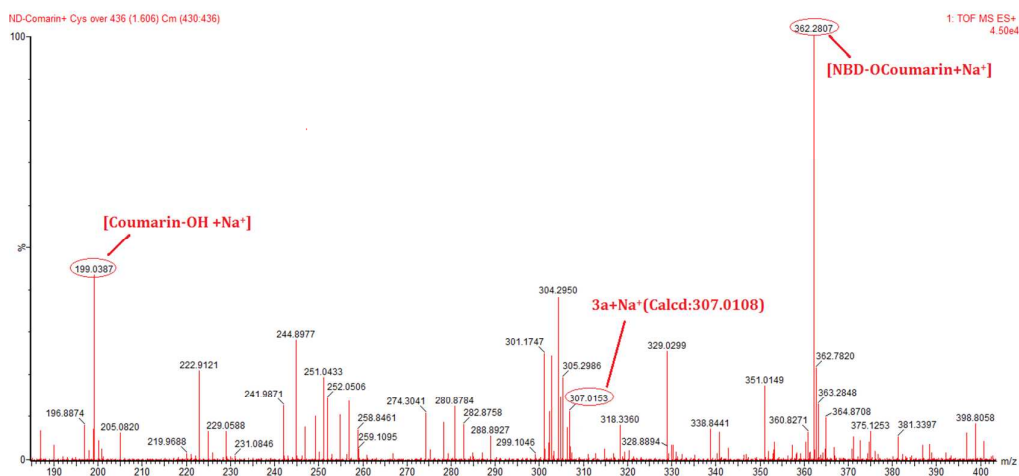


Figure S21. HRMS spectrum of **NBD-OCoumarin** with 5.0 equiv. of Cys in PBS buffer (10 mM, pH 7.4, containing 30% CH₃CN).

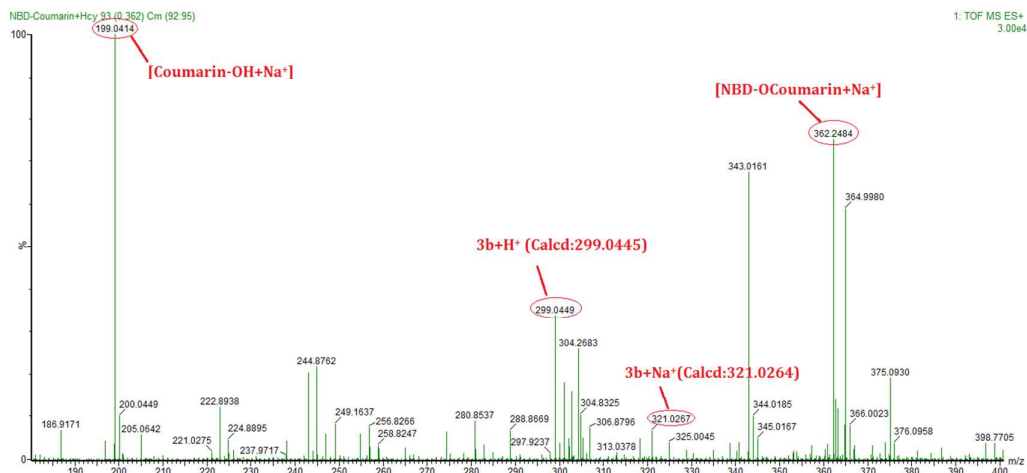


Figure S22. HRMS spectrum of NBD-OCoumarin with 5.0 equiv. of Hcy in PBS buffer (10 mM, pH 7.4, containing 30% CH₃CN).

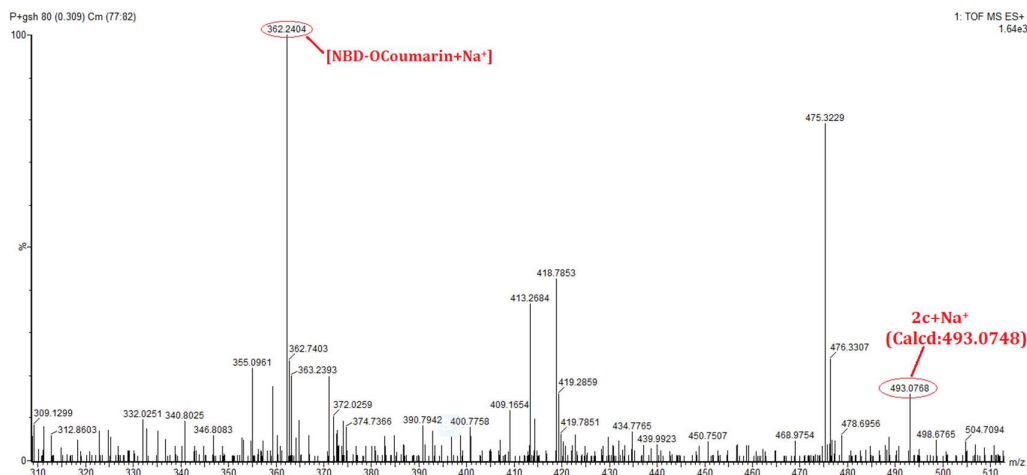


Figure S23. HRMS spectrum of NBD-OCoumarin with 5.0 equiv. of GSH in PBS buffer (10 mM, pH 7.4, containing 30% CH₃CN).

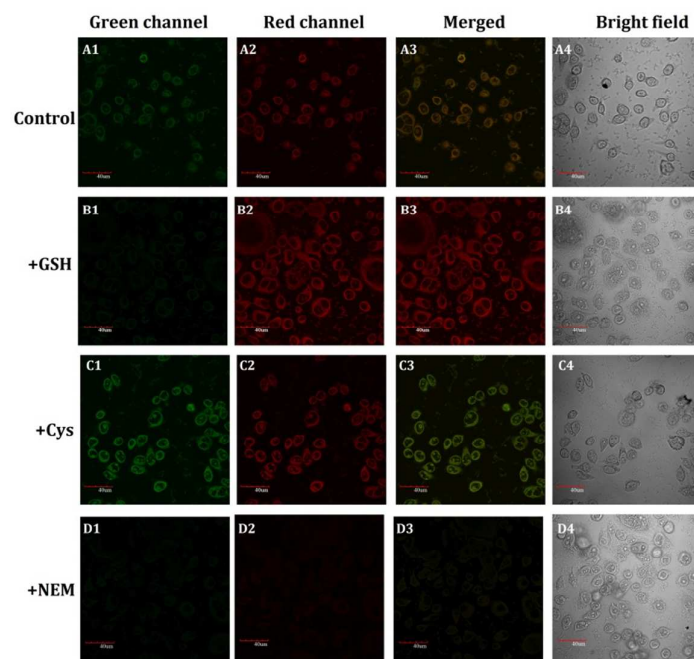


Figure S24. Confocal microscopic images of Cys and GSH in HeLa cells. (A1-A4) HeLa cells incubated with 5.0 μM **NBD-OBodipy**. (B1-B4) HeLa cells pre-treated with 1.0 mM GSH and then incubated with 5.0 μM **NBD-OBodipy**. (C1-C4) HeLa cells pre-treated with 1.0 mM Cys and then incubated with 5.0 μM **NBD-OBodipy**. (D1-D4) HeLa cells pre-treated with 1.0 mM NEM and then incubated with 5.0 μM **NBD-OBodipy**. Emission was collected at 500-550 nm for green channel (excited at 476 nm) and 650-710 nm for red channel (excited at 633 nm). Scale bar: 40 μm .

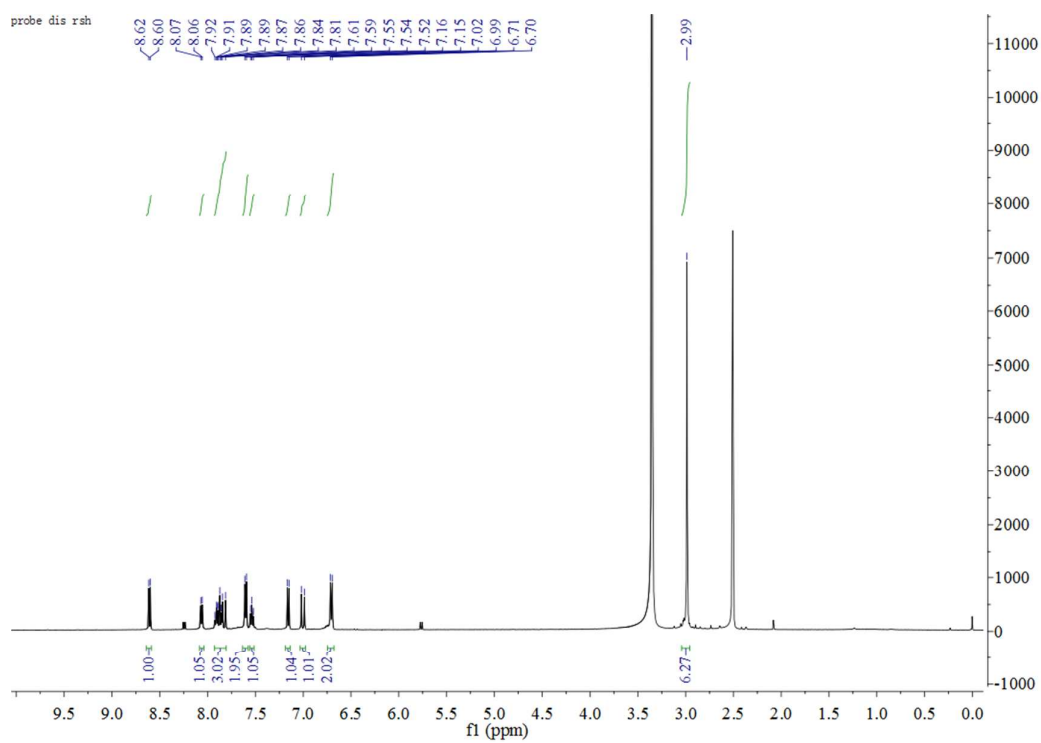


Figure S25. ^1H NMR spectrum of NBD-OF.

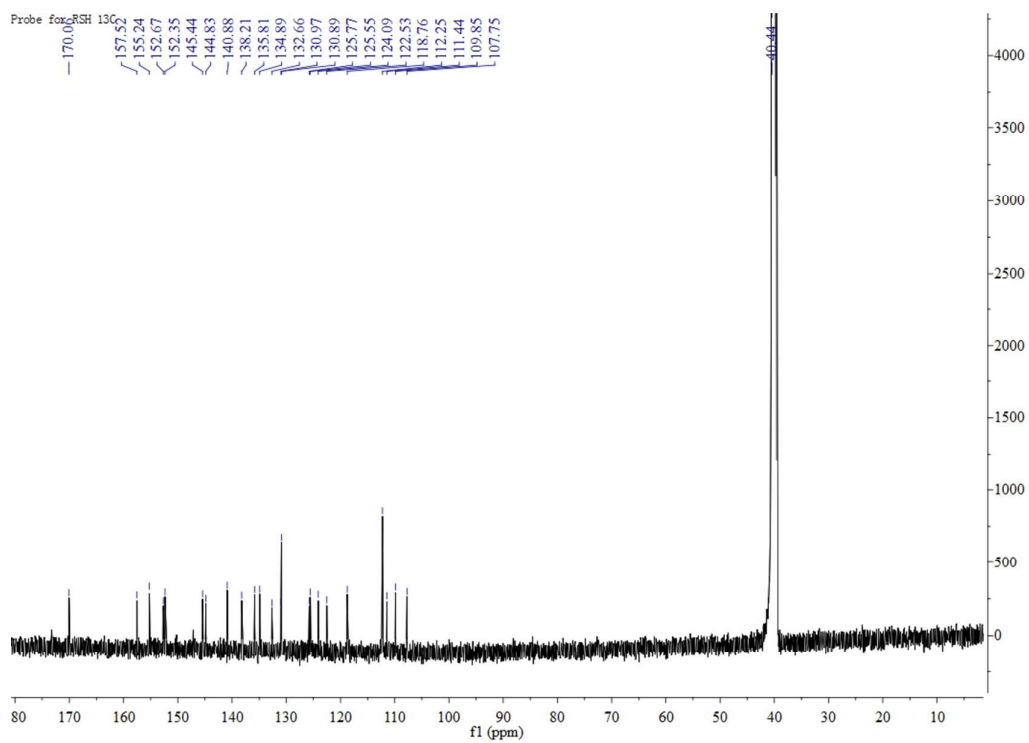


Figure S26. ^{13}C NMR spectrum of NBD-OF.

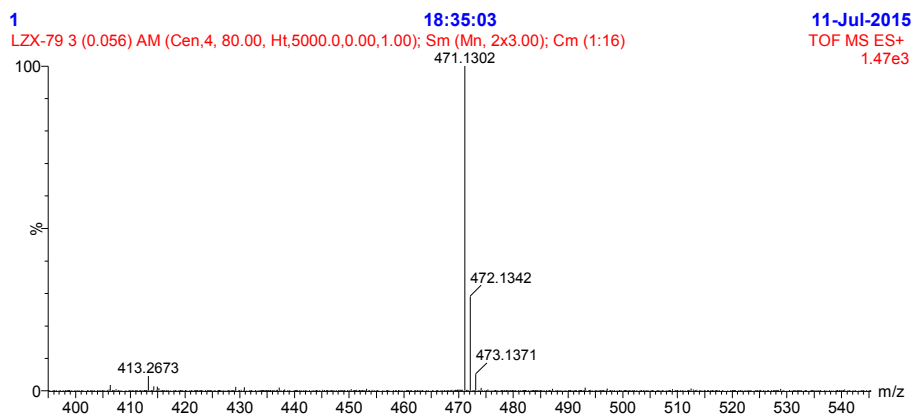


Figure S27. HRMS spectrum of NBD-OF.

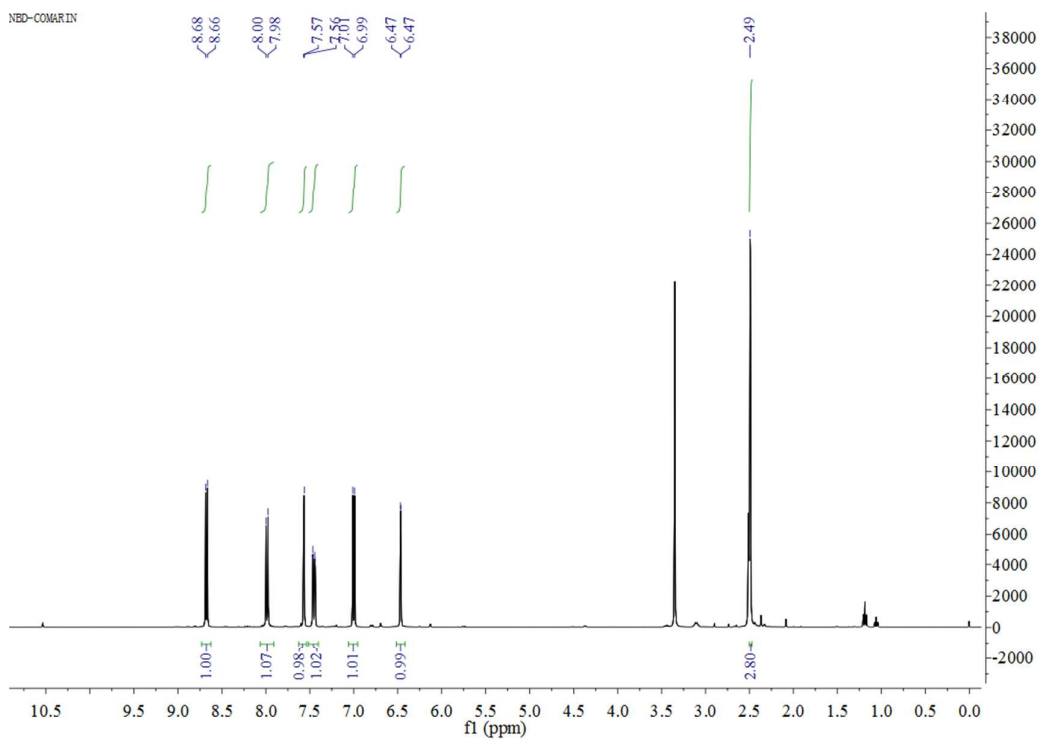


Figure S28. ^1H NMR spectrum of NBD-OCoumarin.

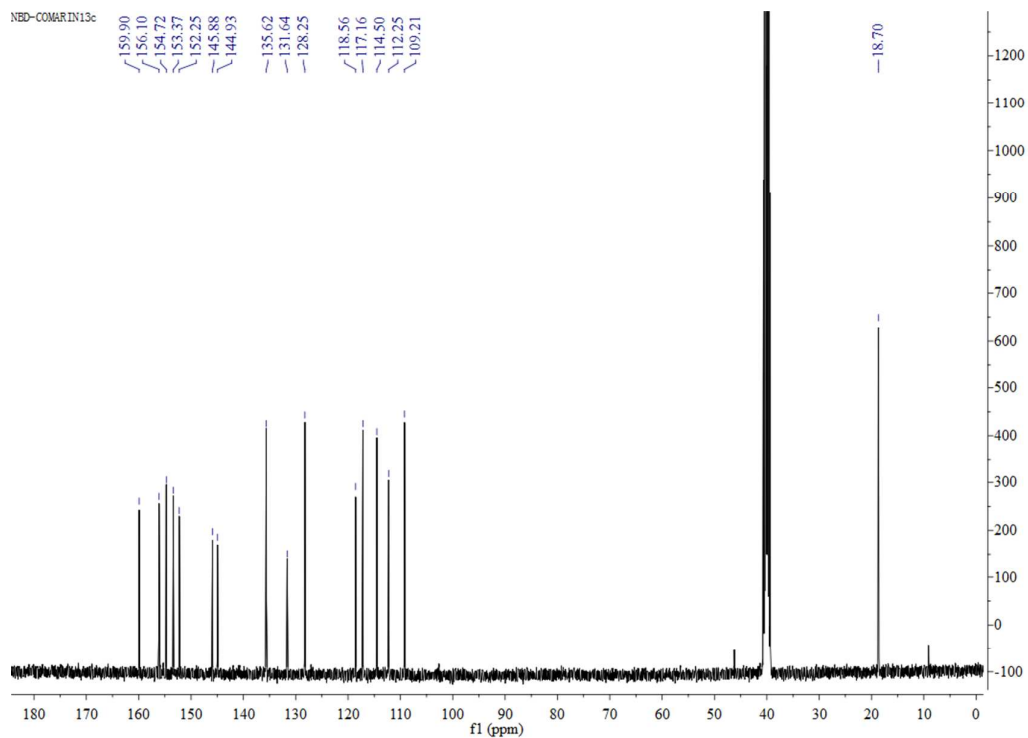


Figure S29. ^{13}C NMR spectrum of NBD-OCoumarin.

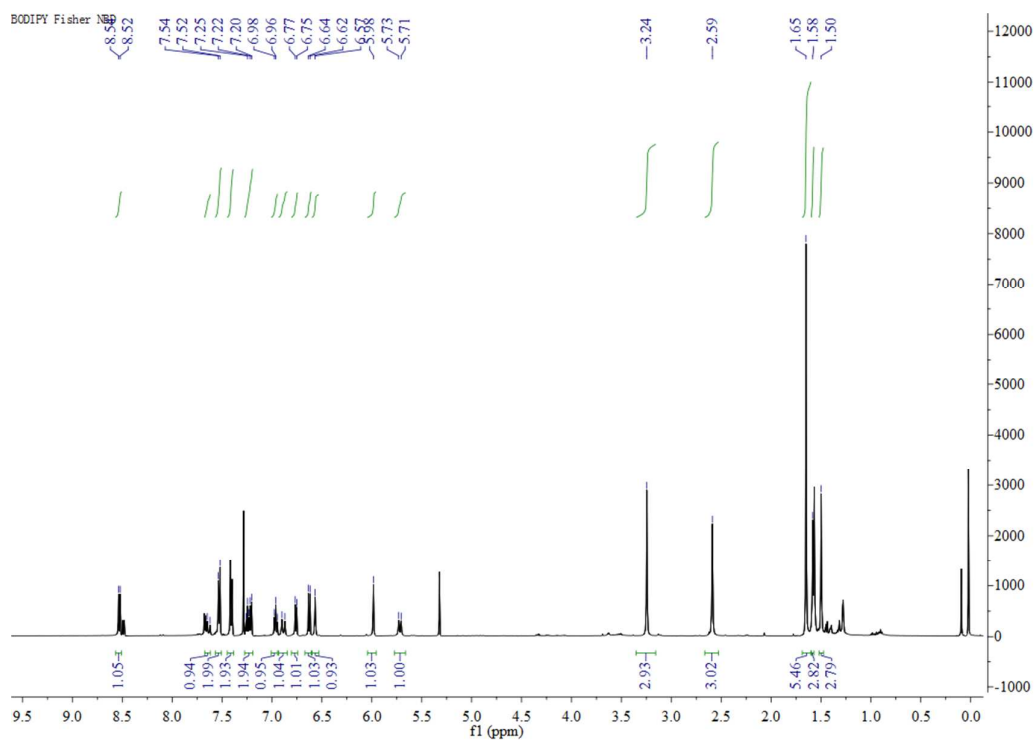


Figure S30. ^1H NMR spectrum of NBD-OBodipy.

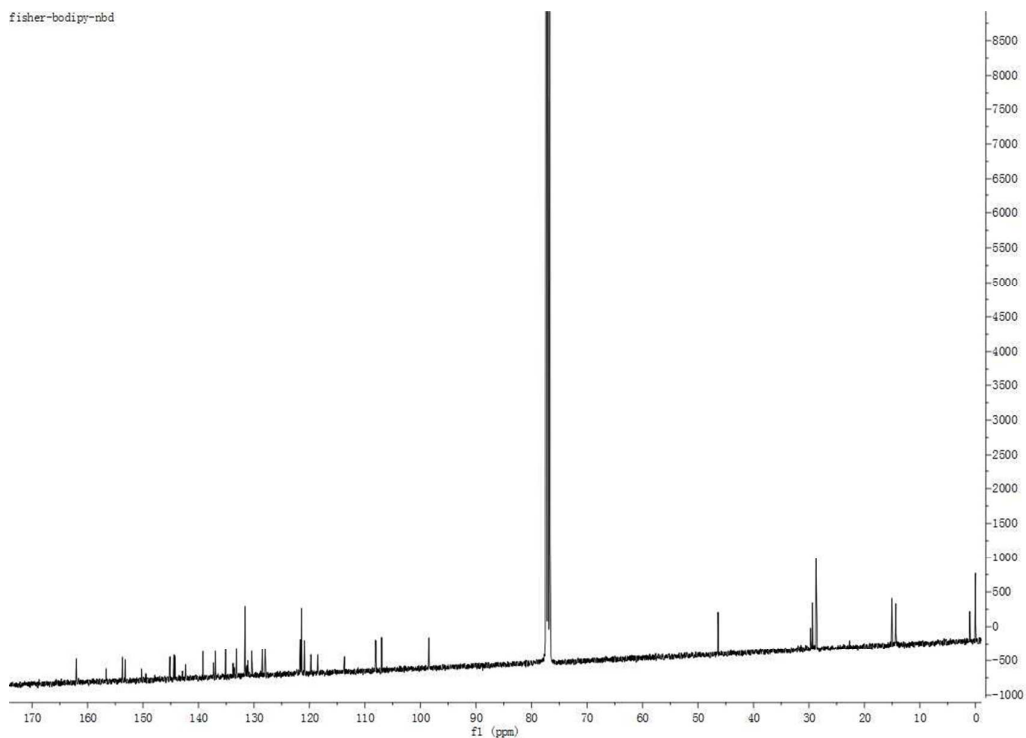


Figure S31. ^{13}C NMR spectrum of NBD-OBodipy.

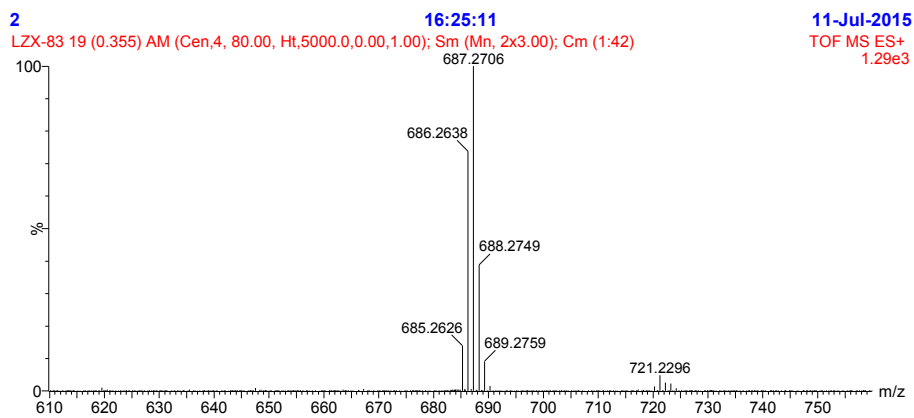


Figure S32. HRMS spectrum of NBD-OBodipy.